参考資料２

血小板製剤に対する感染症因子低減化血小板の市販後調査及び観察研究に関する文献集

A prospective observational cohort safety study of 5106 platelet transfusions with components prepared with photochemical pathogen inactivation treatment

Jean C. Osselaer, Nathalie Messe, Tor Hervig, Jose Bueno, Emma Castro, Aurora Espinosa, Patrizia Accorsi, Klaus Junge, Michele Jacquet, Jocelyne Flament, and Laurence Corash

BACKGROUND: Inactivation of pathogens and white blood cells in platelet (PLT) components with amotosalen and UVA light (INTERCEPT, Cerus Europe BV) has entered clinical practice in European blood centers. A prospective cohort study was implemented to characterize the safety profile of this new PLT component in a broad patient population.

STUDY DESIGN AND METHODS: Apheresis or buffy-coat PLT components were leukoreduced, suspended in approximately 35 percent plasma and 65 percent PLT additive solution, and treated with the INTERCEPT process. Blood centers were requested to complete a safety data form after each transfusion.

RESULTS: Data for 5106 INTERCEPT components administered to 651 patients were monitored. A total of 5051 (98.9%) transfusions and 609 (93.5%) patients had no reported reactions. Fifty-five (1.1%) transfusions were associated with adverse events, and 42 (0.8%) were possibly, probably, or related to the PLT transfusion. Adverse events occurred in 42 (6.4%) patients, but in only 32 (4.9%) patients was a causal relationship to PLT transfusion established. One reaction was serious, and no deaths were related to PLT transfusion. Among the transfusions reactions, the most frequent clinical events in descending frequency were chills, fever, dermatologic reactions, dyspnea, nausea or vomiting, and hypotension. No episodes of transfusion-related acute lung injury were reported.

CONCLUSIONS: In this cohort study, 99.2 percent of transfusions were without reactions attributed to PLTs. INTERCEPT PLTs exhibited a safety profile similar to that previously reported for conventional PLT components.

In late 2002 a photochemical treatment (PCT) process (INTERCEPT Blood Systems, Cerus Europe BV, Leusden, Netherlands) for inactivation of pathogens and white blood cells that may contaminate platelet (PLT) components received CE Mark registration and became available for routine use within certain European countries. During the clinical development of this technology, randomized controlled trials were conducted in selected patient populations frequently supported with PLT transfusions during periods of thrombocytopenia.[1,2] By necessity for conduct of the clinical trials, these studies primarily enrolled patients with hematology–oncology disorders. The trials focused on posttransfusion PLT count increments[1] and on assessments of hemostatic efficacy in

ABBREVIATIONS: DSMB = data and safety monitoring board; HPC(s) = hemovigilance plan coordinator(s); PCT = photochemical treatment.

From the Blood Transfusion Center, Cliniques Universitaires de Mont Godinne, Université Catholique de Louvain, Yvoir, Belgium; Haukeland University Hospital, Bergen, Norway; Centro de Transfusión, Cruz Roja Española, Madrid, Spain; St Olav Hospital, Trondheim, Norway; Centro Trasfusionale, Ospedale di Pescara, Pescara, Italy; Omega Mediation, Offenbach, Germany; Transfusion Therapies, Baxter Healthcare, Nivelles, Belgium; and Medical Affairs, Cerus Corp., Concord, California.

Address reprint requests to: Laurence Corash, MD, Medical Affairs, Cerus Corp., 2411 Stanwell Drive, Concord, CA 94520; e-mail: larry_corash@cerus.com.

Supported by research grants from Cerus Corp.

Disclosures: Jocelyne Flament was an employee of Baxter Healthcare Corp. during conduct of the study. Laurence Corash was an employee of Cerus Corp. during conduct of the study.

Received for publication April 16, 2007; revision received December 3, 2007, and accepted December 6, 2007.

doi: 10.1111/j.1537-2995.2008.01643.x

TRANSFUSION 2008;48:1061-1071.

patients with relatively stable thrombocytopenia requiring repeated PLT transfusions.[2] Based on the design of these clinical trials, the number of patients studied was determined by the statistical power required to assess the primary endpoints. In the European trial of whole blood–derived buffy-coat PLT components, 52 patients received 311 PCT PLT component transfusions. To assess hemostatic efficacy, a larger study was conducted in the United States in which 318 patients received 2678 PCT PLT component transfusions. In both studies patients were assessed specifically for acute transfusion reactions for 6 hours after study transfusions and for other adverse events for either 7[2] or 28 days[1] after the last study PLT transfusion. In both studies the incidence of acute transfusion reactions after receipt of photochemically treated PLTs was low, and the safety profile was similar to that of conventional PLTs.[3]

In general, prospective studies regarding the safety of PLT transfusion have been limited. The largest prospective PLT transfusion study before the SPRINT study[2,3] was the TRAP study of PLT alloimmunization, which enrolled 533 patients treated with 6379 transfusions, but this study did not specifically examine safety.[4] After CE Mark registration of the INTERCEPT system, an observational cohort safety study was implemented to prospectively collect information on at least 5000 PLT transfusions to extend the safety profile of PLT components prepared with PCT administered to a broad patient population.

MATERIALS AND METHODS

General study design

Blood transfusion centers with the INTERCEPT Blood System for PLTs for routine production of PLT components were invited to participate in this study. Patients in clinical care institutions who received PLTs prepared with PCT were specifically monitored for adverse health effects for 24 hours after each transfusion; however, there was no time limitation on reporting adverse events after transfusion. The sole inclusion criterion for enrollment was receipt of at least one PLT component prepared with PCT. Patients who received PLT transfusions administered in an outpatient clinic were observed for approximately 6 hours after transfusion and assessed before discharge. Study personnel contacted outpatient transfusion recipients the following day to complete the assessment with the standard data record form. There were no other inclusion or exclusion criteria. Patients in this study received only PLT components prepared with pathogen inactivation treatment.

The study was designed as a prospective, single cohort observational study to be consistent with European Hemovigilance Network recommendations for surveillance of adverse reactions to transfusion of labile blood components and with those of national transfusion services.[5,6] Study centers transfusing PLT components prepared with PCT for pathogen inactivation (INTERCEPT Blood System for Platelets) in routine clinical practice were requested to complete a report for each PLT transfusion regardless of whether or not an adverse event occurred following transfusion. Transfusions associated with serious adverse events were reported in greater detail. Patients were assigned a center specific study number to preserve anonymity.

Conduct of the study

In each study center blood transfusion service, hemovigilance plan coordinators (HPCs) were designated as responsible persons for the conduct of the hemovigilance plan and coordinated all the related activities on site. These HPCs, with expertise in transfusion medicine, were responsible for oversight of data collection, ensuring data completion, reviewing assessments for relation to PLT transfusion, and completion of reporting information on any adverse event after PLT transfusion regardless of potential relation to the transfusion.

Before initiation of the study, clinical care personnel were trained to the study protocol and the specific form for data collection. For each study PLT component issued for transfusion, a specific transfusion report form was issued with the PLT component (Fig. 1). This form was completed by the primary care physician and returned to the HPC in the blood center. The primary care attending physician was responsible for assessing the relation of adverse events to the PLT transfusion. The HPC reviewed the completed forms and contacted the primary care physician if data were incomplete or assessments of relation did not match the reported clinical data. The HPC had access to patient medical care records to query transfusion reports. The ultimate decision for assessment of the relation of adverse events to the PLT transfusion was the responsibility of the primary treating physician. HPCs were charged with populating the database, by completing electronic data entry. In centers where electronic data entry was not possible, paper forms were submitted and a sponsor representative populated the database. The active HPCs were Dr P. Accorsi, Pescara Italy (Site 02); Dr J.L. Bueno, Madrid Red Cross, Spain (Site 04); Dr A. Espinosa, Trondheim, Norway (Site 03); Dr T. Hervig, Bergen, Norway (Site 08); and Dr J.C. Osselaer, Mont Godinne, Belgium (Site 01).

A data and safety monitoring board (DSMB) was constituted to review the study protocol and provide oversight of the study. The DSMB reviewed an interim analysis of the data after 2500 transfusions and the final report after 5106 transfusions.

Study report forms

The report form used for this study was developed on the basis of hemovigilance report forms already in use and

PLATELET TRANSFUSION REPORT

Patient

Age : Sex : F ❑ M ❑

Hospital name and city :

_____

Location: Surgical ❑ Medical ❑ ICU ❑ Outpatient ❑ Other _____

Reporting

Reporting date :

Reporter name: _____

Signature: _____

INTERCEPT Platelet Characteristics and Transfusion

Type:

Apheresis platelets ❑

Random-donor platelets ❑

Characteristics:

Leucodepleted ❑ Irradiated ❑

Plasma depleted ❑ Other, specify ❑

Antigen matched ❑ _____

Product id #: _____ Blood Bank id #:_____

Date and time of transfusion :

Did the patient experience any acute adverse transfusion reaction within 24 hours after transfusion ?

Yes ❑ No ❑ ***If no, stop here***

If yes **:** Start date and time of adverse reaction:

Causal relation	Grade	
❑	Unrelated	0 ❑ Isolated dysfunction without clinical or biological manifestation
❑	Probably Unrelated	1 ❑ Absence of immediate or long-term life-threatening
❑	Possibly Related	2 ❑ Long-term life-threatening
❑	Probably Related	3 ❑ Immediate life-threatening
❑	Related	4 ❑ Death

Symptoms / Signs

Fever	❑	Urticaria	❑	Dyspnea	❑	Shock	❑
Chills	❑	Skin rash	❑	Nausea/vomiting	❑	Platelet refractoriness	❑
Cardiac arrhythmia	❑	Jaundice	❑	Lower back pain	❑	TRALI	❑
Hypotension	❑	Pulmonary oedema	❑	Chest / abdominal pain	❑	Other	❑
Itching	❑	Bronchospasm	❑			Specify:_____	

Clinical signs

Parameters	Before transfusion	After transfusion
Temperature	• °C ❑ ND	• °C ❑ ND
Blood pressure (Systolic/Diastolic)	- mmHg ❑ ND	- mmHg ❑ ND
Pulse	per min ❑ ND	per min ❑ ND

Transfusion-related laboratory abnormalities

Parameters (specify)	Date	Value	Unit

Bacteriological assessments

Blood culture ❑	Date	❑ Negative or ❑ Positive If positive, strain: _____
Platelet culture ❑	Date	❑ Negative or ❑ Positive If positive, strain: _____

Conclusion/Diagnosis/Additional examinations (e.g. X-Ray, …)

Please keep a copy of this report in the patient file and do not forget to complete a "Delayed Transfusion Reaction Report" if any delayed transfusion reaction occurs (e.g. GVHD, …)

Fig. 1. Case report form for reporting responses to PLT transfusions and classification of adverse events after transfusion.

DELAYED TRANSFUSION REACTION REPORT

Patient

Age : Sex : F ❑ M ❑

Hospital name and city :

_____

Location: Surgical ❑ Medical ❑ ICU ❑
Outpatient ❑ Other _____

Reporting

Reporting date :

Reporter name: _____

Signature: _____

Start date of the delayed transfusion reaction:

Suspected or confirmed diagnosis

Products transfused that can be related to this event (by decreasing imputability order)

Type of product (RBC/SDP platelets/random donor platelets/plasma/other)	Product number	Date of transfusion	Blood bank ID

Comments

Please keep a copy of this report in the patient file

Fig. 1. ***Continued.***

was reviewed by the DSMB before utilization (Fig. 1). Information was collected in several specific categories: patient demographics, PLT component characteristics, transfusion time, transfusion-related events, documentation of adverse events, causal relation, severity, symptoms, clinical findings, and laboratory data. For each transfusion the following signs, symptoms, and specific clinical syndromes were evaluated with a checklist format: fever, chills, cardiac arrhythmia, hypotension and/or hypertension, itching, urticaria, skin rash, jaundice, pulmonary edema, bronchospasm, dyspnea, respiratory distress, nausea, vomiting, lower back pain, chest pain, abdominal pain, clinical shock, refractoriness to PLT transfusion, and transfusion-related acute lung injury (TRALI). Criteria were provided for the diagnosis of TRALI.[7] Any other findings classified as adverse events were entered as free text. The following clinical signs were recorded before and after each transfusion: temperature, blood pressure, and heart rate. After study transfusions, abnormal clinical laboratory values, results of diagnostic procedures, chest radiographs, and bacterial cultures from patient and blood component sources were recorded from the medical record as required to verify the adverse event and relation to transfusion. A supplemental form was provided, on which adverse events occurring more than 24 hours after the transfusion, considered as potential delayed transfusion reactions, were reported with free text to define the event and the relationship to the imputed PLT component.

The relation of the adverse event to the PLT transfusion was classified within the following categories: unrelated, probably unrelated, possibly related, probably related, and related. Adverse events classified as possibly, probably, or related to transfusion were defined as transfusion reactions. Adverse events and transfusion reactions were graded for clinical severity by the HPC within the following categories: Grade 0 = isolated dysfunction without clinical or biologic manifestation; Grade 1 = absence of immediate or long-term life-threatening effects; Grade 2 = long-term life-threatening effects; Grade 3 = immediate life-threatening effects; and Grade 4 = death.

Preparation of PLT components

PLT components were collected by apheresis or by whole blood–derived buffy-coat procedures from volunteer donors according to standard operating procedures at each center. Donors were screened and tested for transfusion-transmitted pathogens according to each center's standard operating procedures in compliance with respective national regulations. All components were leukoreduced, either by filtration or by process leukodepletion. PLT components (containing 2.5×10^{11}-6.0×10^{11} PLTs) were suspended in approximately 35 percent plasma and 65 percent PLT additive solution (AS; Intersol, Baxter Transfusion Therapies, La Chatre, France) and prepared with amotosalen-HCl (nominal final concentration 150 µmol/L) and a 3 J per cm^2 UVA light treatment (320-400 nm) according to the manufacturer's instructions (Cerus Europe BV).[8] Treated PLT components were stored for up to 5 days with temperature control (22-24°C) according to standard operating procedures for each center in compliance with national regulations. Photochemical pathogen inactivation treatment was used in place of bacterial detection to prevent bacterial contamination and in place of gamma irradiation for prevention of transfusion associated graft-versus-host disease (GVHD) at all centers except Trondheim, Norway.

PLT transfusion

Primary care physicians ordered PLT components for transfusion according to standard indications within each institution. Primary care physicians prescribed pretransfusion medication per standard of care. Participating primary care physicians were requested to report all adverse health effects for a period of 24 hours after each transfusion with the standard report form and could report any adverse health effects after a study transfusion without time limitation.

Statistical analyses

A detailed statistical analysis plan for the study was prepared and approved before analysis. All statistical analyses, summary tables, and data listings were generated with computer software (SAS Version 8.2, SAS Institute, Cary, NC). The primary assessment was the incidence of transfusion reactions. The number and proportion (%) of transfusions and of patients with one or more transfusion reactions were summarized overall, by seriousness and by relationship to PLT transfusion. Corresponding 95 percent confidence intervals (CIs) were calculated for the overall summaries on a per-transfusion basis. In addition, the patient population profile, the characteristics of the PLT transfusions, and the characteristics of the adverse events after PLT transfusion were analyzed. Analyses to identify risk factors potentially associated with transfusion reactions were conducted with multivariate logistic regression analysis and by assessing association at a 10 percent significance level.

Data were analyzed on a per-transfusion or a per-patient basis as appropriate. All INTERCEPT PLT transfusions administered to patients were part of the full analysis population and were analyzed, whether or not an adverse event was observed. All analyses were conducted with this full analysis population.

RESULTS

A total of 5106 transfusions with PLT components prepared with PCT were documented during the study from October 1, 2003, to December 16, 2005, and constitute the full analysis population of this study. Overall, 4494 transfusion reports (88.0%) were issued from Mont Godinne, Belgium; 282 transfusion reports (5.5%) from Bergen, Norway; 189 transfusion reports (3.7%) from Madrid, Spain; 139 transfusion reports (2.7%) from Trondheim, Norway; and 2 reports from Pescara, Italy.

Study patient demographics

A total of 651 patients received transfusions during the conduct of this hemovigilance plan (Table 1). Slightly more patients were male. The median age for all patients was 65 years (range, <1-93 years). The majority of patients received PLT transfusions in nonintensive care hospital locations, although a substantial number of study PLT components were transfused in intensive care units and a small proportion in outpatient clinics (Table 1). Hematooncology diseases with or without chemotherapy and/or stem cell transplant constituted 58.1 percent of the primary diagnoses among the transfused patient population (Table 1). A significant number of patients receiving PLT transfusion (26.9%) underwent cardiovascular surgery. Other diagnoses included surgical interventions (such as orthopedic, neurologic, obstetric, organ transplant, and multiple trauma). Additional primary indications for PLT transfusions were systemic sepsis due to unspecified sources, gastrointestinal bleeding, and sepsis secondary to localized infections.

TABLE 1. Patient demographics at first study transfusion

Patient characteristics (n = 651)	
Sex n (%)*†	
Male	385 (59.1)
Female	262 (40.2)
Age (years)	
Mean ± SD	61.2 ± 17.0
Median (range)	65 (<1-93)
Patient location†	
Intensive care unit	214 (32.9)
Outpatient care unit	46 (7.1)
Nonintensive care unit	391 (60.1)
Hematology-oncology patients	378 (58.1)
Conventional chemotherapy	315 (48.4)
Stem cell transplant (SCT)	47 (7.2)
No chemotherapy or SCT	16 (2.5)
Surgery patients	221 (33.9)
Cardiovascular	175 (26.9)
Organ transplant	8 (1.2)
Other surgical procedures	38 (5.8)
Other diagnoses	52 (8.0)

* Four patients had missing data for sex.
† Data are reported as number (%).

Overall, 223 patients (34.3%) had no previous transfusion history at time of the first study PLT transfusion, and 362 patients (55.6%) had already received another blood product before the first study transfusion. Information about transfusion history was missing for 66 patients (10.1%). Among the 362 patients with a transfusion history, 22 patients (3.4%) reported experiencing a transfusion reaction of some type in association with prior transfusions. The majority of the PLT components (3525/69.0%) were administered to patients who had already received another blood component before the first study PLT transfusion. Among these transfusions, 634 (12.4%) PLT products were transfused to patients reported to have experienced at least one transfusion reaction in the past.

PLT component demographics

A large proportion of the PLT components were transfused on nonintensive hospital care units. Most of the PLT components (90.3%) were administered to hematooncology patients. While a significant number of patients receiving study PLT components (26.9%) were undergoing cardiovascular surgery, they used only 5.5 percent of the total PLT components, because most of these patients required only one PLT transfusion episode.

Most of the study components were manufactured from apheresis collections (92.0% vs. 8.0% for buffy-coat products). All centers, except Trondheim, elected to use PCT PLTs without gamma irradiation (97.3%) for patients at risk of transfusion-associated GVHD based on reported data[9] showing that the photochemical process effectively inactivates T cells. Among the 5106 study products transfused, 158 PLT units (3.1%) were human leukocyte antigen (HLA) matched.

Extent of exposure

During the observational period, the range of study transfusions per patient was 1 to 156, with a mean of 7.8 ± 16.2 transfusions per patient. The median value was 2 transfusions per patient. A substantial proportion of patients (58.4%) received between 2 and 10 PLT components (Table 2).

Adverse events and transfusion reactions after PLT transfusions

On a per-transfusion basis, 55 of 5106 transfusions (1.1%; 95% CI, 0.81-1.40) were reported with adverse events after PLT transfusion and 3 of these (0.1%) were reported with serious adverse events. Forty-two transfusions (0.8%; 95% CI, 0.59-1.11) were reported with adverse events (Table 3) causally related to the proximate PLT transfusion; thus these transfusions were associated with a transfusion reaction. These adverse events were within the spectrum

of adverse events associated with transfusion reactions (Table 3). One of 42 transfusions was reported with a serious adverse event, Grade 3, causally related to PLT transfusion. All of the other transfusion-related adverse events were Grade 1. Thirteen transfusions had adverse events reported that were excluded as related to the transfusion (Table 4), and 2 of these transfusions had serious adverse events reported that were excluded as related to the transfusion.

On a per-patient basis, 42 patients (6.5%) experienced adverse events after study transfusions. Eight patients (1.2%) experienced adverse events after two different study transfusions, and 1 patient (0.15%) had adverse events after six different study transfusions. Among the patients experiencing adverse events after transfusion, 32 patients (4.9%) experienced adverse events attributed to the study PLT transfusion (possibly related, probably related, or related) and were classified as patients with a transfusion reaction. Three of the 42 patients had serious adverse events after PLT transfusion, but for only 1 patient was the serious adverse event attributed to the PLT transfusion. Of the 42 transfusion reactions in 32 patients, 33 transfusions were associated with a single symptom and/or sign, 8 with two, and 1 with six. Among these 42 transfusions classified as resulting in transfusion reactions, the time to the first reaction was variable (Table 5). Only 4 transfusions were preceded by medication to reduce potential transfusion reactions (antihistamines and corticosteroids for 3 and corticosteroids alone for 1 transfusion).

TABLE 2. Patient exposure to study PLT components

Number of study transfusions	Number of patients and proportion (%)
Received at least one study transfusion	651 (100)
Received only 1 transfusion	271 (41.6)
Received more than 1 transfusion	
From 2 to 10 transfusions	271 (41.6)
From 11 to 20 transfusions	47 (7.2)
From 21 to 40 transfusions	33 (5.1)
From 41 to 60 transfusions	11 (1.7)
From 61 to 80 transfusions	8 (1.2)
From 81 to 100 transfusions	7 (1.1)
More than 100 transfusions	3 (0.5)
Number of transfusions per patient	
Mean ± SD	7.8 ± 16.2
Range	1-156
Median	2

TABLE 3. Adverse events related to PLT transfusions classified as transfusion reactions*

Adverse event (clinical observation)	Number of events (%)
Chills	27 (36.0)
Fever	14 (18.6)
Urticaria	14 (18.6)
Dyspnea	4 (5.3)
Skin rash (not otherwise specified)	4 (5.3)
Nausea/vomiting	3 (4.0)
Itching	3 (4.0)
Other: flushing	3 (4.0)
Hypotension	3 (4.0)

* Adverse events (n = 75) reported after PLT transfusions attributed to the transfusion and classified as part of a transfusion reaction involving 42 of 5106 transfusions.

Transfusions associated with suspected bacterial sepsis

Five transfusions were associated with chills and fever or hypotension that met institutional criteria for suspicion of transfusion-associated sepsis, resulting in bacterial cultures of PLT components and patients (Table 6). Twenty-one other PLT components were cultured based on blood center surveillance practice, but were not associated with suspected sepsis, and all of these were sterile.

TABLE 4. Adverse events classified as unrelated to PLT transfusions

Patient	Adverse events*	Causality†	Grade‡	Basis for causality assessment
01-010	Fever, chills, nausea, vomiting	Probably unrelated	1	Prior infection under treatment
01-008	Cardiac arrhythmia	Probably unrelated	1	Condition before transfusion
01-096	Hypotension	Probably unrelated	3	Hypotension before transfusion
01-099	Chills	Probably unrelated	1	Anxiety crisis, not verified as chills, no fever
01-168	Chills, headache	Probably unrelated	1	Fever before transfusion with prior infection
01-098	Fever, chills, hypotension, flushing	Unrelated	3	Prior sepsis due to dental abscesses
01-106	Dyspnea, nausea, vomiting	Probably unrelated	1	Indwelling catheter infection documented
01-178	Fever, chills	Probably unrelated	1	Febrile neutropenia before transfusion
01-230	Chills	Probably unrelated	1	Event after RBC transfusion
01-389	Chills, nausea, vomiting	Probably unrelated	1	Coincident with other medications
01-421	Fever, dyspnea, chest-abdominal pain	Probably unrelated	1	Onset 2 hr after transfusion, blood cultures negative
01-427	Chills	Probably unrelated	1	Onset 76 min after transfusion, blood cultures negative
01-395	Chills	Unrelated	1	No fever increase, PLT unit culture negative

* Adverse events reported after PLT transfusion.
† Causal relation to transfusion as assessed by primary care physician.
‡ Adverse event severity grade where: Grade 0 = isolated dysfunction without clinical or biological manifestation; Grade 1 = absence of immediate or long-term life-threatening consequence; Grade 2 = long-term life-threatening consequence; Grade 3 = immediate life-threatening consequence; Grade 4 = death.

TABLE 5. Number of study transfusions before the first transfusion reaction

Transfusions before first reaction	Transfusions with reactions (n = 42)
1	8
2	6
3	5
4	5
5	2
6-11	7
11-20	3
>20	6

TABLE 6. Transfusions associated with suspected bacterial sepsis

Patient ID	Vital signs	Culture result
01-007	36.8/38.6°C BP* 150/80	Patient blood culture negative
01-039	36.1/36.1°C BP 140/80	Patient blood culture negative
01-464	Afebrile, severe hypotension	PC culture negative
01-178	39°C 12 hr after BP 120/80	PC culture negative
01-098	37.3/39.9°C	PC negative, dental abscess positive
	BP 60/40	

* BP = blood pressure; PC = platelet component.

Patient 01-007 had chills, fever, and urticaria after a transfusion. Culture of a tubing segment was positive for micrococcus, but blood culture from the patient was negative. The urticaria was attributed to antibiotic medication. The tubing segment culture result was considered a laboratory contaminant. Patient 01-039 experienced chills without fever, but with dyspnea after a transfusion. No other symptoms or signs were reported. Culture of the administration tubing set was positive for coagulase-negative staphylococcus, but blood cultures from the patient were negative. Patient 01-464 experienced posttransfusion hypotension. Culture of a detached tubing segment was positive for *Staphylococcus warneri,* but culture of the PLT component and blood cultures were negative. Patient 01-178 developed fever and chills after a PLT transfusion with a positive blood culture for *Escherichia coli*. Culture of the PLT component, however, was negative. Patient 01-098 developed fever (39.9°C) with chills and hypotension 12 hours after a PLT transfusion. Blood culture was positive for the presence of *Actinomyces,* but culture of the associated PLT component was negative. Subsequently, the source of sepsis was identified as a dental abscess. In summary, no posttransfusion adverse events suspicious for transfusion-associated sepsis were confirmed with concomitant-positive PLT component and patient blood cultures.

Serious adverse events after PLT transfusion

Three serious adverse events were reported after PLT transfusion. Patient 01-096 had severe hemodynamic instability after liver biopsy associated with bleeding and was transferred to intensive care. After the onset of hemorrhage, she received a study PLT transfusion followed by severe hypotension (blood pressure, 42/22; heart rate, 92 beats/min). She was treated with fresh-frozen plasma (FFP), vasopressors, and fibrinogen and recovered. The primary care physician assessed the event as probably unrelated to the PLT transfusion and attributed the hypotension secondary to hepatic hemorrhage with hemodynamic instability.

Patient 01-098, receiving chemotherapy for acute leukemia, experienced fever, chills, and hypotension 12 hours after his 31st PLT transfusion. Subsequent blood cultures were positive for the presence of *Actinomyces,* but the PLT component culture was negative. The source of sepsis was attributed to a dental abscess and was classified as unrelated to the PLT transfusion.

Patient 01-464 developed hemorrhage during mitral valve surgery and was treated with PLT transfusions and methylene blue FFP. He experienced hypotension after the second study transfusion. Cultures of the PLT component and blood cultures were negative. One day later the patient experienced a second hypotension episode after transfusion of red blood cells (RBCs). The investigator attributed the event as an allergic adverse event related to the PLT transfusion. The patient had no other allergic symptoms. The patient recovered and was discharged in good condition.

Risk factors associated with transfusion reactions

Both patient and PLT component characteristics were analyzed for association with transfusion reactions. The analyses showed that 6.0 percent of male patients experienced at least one transfusion reaction and 7.3 percent of the female patients experienced at least one transfusion reaction ($p = 0.59$; odds ratio [OR], 1.19). Stratification by age showed that 4.6 percent of the patients older than 64 years of age presented with at least one transfusion reaction compared to 8.3 percent of patients below 64 years of age ($p = 0.06$; OR, 0.54). Finally, 8.8 percent of patients with a prior history of transfusion experienced at least one reaction while only 4.5 percent of patients with no prior transfusion history experienced at least one reaction ($p = 0.07$; OR, 1.99). These factors may be confounded with the diagnostic category of the patients; however, there were no significant associations between diagnostic category and transfusion reactions. Most of the transfusions with attributed reactions were associated with apheresis preparations. Only one random-donor PLT component was reported with a transfusion reaction, but this low incidence of reactions was most likely due to the

disproportionate use of apheresis components in this study and not a true effect of preparation method.

Extent of exposure before the first transfusion reaction

Among the 42 transfusions associated with a transfusion reaction, reactions occurred after single and multiple transfusions (Table 5). These data suggest that repeated exposure to INTERCEPT PLTs did not increase the likelihood of a transfusion reaction. Among the 10 patients without a previous transfusion history who experienced a transfusion reaction, 3 patients had an event at the first PLT transfusion, 1 after the 4th, and 6 patients after more than 5 transfusions. Patient 01-113 experienced the first reaction (urticaria and flushing) after the 139th INTERCEPT transfusion. Thus, for this patient population without prior blood product exposure, the risk of a transfusion reaction after INTERCEPT PLTs did not appear to increase with increased exposure.

DISCUSSION

Generally when a new type of labile blood component is introduced into routine clinical practice, initial information characterizing the safety profile is derived from a limited number of observations during the clinical development phase.[3] The introduction of PCT PLTs into routine clinical practice provided an opportunity to collect more information on the tolerability and safety of PCT PLTs in a broader patient population and under routine clinical conditions in contrast to a clinical trial environment. This approach is consistent with the recent recommendation from a consensus conference that new blood safety technologies should be evaluated with postmarketing hemovigilance studies.[10]

A prospective observational study with obligatory reporting for all transfusions regardless of outcome was designed to assess the safety profile of PCT PLTs in routine clinical practice. The data from the present study represent the largest prospective experience to date for recording potential adverse events associated with PLT transfusions compared to prior studies of retrospective design and limited size.[11-14] This study was planned to be consistent with European hemovigilance practices in which reporting of all grades of transfusion-associated reactions has been emphasized.[5,6] In contrast to passive hemovigilance studies, in this study obligatory reporting for all PLT transfusions was required irrespective of outcome. This study focused on adverse events that could be linked to PLT transfusions, specifically in the first 24 hours after transfusion, but there were no specific limitations on when adverse events could be reported after transfusion. This study captured information on repeated transfusions within patients to determine potential effects of repeated exposure to this new type of PLT component.

A potential limitation of this study was the absence of a concurrent control group receiving conventional PLT components with which to determine a comparative incidence of acute transfusion reactions. Another limitation was the potential for overreporting due to the absence of a blinded design and the increased awareness among observers that a new type of PLT component was under evaluation.

These potential limitations were addressed in several ways. A large portion of the transfusions were administered at the Mont Godinne Blood Transfusion Center, which had prospectively collected data for both PLT and RBC transfusions during an 18-month period before routine implementation of PLT components treated with pathogen inactivation. After the universal introduction of treated PLT components into clinical use at this center, the methods for RBCs did not change. During both periods of observation, PLT ASs were used to reduce exposure to allogeneic plasma.[15] Thus, in this center, we were able to compare the prevalence of transfusion associated adverse events with the same group of observers for one component with a new intervention (PCT PLTs) and another component that was unchanged (RBCs). Based on a comparison of the two observation periods, Osselaer and coworkers[15] reported a significant reduction in reactions to treated PLT components, from 1.3 to 0.9 percent ($p = 0.02$), while the incidence of reactions to RBCs was equal in both periods (0.4%). The experience from this two-period, two-component analysis suggested that observer sensitivity for overreporting did not occur. In addition, these data provided a background rate for acute transfusion reactions for leukoreduced PLT components with PLT AS (1.3% of transfusions).

Other estimates on the background prevalence of transfusion reactions can be obtained from the literature. On a per-transfusion basis, the prevalence has been reported to range from 18 to 31 percent; however, these studies were conducted some years ago with variable methods of PLT preparation.[11,16-18] More recently, the incidence of moderate and severe transfusion reactions has been reported from the TRAP study, which examined 8769 PLT transfusions in 598 patients during induction therapy for acute leukemia.[19] The overall incidence of reactions was 2.2 percent of transfusions, and 22 percent of patients experienced at least one transfusion reaction. In comparison to the TRAP trial, in this study in which all grades of reactions were reported, both the proportion of transfusions associated with a reaction (0.8%) and the proportion of patients (4.9%) experiencing at least one transfusion reaction causally attributed to a PLT component were lower.

Another comparison can be made with data from the hemovigilance network in France.[5] In that study, which

reported data for transfusion reactions during 2 years in which the reporting system was first implemented, an incidence of four events per 1000 PLT components (0.4%) was reported. This may be an underestimate, however, since each whole-blood PLT concentrate in a pool was tabulated as an individual component. More recently, Kerkhoffs and colleagues[14] compared the incidence of transfusion reactions for leukoreduced pooled PLT components in plasma and plasma with AS in a study of 168 patients and 765 transfusions. They observed an incidence of 5.5 percent of transfusions with reactions for PLTs in plasma versus 2.4 percent of transfusions for PLTs in a mixture of plasma and AS. On a per-patient basis, 9.5 percent of patients transfused with PLTs in plasma-ASs had reactions compared to 15.5 percent of patients supported with PLTs suspended in plasma.

In this study, which is the largest prospective PLT transfusion study to date specifically designed to capture all grades of transfusion reactions, the prevalence of reactions per transfusion and per patient was at the lower range of those reported in studies with conventional components. Younger patient age and prior exposure to blood transfusions were risk factors trending to a higher incidence of transfusion reactions. Recently, a higher rate of transfusion reactions also was reported in a hemovigilance survey of pediatric hematology patients.[20]

Prior exposure to INTERCEPT PLT transfusions did not increase the likelihood of a transfusion reaction. In comparison to other studies of PLT components in plasma-AS mixtures, the incidence of transfusion reactions on a per-patient basis for components prepared with PCT was reduced further. Importantly, this study enrolled a substantial number of patients with hematology–oncology disorders treated with complex therapies and supported with repeated PLT transfusions as well as surgical patients requiring PLT support. No incidents of TRALI, transfusion-transmitted bacterial sepsis, or death associated with acute transfusion reactions were observed in this study. Based on this experience in a broad patient population, PLT components prepared with PCT were well tolerated in routine clinical practice. The types and severity of acute reactions to PLT components prepared with pathogen inactivation treatment were consistent with previous reports of adverse events to conventional PLT components, and the data from this study provide additional data on the safety of PLT components treated with amotosalen and UVA light.

ACKNOWLEDGMENTS

The Data and Safety Monitoring Board was composed of the following members: Dr Harvey Klein, Division of Transfusion Medicine, National Institutes of Health, Bethesda, MD; Dr Paul Ness, Transfusion Medicine Division, The Johns Hopkins Hospital, Baltimore, MD; Dr Jacques Cinqualbre, CHU de Hautepierre, Strasbourg, France; Dr Miguel Lozano, Hospital Clinic Provincial, Hemotherapy and Hemostasis, Barcelona, Spain; Dr Daniele Sondag, Service Francophone du Sang, Brussels, Belgium; Dr Paul Strengers, International Society for Blood Transfusion, Amsterdam, Netherlands; and Dr Roger Dodd, Holland Laboratories, American Red Cross, Rockville, MD. The authors thank Dr Antonio Iacone, Centro Trasfusionale, Ospedale di Pescara, for reviewing the manuscript.

REFERENCES

1. van Rhenen DJ, Gulliksson H, Cazenave JP, Pamphilon D, Ljungman P, Kluter H, Vermeij H, Kappers-Klunne M, de Greef G, Laforet M, Lioure B, Davis K, Marblie S, Mayaudon V, Flament J, Conlan M, Lin L, Metzel P, Buchholz D, Corash L; euroSPRITE trial. Transfusion of pooled buffy coat platelet components prepared with photochemical pathogen inactivation treatment: the euroSPRITE trial. Blood 2003;101:2426-33.
2. 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 4th, Davis K, Lin JS, Metzel P, Corash L, Koutsoukos A, Lin L, Buchholz DH, Conlan MG. Therapeutic efficacy and safety of platelets treated with a photochemical process for pathogen inactivation: the SPRINT trial. Blood 2004;104:1534-41.
3. Snyder E, McCullough J, Slichter SJ, Strauss RG, Lopez-Plaza I, Lin J-S, Corash L, Conlan MG for the SPRINT Study Group. Clinical safety of platelets photochemically treated with amotosalen HCl and ultraviolet A light for pathogen inactivation: the SPRINT trial. Transfusion 2005;45:1864-75.
4. Slichter SJ, Davis K, Enright H, Braine H, Gernsheimer T, Kao KJ, Kickler T, Lee E, McFarland J, McCullough J, Rodey G, Schiffer C, Woodson R. Factors affecting post-transfusion platelet increments, platelet refractoriness, and platelet transfusion intervals in thrombocytopenic patients. Blood 2005;105:4106-14.
5. Andreu G, Morel P, Forestier F, Rebibo D, Janvier G, Herve P. Hemovigilance network in France: organization and analysis of immediate transfusion incident reports from 1994 to 1998. Transfusion 2002;42:1356-64.
6. Faber JC. Haemovigilance in Europe: now and tomorrow. In: Rouger P, Hossenlopp C, editors. Blood transfusion in Europe. Paris: Elsevier SAS; 2005. p. 213-32.
7. Ware L, Matthay M. The acute respiratory distress syndrome. N Engl J Med 2000;342:1334-49.
8. Janetzko K, Cazenave JP, Kluter H, Kientz D, Michel M, Beris P, Lioure B, Hastka J, Marblie S, Mayaudon V, Lin L, Lin JS, Conlan MG, Flament J. Therapeutic efficacy and safety of photochemically treated apheresis platelets processed with an optimized integrated set. Transfusion 2005; 45:1443-52.
9. Grass JA, Wafa T, Reames A, Wages D, Corash L, Ferrara. JL, Lin L. Prevention of transfusion-associated graft-versus-

host disease by photochemical treatment. Blood 1999;93: 3140-7.
10. Klein HG, Anderson D, Bernardi MJ, Cable R, Carey W, Hoch JS, Robitaille N, Sivilotti ML, Smaill F. Pathogen inactivation: making decisions about new technologies—preliminary report of a consensus conference. Vox Sang 2007;93:179-82.
11. 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.
12. deWildt-Eggen J, Nauta S, Schrijver JG, Kooy MvM, 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.
13. Heddle NM, Blajchman MA, Meyer RM, Lipton JH, Walker IR, Sher GD, Constantini LA, Patterson B, Roberts RS, Thorpe KE, Levine MN. A randomized controlled trial comparing the frequency of acute reactions to plasma-removed platelets and pre-storage WBC-reduced platelets. Transfusion 2002;42:556-66.
14. Kerkhoffs JH, Eikenboom JC, Schipperus MS, van Wordragen-Vlaswinkel RJ, Brand R, Harvey MS, de Vries RR, Barge R, van Rhenen DJ, Brand A. A muticenter randomized study of the efficacy of transfusions with platelets stored in platelet additive solution II versus plasma. Blood 2006;108:3210-5.
15. Osselaer JC, Doyen C, Messe N, Van Hooydonk M, Jacquet M, Sonnet A, Bosly A, Flament J, Corash L. Routine use of platelet components prepared with photochemical treatment (INTERCEPT platelets): impact on clinical outcomes and resources. Blood 2005;106:129a.
16. Heddle NM, Kalma L, Singer J, Richards C, Fedak P, Walker I, Kelton JG. The role of plasma from platelet concentrates in transfusion reactions. N Engl J Med 1994;331:625-8.
17. Mangano MM, Chambers LA, Kruskall MS. Limited efficacy of leukopoor platelets for prevention of febrile transfusion reactions. Am J Clin Pathol 1991;95:733-8.
18. Heddle NM, Klama LN, Griffith L, Roberts R, Shukla G, Kelton JG. A prospective study to identify risk factors associated with acute reactions to platelet and red cell transfusions. Transfusion 1993;33:794-7.
19. Enright H. Factors influencing moderate to severe reactions to PLT transfusions: experience of the TRAP multicenter clinical trial. Transfusion 2003;43:1545-52.
20. Angelini-Tibert MF, Currie C, Slaedts M, Corash LM, Rasongles P. Safety of platelet components prepared with photochemical treatment (INTERCEPT) transfused to pediatric oncology-hematology patients. Transfusion 2007;43(S3):22A.

市販後調査及び観察研究の報告②

Vox Sanguinis (2008) **94**, 315–323

ORIGINAL PAPER

DOI: 10.1111/j.1423-0410.2007.01035.x

An active haemovigilance programme characterizing the safety profile of 7437 platelet transfusions prepared with amotosalen photochemical treatment

J. C. Osselaer,[1] J. P. Cazenave,[2] M. Lambermont,[3] O. Garraud,[4] M. Hidajat,[5] L. Barbolla,[6] R. Tardivel,[7] L. Defoin,[1] C. Waller,[2] I. Mendel,[2] J. P. Raidot,[2] G. Kandel,[2] R. De Meuter,[3] P. Fabrigli,[4] D. Dehenau,[5] J. L. Arroyo,[6] F. Padrón,[6] H. Gouezec,[7] M. Corral,[8] M. Jacquet,[9] D. Sundin,[9] L. Lin[9] & L. Corash[9]

[1] *Blood Transfusion Center, Cliniques Universitaires de Mont Godinne, Universite Catholique de Louvain, Yvoir, Belgium*
[2] *EFS Alsace, Strasbourg, France*
[3] *Service du Sang, Belgian Red Cross, Erasme University Hospital, Brussels, Belgium*
[4] *EFS Auvergne Loire, St. Etienne, France*
[5] *Blood Transfusion, AZ St Jan AV, Brugge, Belgium*
[6] *Centro de Hemoterapia y Hemodonacion de Castilla y Leon, Valladolid, Spain*
[7] *EFS Bretagne, Rennes, France*
[8] *Hospital Universitario de Salamanca, Salamanca, Spain*
[9] *Cerus Corporation, Concord, CA, USA*

Vox Sanguinis

Background An active haemovigilance programme was implemented to survey adverse events (AE) associated with transfusion of platelets photochemically treated with amotosalen and ultraviolet A (PCT-PLT). The results of 5106 transfusions have already been reported. Here we report the results of an additional 7437 PCT-PLT transfusions.

Methods The focus of this ongoing haemovigilance programme is to document all AEs associated with PCT-PLT transfusion. Data collected for AEs include: time of event after starting transfusion, clinical descriptions, vital signs, results from radiographs and bacterial cultures, event severity (Grade 0–4) and causal relationship to PCT-PLT transfusion.

Results One thousand four hundred patients (mean 60 years, range 1–96) received PCT-PLT transfusions. The majority of the patients (53·4%) had haematology–oncology diseases and required conventional chemotherapy (44·8%) or stem cell transplantation (8·6%). Sixty-eight PCT-PLT transfusions were associated with AE. Acute transfusion reactions (ATR), classified as an AE possibly related, probably related, or related to PCT-PLT transfusions were infrequent ($n = 55$, 55/7437 = 0·7%) and most were of Grade 1 severity. Thirty-nine patients (39/1400 = 2·8%) experienced one or more ATRs. The most frequently reported signs/symptoms were chills, fever, urticaria, dyspnoea, nausea and vomiting. Five AEs were considered severe (≥ Grade 2); however, no causal relationship to PCT-PLT transfusion was found. Repeated exposure to PCT-PLT did not increase the likelihood of an ATR. No cases of transfusion-related acute lung injury and no deaths due to PCT-PLT transfusions were reported.

Conclusions Routine transfusion of PCT-PLT is well-tolerated in a wide range of patients. ATRs related to PCT-PLT transfusion were infrequent and most were of mild severity.

Received: 25 October 2007,
revised 24 December 2007,
accepted 24 December 2007,
published online 30 January 2008

Key words: PCT, platelets, haemovigilance, safety, INTERCEPT.

Correspondence: Lily Lin, Cerus Corporation, 2411 Stanwell Drive, Concord, CA 94520, USA
E-mail: llin@cerus.com

Introduction

INTERCEPT Blood System™ uses a photochemical treatment methodology [PCT: amotosalen plus ultraviolet A (UVA) light] to inactivate viruses, bacteria, protozoa, and leucocytes in platelet (PLT) and plasma components. The PLT system received CE Mark registration in Europe in 2002. Several centres in Belgium, Spain, Norway and Italy began routine production of PCT-PLT in 2003. An active haemovigilance programme was immediately implemented to prospectively collect information on PCT-PLT transfusions administered to patients in routine clinical settings. Prior to CE Mark registration, the safety data of PCT-PLT were primarily obtained from controlled clinical trials with a limited number of patients and predetermined clinical and safety end-points [1–3]. The postmarketing haemovigilance programme provided a means to extend the characterization of the safety profile of PCT-PLT in routine use and in a broad patient population. The results of the first 5106 PCT-PLT transfusions have already been reported [4]. With additional centres in Belgium, Spain and France starting with the routine production of PCT-PLT, the database of this haemovigilance programme has been expanded [5].

In March 2007, the Canadian Blood Services and Héma-Québec organized a consensus conference to provide recommendations and guide decision-making about new pathogen inactivation technologies [6]. The panel, consists of nine healthcare professionals and members of the public, stressed the importance of postmarketing surveillance studies in the introduction of new technologies for blood safety. The panel recommended that specific studies should be mandated by the regulatory authorities and supported by the manufacturers and/or the blood suppliers. Postmarketing surveillance for adverse reactions to pathogen inactivation products should be linked to the national haemovigilance systems if possible. Depending on the new pathogen inactivation technologies implemented, specific additional surveillance outcomes may be identified. The panel also suggested that chronically transfused patients might serve as an ideal surveillance population to identify long-term toxicities of pathogen-inactivated products.

The active haemovigilance programme described in this study is in concordance with these recommendations. Although this programme is not directly linked to a specific country haemovigilance system nor designed to replace any existing haemovigilance system, the format of data collection is modelled after the data collection format of the French haemovigilance system for documentation of transfusion incidents [7]. The focus of the current programme is on all adverse events (AE), serious or non-serious, occurring after the start of PCT-PLT transfusion. Following the recent report of 5106 PCT-PLT transfusions [4], here we report the results of an additional 7437 transfusions of PCT-PLT.

Materials and methods

General study design

This was a prospective observational active haemovigilance study. The objective of this study was to document the transfusion safety profile for approximately 7500 PCT-PLT components prepared with the INTERCEPT Blood System™ for platelets (Cerus Europe BV, Leusden, the Netherlands). These components were prepared in three centres in Belgium (CTS UCL Mont Godinne, CTS Brabant-Hainaut and AZ Sint Jan AV), three centres in France (EFS-Alsace, EFS-Auvergne-Loire and EFS-Bretagne), and one centre in Spain (CHEMCYL Valladolid) and administered to thrombocytopenic patients under standard clinical practice in hospitals. There were no randomization requirements, no inclusion criteria and no exclusion criteria of patients other than the need to receive a platelet transfusion. Baseline demographical information was collected on all study participants. Patients were assigned a centre-specific study number to preserve anonymity.

Patients who received transfusions of PCT-PLT were monitored for any AEs after the start of each platelet transfusion, which is consistent with European Haemovigilance Network recommendations for surveillance of AE to transfusion of labile blood components, and with those of national transfusion services [7,8]. However, in this study, reporting was obligatory for all PCT-PLT transfusions in each participating clinical site. A transfusion report was required for each PLT transfusion regardless of whether or not an AE occurred. In case of occurrence of an AE, additional clinical and biological information was collected to allow diagnosis and assessment of causality and severity. The data in the final database were anonymous and were reported on a per-transfusion basis as well as on a per-patient basis. Transfusions associated with serious AEs were reported in greater detail.

Study report forms

The report form used for this haemovigilance programme was developed on the basis of haemovigilance report forms already in use. Information was collected in several broad categories: patient demographic/diagnosis data, platelet component characteristics, transfusion events and documentation of all AEs following transfusion. An acute transfusion reaction (ATR) was defined as an AE possibly related, probably related, or related to a PCT-PLT transfusion.

AEs were graded for clinical severity within the following categories: Grade 0, isolated dysfunction without clinical or biological manifestation; Grade 1, absence of immediate or long-term life-threatening effects; Grade 2, long-term life-threatening effects; Grade 3, immediate life–threatening effects; and Grade 4, death. For each transfusion, the following

signs, symptoms and specific clinical syndromes were evaluated: fever, chills, cardiac arrhythmia, hypotension, itching, urticaria, skin rash, jaundice, pulmonary oedema, bronchospasm, dyspnoea, respiratory distress, nausea, vomiting, lower back pain, chest pain, abdominal pain, and shock. Any other findings could be entered as free text including refractoriness to platelet transfusion and transfusion-related acute lung injury. The following available clinical signs were recorded before and after each transfusion: temperature, blood pressure and heart rate. Abnormal clinical laboratory values, results of diagnostic procedures (chest X-ray) and bacterial cultures from patient and blood component sources were recorded when associated with an AE following a PCT-PLT transfusion.

Preparation of platelet components

Platelet components were collected by apheresis or from whole blood-derived buffy-coat procedures according to each centre's standard operating procedures. Volunteer donors were screened and tested for transfusion-transmitted pathogens according to each centre's standard operating procedures in compliance with respective national regulations. All components were leucocyte reduced, either by filtration (Sepacell PLS-5A, Asahi Biomedical, Tokyo, Japan) or process leucodepletion (Amicus Cell Separator, Fenwal, La Chatre, France; Haemonetics MCS+, Haemonetics, Braintree, MA, USA). Platelet components containing 2·5 to $6{\cdot}0 \times 10^{11}$ platelets were suspended in approximately 35% plasma and 65% InterSol™ (Fenwal) and prepared with amotosalen (nominal final concentration 150 μM) and a 3 J/cm^2 UVA light treatment (320–400 nm) according to the manufacturer's instructions for use (Cerus Europe BV). After treatment, PCT-PLTs were stored up to either 5 or 7 days under temperature-controlled conditions (22 ± 2 °C) before release for transfusion depending on country-specific regulations. PCT-PLTs were transfused before the expiration period of 5 days in France and Spain or 7 days in Belgium. PCT-PLTs were not cultured for bacterial contamination prior to release, and PCT was used in place of γ-irradiation for prevention of transfusion-associated graft-versus-host disease in all sites except EFS-Bretagne and EFS-Auvergne-Loire.

Platelet transfusion

PCT-PLT components for transfusion were ordered according to standard indications within each institution. The investigator was requested to report all AEs occurring after starting transfusion without time limitation. The severity of each AE (Grade 0 to 4) and the relationship of each AEs to the preceding platelet transfusion were assessed by the investigator. Serious adverse events were reported in greater detail with a narrative for each event.

Statistical analyses

All statistical analyses, summary tables and data listings were generated using SAS® version 8·2. The primary assessment of safety was the proportion of ATR for the transfusions reported. The safety profile of PCT-PLT transfusions included information on: the number of PCT-PLT transfusions by patient; the patient population profile; the characteristics of the PCT-PLT transfused, and the characteristics of the AE following platelet transfusion.

Data were analysed on a per-transfusion basis as well as on a per-patient basis. All PCT-PLT transfusions administered to a patient were included in the full analysis population, whether or not an AE was observed. Data were summarized for each parameter using descriptive statistics (mean, standard deviation, median, and range).

Statistical tests were performed for the exploration of risk factors only (multivariate logistic regression at 10% significant level). The variables included in the analysis are patient gender, age, previous transfusion history, type of platelet concentrate, γ-irradiation, antigen-matching and primary diagnosis. Variables with descriptive statistics were tested for *P* values and odds ratio. The number and proportion (%) of transfusions with one or more AEs were summarized overall, by seriousness and by relationship to platelet transfusion. Corresponding 95% confidence intervals (CIs) were calculated.

The non-survival analysis method is a univariate analysis of the number of transfusions received before the first occurrence of an AE. Only patients with at least one AE were considered in this analysis.

Results

Distribution of transfusions

A total of 7437 PCT-PLT transfusions were documented between May 2005 and January 2007 and constitute the full analysis population. The distribution of transfusion reports were: 3057 (41·1%) from CTS UCL Mont Godinne, 2048 (27·5%) from EFS-Alsace, 899 (12·1%) from CTS Brabant-Hainaut, 572 (7·7%) from EFS-Auvergne-Loire, 440 (5·9%) from AZ Sint Jan AV, 381 (5·1%) from CHEMCYL, and 40 (0·5%) from EFS-Bretagne.

Patient demographics

A total of 1400 patients underwent transfusion (Table 1). The majority of the patients were male (61·3%) and the mean age was 60 years (range < 1–96 years). Haematology–oncology diseases treated by chemotherapy (44·8%) and stem cell transplantation (8·6%) constituted 53·4% of the primary diagnoses and therapies among the transfused population. A significant number of patients receiving platelet transfusion (17·2%)

Table 1 Patient and transfusion demographics

	Patient characteristics (*n* = 1400)	Transfusion characteristics (*n* = 7437)
Gender (*n*, %)		
Male	858 (61·3%)	4354 (58·5%)
Female	542 (38·7%)	3082 (41·4%)
Unknown		1 (< 0·1%)
Age (years)		
Mean ± SD	60·0 ± 17·8	
Median	63	
(minimum–maximum)	(<1–96)	
Location of transfusion		
Intensive care unit		1145 (15·4%)
Outpatient		382 (5·1%)
Regular ward		5908 (79·4%)
Unknown		2 (< 0·1%)
Haematology–oncology patients	748 (53·4%)	5463 (73·5%)
Conventional chemotherapy	627 (44·8%)	4481 (60·3%)
Stem cell transplant	121 (8·6%)	982 (13·2%)
Surgery patients	241 (17·2%)	480 (6·5%)
Cardiovascular surgery	209 (14·9%)	349 (4·7%)
Solid organ transplantation	32 (2·3%)	131 (1·8%)
Other diagnoses	397 (28·4%)	859 (11·6%)
Missing diagnosis	14 (1·0%)	635 (8·5%)
History of a previous transfusion		
Yes	837 (59·8%)	5029 (67·6%)
No	398 (28·4%)	1927 (25·9%)
Unknown	165 (11·8%)	481 (6·5%)
If 'Yes' – did they experience a transfusion-related adverse event?[a]		
Yes	53 (6·3%)	382 (7·6%)
No	779 (93·0%)	4639 (92·2%)
Unknown	5 (0·6%)	8 (0·2%)

[a]For per-patient basis, the denominator is 837; for per-transfusion basis, the denominator is 5029.

were undergoing cardiovascular surgery or solid organ transplantation. Other diagnoses included haematology–oncology diseases not treated by chemotherapy and/or stem cell transplantation and surgery other than cardiovascular surgery and solid organ transplantation.

Of all patients, 837 patients (59·8%) had already received another blood product before the first PCT-PLT transfusion (Table 1). Among these patients, 53 patients (6·3% of 837) had a history of a transfusion reaction of some type in the past.

Platelet component demographics

Most of the PCT-PLT units were manufactured from apheresis platforms (4822, 64·8% vs. 2615, 35·2% for buffy-coat products). The majority of the PCT-PLTs (7357, 98·9%) were not treated with γ-irradiation [9]. Among the 7437 PCT-PLTs transfused, only 2·5% (189 units) of platelet units were human leucocyte antigen-matched products.

A large proportion of the PCT-PLT components (5908, 79·4%) were transfused in non-intensive care hospital wards (Table 1). Intensive care units and day-hospital units were the location for 15·4 and 5·1% of the PCT-PLT transfusions (1145 and 382 units, respectively). While most of the PCT-PLT components (5463, 73·5%) were administered to haematology–oncology patients, only 480 PCT-PLT components (6·5%) were administered to surgery patients.

The majority of the PCT-PLT components (5029, 67·6%) were administered to patients who had already received another blood component before the first PCT-PLT transfusion (Table 1). Among these transfusions, 382 (7·6% of 5029) PCT-PLT components were transfused to patients reported to have experienced at least one transfusion reaction in the past.

Number of transfusions per patient

The range of PCT-PLT transfusions per patient was 1 to 129, with an average of 5·3 ± 10·8 (median: 2) transfusions per patient. Of the 1400 patients who received PCT-PLT transfusions, 529 patients (37·8%) received only one PCT-PLT transfusion during this study period, 418 patients (29·9%) received two to three transfusions, and 453 patients (32·4%) received more than four PCT-PLT transfusions during the study. The majority of patients who received multiple transfusions had a primary diagnosis of haematology–oncology diseases treated by chemotherapy and/or stem cell transplantation.

Two patients from CTS UCL Mont Godinne received more than 100 transfusions analysed in this haemovigilance plan. One 56-year-old man (J01-636) who was treated by conventional chemotherapy for haematology–oncology disease received 129 PCT-PLT components within an 8-month period (from April 2006 to November 2006). One 72-year-old woman (J01-071) who was also treated by conventional chemotherapy for haematology–oncology disease received 107 PCT-PLT components within a 10-month period (from August 2005 to November 2006).

Adverse events following PCT-PLT transfusion

On a per-transfusion basis, 68 (0·9% of 7437 transfusions, 95% CI: 0·7–1·2%) transfusions were associated with an AE (Table 2). Of which, 55 (0·7% of 7437 transfusions, 95% CI: 0·6–1·0%) were classified as ATR possibly related, probably related, or related to PCT-PLT transfusion. Only five events were classified as serious AEs (0·07%, 95% CI: 0·0–0·2%), and were judged as probably unrelated to the PCT-PLT transfusion based on the observation of alternative causes for symptoms and no evidence of causal relationship to the platelet transfusion. No cases of transfusion-related acute lung injury and no deaths due to PCT-PLT transfusions were reported.

Table 2 Clinical characteristics of adverse events (AE)

	On a per-transfusion basis *n* (% = *n* × 100/7437)				On a per-patient basis *n* (% = *n* × 100/1400)			
	Any AEs	AE attributed to platelets (ATR)[b]	SAE[a]	SAE attributed to platelets[a,b]	Any AEs	AE attributed to platelets (ATR)[b]	SAEs[a]	SAE attributed to platelets[a,b]
Number with at least one event	68 (0·9%)	55 (0·7%)	5 (< 0·1%)	0 (0·0%)	45 (3·2%)	39 (2·8%)	4 (0·3%)	0 (0·0%)
Signs/Symptoms[c]								
Fever	8 (0·1%)	6 (< 0·1%)	0 (0%)	–	7 (0·5%)	5 (0·4%)	0 (0%)	–
Chills	45 (0·6%)	40 (0·5%)	2 (< 0·1%)	–	31 (2·2%)	28 (2·0%)	1 (< 0·1%)	–
Itching	2 (< 0·1%)	2 (< 0·1%)	0 (0%)	–	1 (< 0·1%)	1 (< 0·1%)	0 (0%)	–
Hypotension	1 (< 0·1%)	0 (0%)	1 (< 0·1%)	–	1 (< 0·1%)	0 (0%)	1 (< 0·1%)	–
Urticaria	14 (0·2%)	14 (0·2%)	0 (0%)	–	13 (0·9%)	13 (0·9%)	0 (0%)	–
Skin rash	5 (< 0·1%)	5 (< 0·1%)	0 (0%)	–	4 (0·3%)	4 (0·3%)	0 (0%)	–
Dyspnoea	8 (0·1%)	6 (< 0·1%)	1 (< 0·1%)	–	8 (0·6%)	6 (0·4%)	1 (< 0·1%)	–
Respiratory distress	1 (< 0·1%)	0 (0%)	1 (< 0·1%)	–	1 (< 0·1%)	0 (0%)	1 (< 0·1%)	–
Nausea/vomiting	8 (0·1%)	5 (< 0·1%)	3 (< 0·1%)	–	5 (0·4%)	3 (0·2%)	2 (0·1%)	–
Lower back pain	6 (< 0·1%)	1 (< 0·1%)	0 (0%)	–	2 (0·1%)	1 (< 0·1%)	0 (0%)	–
Chest/abdominal pain	1 (< 0·1%)	1 (< 0·1%)	0 (0%)	–	1 (< 0·1%)	1 (< 0·1%)	0 (0%)	–
Shock	4 (< 0·1%)	0 (0%)	4 (< 0·1%)	–	3 (0·2%)	0 (0%)	3 (0·2%)	–
Tachycardia	4 (< 0·1%)	3 (< 0·1%)	1 (< 0·1%)	–	3 (0·2%)	2 (0·1%)	1 (< 0·1%)	–
Other	14 (0·2%)	11 (0·1%)	3 (< 0·1%)	–	12 (0·9%)	10 (0·7%)	3 (0·2%)	–

[a]Serious adverse event (SAE): long-term life threatening, immediate life threatening or death.

[b]Causal relationship that was possibly related, probably related, or related to PCT-PLT transfusion.

[c]Number of signs/symptoms can exceed number of AE due to multiple observed signs/symptoms per AE.

On a per-patient basis, 45 patients (3·2% of 1400 patients) who received at least one transfusion of PCT-PLT experienced the 68 AEs following PCT-PLT transfusions (Table 2). Only 39 patients (2·8% of 1400 patients) experienced the 55 ATRs attributed to the PCT-PLT transfusion. Four patients experienced serious AEs following transfusion; however, no causal relationship to PCT-PLT transfusion could be established.

All AEs regardless of the relationship with the PCT-PLT transfusion occurred within 4 h after the start of the platelet transfusion (mean time: 0·3 ± 0·51 h, 0–3·3 h). The majority of AEs (64, or 94·1% of 68 AEs) occurred in patients who were not premedicated. The other four AEs occurred in patients who were premedicated with antipyretic or antihistaminic drugs, or corticosteroids.

Characteristics of clinical signs and symptoms associated with adverse event

On a per-transfusion basis, the most frequently observed symptoms/signs (≥ 0·1% of the total 7437 transfusions) were fever, chills, urticaria, dyspnoea, nausea and/or vomiting (Table 2). The individual incidence of each of the following signs/symptoms was < 0·1%: itching, hypotension, skin rash, respiratory distress, lower back pain, chest or abdominal pain, shock and tachycardia. All additional symptoms included in the category of other, such as refractoriness to platelet transfusion, hypertension, cephalea, pain in the leg, flush, malaise, cyanosis, oxygen desaturation and volume overload were also reported but with an individual incidence of less than 0·1%. Most of ATRs were described principally as Grade 1 chills and urticaria (Table 2).

On a per-patient basis, the most frequently observed symptoms/signs (≥ 0·5% of the total 1400 patients) were fever, chills, urticaria and dyspnoea (Table 2). Approximately 0·1–0·4% of the population (from 2 to 5/1400) experienced the following signs/symptoms: skin rash, nausea/vomiting, shock, lower back pain and tachycardia. Clinical refractoriness to transfusion, hypertension, headache and flushing were additional symptoms reported in the category of 'other'. Less than 0·1% of the study population (only 1/1400) experienced the following signs/symptoms such as hypotension, itching, respiratory distress and chest/abdominal pain. Symptoms such as pulse increase, leg pain, cyanosis, oxygen desaturation, malaise and/or volume overload were also reported in the category of 'other'. Most of the ATRs consisted of various combinations of fever (0·4%), chills (2·0%), urticaria (0·9%), skin rash (0·3%), dyspnoea (0·4%), nausea/vomiting (0·2%), tachycardia (0·1%) and others symptoms (0·7%) (Table 2).

Serious adverse events following platelet transfusion

During the course of this surveillance, five serious AEs were reported following transfusion of PCT-PLT (0·07%, 95% CI: 0·0–0·2). These serious AEs were assessed by the investigators as being 'unrelated or probably unrelated' to the PCT-PLT transfusions and were attributed to progression of underlying illness.

Patient B01-201 was admitted to hospital for a presumed pulmonary infection postchemotherapy. Additional co-morbidities at the time of admission were septic shock, acute renal insufficiency, neutropenia and thrombocytopenia. Intravenous (i.v.) antibiotic therapy was initiated and multiple transfusions of blood products (including PCT-PLT) were administered. One hour after administration of the second platelet unit, the patient complained of dyspnoea, respiratory distress was found to be hypotensive and tachycardic. Severe volume overload was determined to be the aetiology and treatment with oxygen, diuretics, and dialysis was initiated. The event was assessed by the investigator to be unrelated to the PCT-PLT transfusion.

Patient J01-382 experienced chills, nausea and sudden hypotension during transfusion with PCT-PLT. Prior to this, the patient had received at least four PCT-PLT transfusions with no AE. The transfusion was stopped and the patient was treated with i.v. fluids and recovered. Four days later, the patient experienced a second hypotensive episode after transfusion, which was spontaneously resolved. Subsequent to this, the patient received 19 additional PCT-PLT transfusions without any clinical sequelae. This patient did not receive any angiotensin-converting enzyme (ACE) inhibitors. Based on the patient's history and the lack of transfusion reaction with the subsequent transfusions, the investigator assessed both of these events as probably unrelated to PCT-PLT transfusion.

Patient J01-516 was admitted for ischaemic cardiomyopathy and underwent double vessel coronary artery bypass graft (CABG). The patient's postoperative recovery was complicated by a significant decrease in blood pressure, which occurred 10 min after start of transfusion of PCT-PLT. Despite vasopressor support and a 6-min period of circulatory arrest, the patient's condition continued to deteriorate and he died. Cause of death was attributed to an aortic dissection with major disseminated intravascular coagulopathy and mesenteric infarct and was assessed by the investigator as unrelated to the PCT-PLT transfusion.

Patient J01-780 experienced a hypotensive episode, cyanosis, oxygen desaturation and nausea approximately 30 min after receipt of PCT-PLT. The patient received oxygen therapy to treat the event and recovered. The patient had received two units of PCT-PLT before and one unit after this event with no adverse reactions. The patient had a history of hypotensive episodes, which occurred in the absence of transfusions. Based on the patient's history, the event was assessed by the investigator as probably unrelated to the PCT-PLT transfusion.

Risk factors associated with adverse event

The risk for AE was not correlated with the patient gender, age, or antigen-matching. The risk for AE for patients who already had been transfused before the first PCT-PLT transfusion appeared trending higher compared to patients who did not have any transfusion history; however, the difference did not reach statistical significance ($P = 0{\cdot}0675$; odds ratio: 1·875; 95% CI: 0·956–3·648). Buffy-coat-derived platelets were associated with a lower risk for AE compared to apheresis products ($P = 0{\cdot}0305$; odds ratio: 0·473; 95% CI: 0·240–0·932). Irradiated PCT-PLTs were of similar risk for AE compared to non-irradiated PCT-PLTs ($P = 0{\cdot}0848$; odds ratio: 6·344; 95% CI: 0·776–51·862). No trending can be concluded because, of the total 7437 platelet transfusions, only 80 PCT-PLT components were γ-irradiated in EFS-Bretagne and EFS-Auvergne-Loire. Haematology–oncology patients treated with conventional chemotherapy were at a higher risk for AE compared to the other patients ($P \leq 0{\cdot}0001$; odds ratio: 7·660; 95% CI: 3·014–19·467).

Number of transfusions prior to the first adverse event

Among the 45 patients who experienced at least one AE, repeated exposure to PCT-PLT did not appear to increase the likelihood of a transfusion reaction (Table 3). By using the non-survival analysis method (a subset analysis for patients with any AE only), the mean number of transfusions before first AE occurrence was 8·8 ± 10·1 (median = 4, minimum = 0 and maximum = 37).

Discussion

In accordance with the recommendations made by the panel of the Canadian Consensus Conference, an active haemovigilance programme has been implemented in Europe to document the occurrence of AE following transfusion of PCT-PLT [6]. To date, two reports have been prepared. The first report was on the transfusion of 5106 PCT-PLT components administered to patients in five European centres from October 2003 to December 2005 [4]. The second report as described here was on additional 7437 transfusions of PCT-PLT administered to patients in seven European centres between May 2005 and January 2007. This represents a total of 12 543 independent transfusions documented to date. There are no overlaps of PCT-PLT transfusions reported in this haemovigilance programme.

Overall, the incidence of ATR attributed to transfusion of PCT-PLT in both of the haemovigilance reporting periods was infrequent either on a per-transfusion basis (0·8% first period

Table 3 Number of PCT-PLT transfusions per patient prior to the first adverse event (AE)

Number of PCT-PLT transfusions per patient until first occurrence of AE	Full analysis population (*n* = 1400)
1	11 (0·79%)
2	6 (0·43%)
3	3 (0·21%)
4	3 (0·21%)
5	1 (0·07%)
6–10	9 (0·64%)
11–19	6 (0·43%)
≥ 20	6 (0·43%)
N (non survival analysis method)	45
Mean ± SD	8·8 ± 10·1
Median	4
Minimum–maximum	0–37

vs. 0·7% second period) or on a per-patient basis (4·9% first period vs. 2·8% second period). The slightly higher occurrence of ATR per patient in the first reporting period was not surprising, because the mean number of transfusions per patient (7·8 ± 16·2) [4] was greater than those observed in the second period (5·3 ± 10·8). All ATRs were mild in severity and of Grade 1 or lower. No serious AE from both study periods were attributed specifically to transfusion of PCT-PLT.

On a per-transfusion basis, the prevalence of ATR has been reported in the literature to range from 18 to 31%; however, these studies were conducted some years ago with variable methods of platelet preparation [10–13]. More recently, the incidence of moderate and severe ATR has been reported from the trial to reduce alloimmunization to platelets (TRAP) study, which examined 8769 platelet transfusions in 598 patients during induction therapy for acute leukaemia [14]. In the TRAP study, platelet components were prepared by four methods: unfiltered pooled whole blood-derived platelets in plasma; filtered pooled whole blood-derived platelets in plasma; unfiltered pooled whole blood-derived platelets in plasma treated with ultraviolet B illumination to reduce human leucocyte antigen sensitization; and filtered apheresis platelets in plasma. None of these components were prepared with additive solutions. The overall incidence of ATR was 2·2% of transfusions, and 22% of patients experienced at least one ATR. In comparison to the TRAP trial, the current study in which all grades of reactions were reported, both the proportion of transfusions associated with a reaction was lower (0·7%) as well as the proportion of patients (2·8%) experiencing at least one ATR. The use of 65% InterSol, a platelet additive solution, in the preparation of PCT-PLT may partially contribute to the reduction in the observed incidence of ATR [15].

The incidence of ATR in this study can be compared to data from the haemovigilance network in France [7]. In France, data were reported for transfusion reactions, with an incidence of four events per 1000 platelet components (0·4%), during 2 years in which the reporting system was first implemented. However, this may be an underestimate since each whole blood platelet concentrate in a pool was tabulated as an individual component transfusion. More recently, Kerkhoffs *et al.* [16] compared the incidence of transfusion reactions for leucoreduced pooled platelet components in plasma and plasma with additive solution in a study of 168 patients and 765 transfusions. They observed an incidence of 5·5% of transfusions with reactions for platelets in plasma vs. 2·4% of transfusions for platelets in a mixture of plasma and additive solution. On a per-patient basis, 9·5% of patients transfused with platelets in plasma plus additive solutions had reactions compared to 15·5% of patients supported with platelets suspended in plasma. These results further support the role of the platelet additive solution, InterSol, in the reduction of ATR observed in this study.

During the conduct of this study, an interim analysis of 2497 PCT-PLT transfusions administered to 606 patients in the three regions of France (EFS-Alsace, EFS-Auvergne-Loire and EFS-Bretagne) was performed [5]. Of the 606 patients, the predominant recipients of PCT-PLT were haematology–oncology patients (46·2%); 39·9% treated with chemotherapy and 6·3% treated with stem cell transplantation. These proportions were only slightly lower than those in the overall study population of 1400 patients, yet only four of the 606 patients (0·7%) reported an AE, including one serious AE of volume overload classified as unrelated to PCT-PLT transfusion. This low rate of AE observed in the French regions could contribute to the overall low incidence of ATR per patient in this study.

Premedication in patients did not play a role in the overall low incidence of ATR reported in this study. Information on premedication was only requested in case of AE occurrence. Of the 68 transfusions with occurrence of at least one AE, only two antipyretic, two antihistaminic and one corticosteroid were prescribed to patients. For the majority (64/68, or 94·1%) of these transfusions, patients were not premedicated.

The active haemovigilance programme described here is a prospective observational study, which was designed to assess the safety profile of PCT-PLT in routine clinical practice. The data from this programme represent the largest prospective experience to date for recording potential AE associated with platelet transfusions compared to prior studies of retrospective design and limited in size [10,16–18]. The present study was designed to be consistent with European haemovigilance practices in which reporting of all grades of transfusion-associated reactions has been emphasized [7,8]. In contrast to other haemovigilance studies, obligatory reporting for all platelet transfusions was required irrespective of whether or not an AE was observed. The current study focused on AE that could be linked to PCT-PLT transfusions after starting transfusion, but there were no specific limitations

on when adverse events could be reported following transfusion. Based on the patient population supported with platelet transfusion, the study was designed to capture repeated transfusions of PCT-PLT within patients to determine potential effects of repeated exposure to this new type of platelet component.

A limitation of the present study is the absence of a concurrent control group receiving conventional platelet components with which to determine a comparative baseline incidence of ATR. However, because reporting is obligatory, the expected outcomes of this active haemovigilance study are the increase in clinical experience with transfusion of PCT-PLT, the detection of unexpected AE following PCT-PLT transfusions in patient populations and for indications that were not studied previously in a formal clinical trial environment, and the establishment of a safety database for future reference.

In the current study, which was specifically designed to capture all grades of transfusion reactions, the prevalence of ATR per transfusion, was at the lower range of those reported in studies with conventional components. Prior exposure to PCT-PLT transfusions did not increase the likelihood of an ATR. The overall incidence of ATR was lower than that previously reported either on a per-transfusion or on a per-patient basis. Based on experience in a broad patient population, platelet components prepared with amotosalen photochemical treatment were well-tolerated in routine clinical practice.

Acknowledgements

The authors would like to thank the following individuals of Fenwal, formerly Baxter R&D Europe, who assisted in the implementation of the INTERCEPT Blood System at the blood centres, provided critical input into the scope and design of the haemovigilance programme, and training of site personnel on data entering: Jocelyne Flament, Jean-Marc Payrat, Nick Moerman, Valentine Franck, and Veronique Mayaudon. Quintiles provided data management and statistics service for this haemovigilance programme. This study was supported in part by Cerus Corporation. Three authors (D. Sundin, L. Lin and L. Corash) were affiliated with and held stock or stock options of Cerus Corporation during the conduct of this study. Three authors (J. C. Osselaer, J. P. Cazenave and O. Garraud) served on Cerus's European Scientific Advisory Board.

References

1 van Rhenen DJ, Gulliksson H, Cazenave JP, Pamphilon D, Ljungman P, Klüter H, Vermeij H, Kappers-Klunne M, de Greef G, Laforet M, Lioure B, Davis K, Marblie S, Mayaudon V, Flament J, Conlan M, Lin L, 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

2 McCullough J, Vesole DH, Benjamin RJ, Slichter SJ, Pineda A, Snyder E, Stadtmauer E, 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 M: Therapeutic efficacy and safety of platelets treated with a photochemical process for pathogen inactivation: the SPRINT trial. *Blood* 2004; **104**:1534–1541

3 Janetzko K, Cazenave JP, 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 optimized integrated set. *Transfusion* 2005; **45**:1443–1452

4 Osselaer J, Messe N, Hervig T, Bueno JL, Castro E, Espinosa A, Lacone A, Junge K, Jacquet M, Flament J, Corash L: A prospective observational cohort safety study of 5106 platelet transfusions prepared with photochemical treatment. *Transfusion* 2008: doi: 10.1111/j.1537-2995.2008.01643.x

5 Garraud O, Fabrigli P, Tardivel R, Gouezec H, Waller C, Mendel I, Raidot JP, Kandel G, Isola H, Cazenave JP: Hemovigilance for transfusion of INTERCEPTTM platelets in routine therapeutic use: experience in Etablissement Francais du Sang (EFS) blood centers. *Vox Sang* 2007; **93**(S1):32

6 Klein HG, Anderson D, Bernardi MJ, Cable R, Carey W, Hoch JS, Robitaille N, Sivilotti MLA, Smaill F: Pathogen inactivation: making decisions about new technologies. Report of a consensus conference. *Transfusion* 2007; **47**:2338–2347

7 Andreu G, Morel P, Forestier F, Debeir J, Rebibo D, Janvier G, Hervé P: Hemovigilance network in France: organization and analysis of immediate transfusion incident reports from 1994–1998. *Transfusion* 2002; **42**:1356–1364

8 Faber JC: Haemovigilance in Europe: now and tomorrow; in Rouger P, Hossenlopp C (eds): *Blood Transfusion in Europe*, Paris, Elsevier SAS, 2005:213–232

9 Corash L, Lin L: Novel processes for inactivation of leukocytes to prevent transfusion-associated graft-versus-host disease. *Bone Marrow Transplant* 2004; **33**:1–7

10 Chambers LA, Kruskall MS, Pacini DG, Donovan LM: Febrile reactions after platelet transfusion: the effect of single versus multiple donors. *Transfusion* 1990; **30**:219–221

11 Heddle NM, Kalma L, Singer J, Richards C, Fedak P, Walker I, Kelton JG: The role of the plasma from platelet concentrates in transfusion reactions. *N Engl J Med* 1994; **331**:625–628

12 Mangano MM, Chambers LA, Kruskall MS: Limited efficacy of leukopoor platelets for prevention of febrile transfusion reactions. *Am J Clin Pathol* 1991; **95**:733–738

13 Heddle NM, Klama LN, Griffith L, Roberts R, Shukla G, Kelton JG: A prospective study to identify the risk factors associated with acute reactions to platelet and red cell transfusions. *Transfusion* 1993; **33**:794–797

14 Enright H: Factors influencing moderate to severe reactions to PLT transfusions: experience of the TRAP multicenter clinical trial. *Transfusion* 2003; **43**:1545–1552

15 Isola H, Kientz D, Wiesel ML, Laforet M, Ohlmann P, Waller C, Mendel I, Raidot JP, Kandel G, Cazenave JP: Implementation of photochemical inactivation (INTERCEPT Blood System for platelets) in a regional blood center. *Vox Sang* 2007; **93**(S1):165

16 Kerkhoffs JH, Eikenboom JC, Schipperus MS, van Wordragen-Vlaswinkel RJ, Brand R, Harvey MS, de Vries RR, Barge R, van Rhenen DJ, Brand A: A muticenter randomized study of the efficacy of transfusions with platelets stored in platelet additive solution II versus plasma. *Blood* 2006; **108**:3210–3215

17 deWildt-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

18 Heddle NM, Blajchman MA, Meyer RM, Lipton JH, Walker IR, Sher GD, Constantini LA, Patterson B, Roberts RS, Thorpe KE, Levine MN: A randomized controlled trial comparing the frequency of acute reactions to plasma-removed platelets and prestorage WBC-reduced platelets. *Transfusion* 2002; **42**:556–566

Transfusion of platelet components prepared with photochemical pathogen inactivation treatment during a Chikungunya virus epidemic in Ile de La Réunion

Patrice Rasonglès, Marie France Angelini-Tibert, Philip Simon, Caroline Currie, Herve Isola, Daniel Kientz, Marc Slaedts, Michele Jacquet, David Sundin, Lily Lin, Laurence Corash, and Jean Pierre Cazenave

BACKGROUND: During the Chikungunya virus (CHIKV) epidemic on Ile de La Réunion, France, more than 30% of 750,000 inhabitants were infected. Local blood donation was suspended to prevent transfusion-transmitted infection (TT-CHIKV). To sustain the availability of platelet (PLT) components, the Établissement Français du Sang implemented universal pathogen inactivation (INTERCEPT, Cerus Europe BV) of PLT components (CPAs). The study assessed the safety of PLT components treated with pathogen inactivation transfused in routine clinical practice.

STUDY DESIGN AND METHODS: This was a retrospective observational study using patient medical records and the AFSSAPS hemovigilance database (eFIT) to identify TT-CHIKV and adverse events (AEs) classified as acute transfusion reactions (ATRs) to PLT components prepared with pathogen inactivation.

RESULTS: During 1 year, 1950 INTERCEPT-CPAs were transfused to 335 adult, 51 pediatric, and 41 infant patients. Nineteen AEs were observed in 15 patients and 10 were classified as ATRs. Eight ATRs occurred in 6 pediatric hematology-oncology patients. No ATRs were observed in infants. The most frequently reported signs and symptoms were Grade 1 urticaria, itching, chills, fever, and anxiety. No cases of transfusion-related acute lung injury, TT-sepsis, or TT-CHIKV were detected.

CONCLUSIONS: INTERCEPT-CPAs were well tolerated in a broad range of patients, including infants. ATR incidence was low and when present ATRs were of mild severity.

Starting in 2005, an epidemic of Chikungunya virus (CHIKV) in the overseas French department of Ile de La Réunion, an island in the South Indian Ocean, resulted in the infection of more than one-third of the 750,000 inhabitants by early 2006.[1] CHIKV is an enveloped single-stranded alpha virus from the Togaviridae family transmitted by *Aedes* mosquitoes. It generally causes a mild febrile illness characterized by arthralgias lasting up to 10 days, but the recent epidemic was associated with myalgias, dermatitis, hemorrhage, meningoencephalitis, respiratory failure, cardiovascular decompensation, and fulminant hepatitis with persistent arthralgias in some patients.[2] Subsequently, more than 700 cases of CHIKV infection were reported in metropolitan France among returning travelers, and 1 infection

ABBREVIATIONS: AE(s) = adverse event(s); ATR(s) = acute transfusion reaction(s); CHIKV = Chikungunya virus; CPA(s) = apheresis platelet component(s); CRF(s) = case report form(s); EFS = Établissement Français du Sang; SAE(s) = severe adverse event(s); TT = transfusion transmitted.

From the EFS Ile de La Réunion and CHR Centre Hospitalier Départemental Felix Guyon, St Denis, Ile de La Réunion, France; CHR Groupe Hospitalier Sud Réunion de St Pierre, Ile de La Réunion, France; Cerus Corporation, Concord, California; EFS Alsace, Strasbourg, France; Cerus Europe BV, Amersfoort, The Netherlands; the University of California School of Medicine, San Francisco, California; INSERM U.311 and Université Louis Pasteur, Strasbourg, France.

Address reprint requests to: Laurence Corash, MD, Cerus Corporation, 2411 Stanwell Drive, Concord, CA 94520; e-mail: lcorash@cerus.com.

This study was supported in part by Cerus Corporation.

Received for publication July 28, 2008; revision received December 12, 2008, and accepted December 12, 2008.

doi: 10.1111/j.1537-2995.2009.02111.x

TRANSFUSION 2009;49:1083-1091.

after needle stick of a health care worker.[3,4] Owing to the high prevalence of CHIKV infection and the potential for transfusion-transmitted (TT) infection, the Établissement Français du Sang (EFS [French National Transfusion Service]) suspended blood donation on Ile de La Réunion to prevent TT-CHIKV.[1] To meet the requirements for safe blood components on Ile de La Réunion, red blood cells and plasma components (fresh-frozen plasma) were supplied by EFS from metropolitan France. Because of the limited shelf life (5 days) of platelet (PLT) components, EFS-La Réunion implemented pathogen inactivation preparation of apheresis PLT components (CPAs) to maintain local PLT component supplies.[5]

Prior research studies had demonstrated that CHIKV was inactivated by photochemical treatment with amotosalen HCl and UVA light (INTERCEPT Blood System for platelets, Cerus Europe BV, Amersfoort, The Netherlands).[6] In addition, this system had been shown to inactivate high levels of a broad spectrum of viruses, bacteria, protozoa, and white blood cells (WBC) in PLT components.[7-9] The INTERCEPT system received CE Mark registration as a Class III drug device and as of 2005 received approval from the Agence Française de Sécurité Sanitaire des Produits de Santé (AFSSAPS, French Agency of Medical Safety of Health Products) for use with both apheresis- and whole blood–derived PLT components in France.

The INTERCEPT Blood System was implemented in routine practice as of March 13, 2006, by EFS-Ile de La Réunion. To date, approximately 4000 INTERCEPT-CPAs have been administered to a broad range of patients on Ile de La Réunion. After the first year of routine use of pathogen inactivation to prepare PLT components, we conducted a retrospective analysis of the response to transfusion of 1950 components to determine the incidence of acute transfusion reactions (ATRs) and serious adverse events (SAEs) attributed to use of this novel component. In addition, we determined the incidence of TT-CHIKV infection for the first year after implementation of pathogen inactivation treatment during the CHIKV epidemic.

MATERIALS AND METHODS

Collection of PLT components

Before introduction of the INTERCEPT system, CPAs were the sole type of PLT component provided by EFS-La Réunion. All CPAs were collected in donor plasma with integral filtration leukoreduction (Haemonetics, Braintree, MA). After introduction of INTERCEPT, PLTs were collected in approximately 40% donor plasma and 60% PLT additive solution (InterSol, Fenwal, La Chatre, France) from donors with PLT counts of 250×10^9/L or more using a blood component collection system (Haemonetics MCS+ system with the CSDP software) to allow automatic addition of InterSol. The targeted PLT dose per collection was 4.0×10^{11} or greater. WBC contamination was reduced by filtration with an integral WBC filter (Haemonetics). In addition to standard viral screening tests, donors were tested for CHIKV infection by an investigational reverse-transcriptase polymerase chain reaction assay (RT-PCR).[3,10]

Pathogen inactivation treatment of PLT components

CPAs containing 2.5×10^{11} to 6.0×10^{11} PLTs in 300 to 390 mL of approximately 40% plasma and 60% InterSol were prepared with pathogen inactivation using the INTERCEPT processing system (INT2202, Cerus Europe BV) according to manufacturer's instructions for use. Briefly, a unit of CPA was mixed with amotosalen (nominal final concentration of 150 µmol/L) and illuminated with long-wavelength ultraviolet UVA (320-400 nm) light for a 3 J/cm^2 treatment. The illuminated PLT mixture was incubated in a compound adsorption device in a temperature controlled PLT shaker/incubator (22 ± 2°C) for 6 to 16 hours before transferring to the final storage container. Treated CPAs were stored for up to 5 days under standard blood bank conditions before issue for transfusion.

Hemovigilance surveillance

General study design

This was a retrospective analysis of data recorded prospectively in primary care medical records and as part of the AFSSAPS active hemovigilance surveillance program.[11] There were no patient inclusion or exclusion criteria other than the requirement for PLT transfusion. All patients who received PLT transfusion support during the defined study period were included in the analysis. Case report forms (CRFs) were used to collect patient data[12] on each transfusion of INTERCEPT-CPAs between March 13, 2006, and March 13, 2007, regardless of whether an adverse event (AE) was reported.

The primary endpoint of the study was the proportion of transfusions with ATR after administration of PLT components. ATRs were defined as AEs possibly related, probably related, or related to a PLT transfusion. SAEs were defined as AEs that were fatal, life-threatening, or disabling; resulted in or prolonged hospitalization or morbidity; or were incapacitating. Secondary endpoints included evidence of acute TT-CHIKV infection (based on nucleic acid amplification of viral sequences). All transfused patients were monitored for 7 days after each transfusion for potential TT-CHIKV infection using standard EFS operating procedures.[10] Data also were collected on use of INTERCEPT-CPAs by patient primary diagnosis category and clinical indication for transfusion.

Data collection methods

All patients transfused with PLTs prepared by EFS-La Réunion from March 13, 2006, through March 13, 2007, were identified from the EFS-La Réunion electronic database for the collection, production, and issuance of blood components. Each patient was identified with a unique study number to preserve anonymity. The following data were collected: PLT product code; patient unique identification number associated with the component, patient demographics (age, sex), and primary diagnosis based on clinical care area; primary therapy (chemotherapy, hematopoietic stem cell transplant); surgery (cardiovascular or organ transplant); or other (general medical or multisystem organ failure).

Primary care medical records of each patient were reviewed for the 24 hour period before each transfusion to establish a baseline profile of the patient's clinical condition, for 7 days after each PLT transfusion to identify new AEs arising after transfusion, and to record the relationship of AEs to PLT transfusion in the primary medical record as assessed by primary care physicians. This review was conducted by an observer without knowledge of AEs reported in the AFSSAPS hemovigilance system (eFIT).[11] For the 24 hours before and for the 7 days after each PLT transfusion, medical records were specifically reviewed for evidence of clinical conditions that could be attributed to transfusion-related reactions, including fever (increase in temperature of 2 or 1°C with chills), chills, nausea, skin rash, urticaria, dyspnea, bronchospasm, tachycardia or bradycardia (change in heart rate by >25 bpm), hypotension or hypertension (decrease or increase in systolic or diastolic blood pressure >30 mm Hg, respectively), hemoglobinuria, hemolysis, and change in general well-being. Specific criteria were provided for the diagnosis of transfusion-associated acute lung injury (TRALI).[13] Clinical microbiology laboratory records were reviewed for documentation of transfusion-associated sepsis. The diagnosis of transfusion-associated sepsis required the isolation of the same bacteria species from the patient and the implicated PLT component.

Transfusion CRFs were completed for each PLT transfusion regardless of whether or not an AE was noted in the medical record. In case of the occurrence of an AE, additional clinical and biologic information as well as test results for CHIKV infection (nucleic acid testing [NAT] by RT-PCR) were collected. These data were used by the medical record reviewer for assessment of causality and severity based on the medical record. Clinical severity of AEs was classified according to the following scale: Grade 0 = isolated dysfunction without clinical or biologic manifestation; Grade 1 = absence of immediate or long-term life-threatening effects; Grade 2 = long-term life-threatening effects; Grade 3 = immediate life-threatening effects; and Grade 4 = death. The relationship of AEs to the most proximate PLT transfusion was classified using the same criteria as used by the AFSSAPS hemovigilance system.[11]

The standardized CRFs had been validated in a prior hemovigilance study.[14] Data from the CRF were entered into an independent electronic database used for postmarketing hemovigilance programs[12,14] and reviewed by the principal investigator for incomplete data. At the conclusion of the study, AEs classified as transfusion reactions based on review of the primary care medical records were compared against AEs previously reported under the AFSSAPS hemovigilance program recorded in the eFIT database[11] to determine the total incidence of AEs attributed to PLT transfusion. These data were then analyzed to determine the incidence of ATRs.

Statistical analyses

A statistical analysis plan for the study was prepared and approved before analysis. All statistical analyses, summary tables, and data listings were generated using computer software (SAS, Version 8.2, SAS Institute, Cary, NC). The primary assessment was the incidence of transfusion reactions. The number and proportion (%) of transfusions and the proportion of patients with one or more transfusion reactions were summarized overall, by seriousness and by relationship to PLT transfusion. Corresponding 95% confidence intervals (CIs) for the binomial proportion were calculated using the F distribution method. The 95% CI were based on number of patients with any AE/ATR and the number of transfusions associated with any AE/ATR. In addition, the patient population profile, the characteristics of the PLT components, and the characteristics of the AEs after PLT transfusion were analyzed. Analyses to identify risk factors potentially associated with transfusion reactions were conducted using multivariate logistic regression analysis and by assessing association at a 10% significance level. Data were analyzed on a per-transfusion and a per-patient basis. All INTERCEPT PLT components administered to patients were part of the full analysis population and were analyzed, whether or not an AE was observed. All analyses were conducted using this full analysis population.

RESULTS

PLT component characteristics

Each CPA was treated with pathogen inactivation using the INTERCEPT Blood System on either Day 0 or Day 1 after PLT collection and stored for up to 5 days before release for transfusion. PLT components were released after completion of serologic and NAT. Pathogen inactivation treatment was used without bacteria detection other than routine quality control (QC) assays. Pathogen inactivation treatment replaced cytomegalovirus (CMV)

serology for patients who required CMV-safe PLTs and replaced gamma irradiation for prevention of transfusion-associated graft versus host disease.

The INTERCEPT process resulted in a mean PLT loss of 7.8% due to volume loss during container transfers. The mean PLT yield of INTERCEPT-CPAs was $4.2 \times 10^{11} \pm 0.7 \times 10^{11}$ PLTs per component. The residual WBC count met the national QC requirement ($<0.5 \times 10^{6}$/unit). Approximately 15% of PLT components were divided into 2 units before transfusion to fulfill clinical demand. The proportion of split PLT components was similar to that in the period before implementation of pathogen inactivation.

Patient demographics

Between March 13, 2006, and March 13, 2007, a total of 1950 INTERCEPT-CPAs were transfused to 427 patients (Table 1). Each patient received at least one INTERCEPT-CPA. The patient population consisted of 335 adult patients (>18 years), 51 pediatric patients (≥1 to <18 years), and 41 infants (<1 year). There were more male patients in each age group (Table 1).

Hematology-oncology disorders treated with chemotherapy and stem cell transplantation constituted 29.0% of the primary diagnoses among the transfused patient population and these patients received 61% of the PLT components (Tables 1 and 2). The largest patient group supported with PLT components was the general medical population (58.5%), but they received only 30% of the PLT components. A number of patients receiving PLT transfusions (12.2%) underwent major surgical procedures including cardiovascular surgery or solid organ transplantation. Among the pediatric patient group, the proportion of hematology-oncology patients (66.7%) was significantly higher ($p = 0.001$) than among the adult patient group (26.3%).

Approximately half of the patient population (51.5%) received transfusions in intensive care units and the other half (48.5%) were transfused on non–intensive care hospital services (Table 1). There were no outpatient transfusions in the current surveillance program. Subgroup analysis showed that, while most of the pediatric patients (78.4%) were transfused in non–intensive care hospital wards, the majority of infants (90.2%) were transfused on intensive care units.

PLT transfusion exposure

Approximately 53% of patients had a prior history of transfusion exposure to some blood component. The median number of PLT transfusions per patient was 2.0 (range, 1-66; Table 2). Of 1950 PLT transfusions, 1372 transfusions were administered to adult patients while 487 and 91 transfusions were administered to pediatric patients and infants, respectively. Based on the respective patient population, 36 to 47% of patients received two or more PLT transfusions. The number of transfusions per pediatric patient (9.5 ± 14.7) was significantly higher ($p < 0.001$) compared to those in the adult population (4.1 ± 6.2) while the opposite was true for infants (2.2 ± 2.4, $p < 0.002$). Based on primary diagnosis category, hematology-oncology patients in all age groups received a higher proportion of PLT transfusions per patient than those in other diagnosis groups (Table 2).

TABLE 1. Demographics of patients transfused with INTERCEPT-CPAs*

Demographic	Patients (n = 427)	Adult (n = 335)	Pediatric (n = 51)	Infants (n = 41)
Gender				
Male	262 (61.4)	202 (60.3)	35 (68.6)	25 (61.0)
Female	165 (38.6)	133 (39.7)	16 (31.4)	16 (39.0)
Age (years)				
Mean ± SD	42.4 ± 24.8	52.6 ± 17.1	9.4 ± 5.3	NA†
Median	46.0	53.0	10.0	NA†
Range	<1 to 87	>18 to 87	1 to 18	<1
Care location				
Intensive	220 (51.5)	172 (51.3)	11 (21.6)	37 (90.2)
Nonintensive	207 (48.5)	163 (48.7)	40 (78.4)	4 (9.8)
Hematology-oncology primary therapy	124‡ (29.0)	87 (26.3)	34 (66.7)	3 (7.3)
Conventional chemotherapy	102 (82.2)	69 (79.3)	30 (88.2)	3 (100)
Stem cell transplant	14 (11.3)	10 (11.5)	4 (11.8)	0 (0)
Surgery	52 (12.2)	48 (14.3)	3 (5.9)	1 (2.4)
Cardiovascular	49 (94.2)	45 (93.8)	3 (100)	1 (100)
Solid organ transplant	3 (5.8)	3 (6.2)	0 (0)	0 (0)
General medical	250 (58.5)	199 (59.4)	14 (27.4)	37 (90.3)
Missing diagnosis	1 (0.2)	1 (0.3)	0 (0)	0 (0)

* The number of patients (n) and the proportion (%) within each category are presented.
† Age for infants was only recorded as <1 year. NA = not applicable.
‡ Eight adult patients had no active therapy specified at time of transfusion.

TABLE 2. Transfusion exposure among patient populations

Population	All patients (n = 427)	Adult patients (n = 335)	Pediatric patients (n = 51)	Infant patients (n = 41)
All patients				
Transfusions (n)	1950	1372	487	91
Mean ± SD	4.6 ± 7.7	4.1 ± 6.2	9.5 ± 14.7	2.2 ± 2.4
Median	2.0	2.0	4.0	1.0
Range	1-66	1-46	1-66	1-11
Hematology-oncology				
Transfusions (n)	1192	738	446	8
Mean ± SD	9.6 ± 11.7	8.5 ± 8.9	13.1 ± 16.8	2.7 ± 2.9
Median	6.0	6.0	6.5	1.0
Range	1-66	1-46	1-66	1-6
Surgical				
Transfusions (n)	149	135	8	6
Mean ± SD	2.9 ± 3.6	2.8 ± 3.7	2.7 ± 1.5	6.0 ± 0.0
Median	2.0	2.0	3.0	6.0
Range	1-24	1-24	1-4	6.0
General medical				
Transfusions (n)	596	486	33	77
Mean ± SD	2.4 ± 3.6	2.4 ± 3.8	2.4 ± 2.8	2.1 ± 2.3
Median	1.0	1.0	1.0	1.0
Range	1-37	1-37	1-11	1-11
Missing diagnoses				
Transfusions (n)	13	13	0	0
Mean ± SD	13.0 ± 0.0	13.0 ± 0.0		
Median	13.0	13.0		
Range	13	13		

TABLE 3. Clinical characteristics of AEs and ATRs among patient populations*

	All patients (n = 427) Transfusions (n = 1950)		Adult patients (n = 335) Transfusions (n = 1372)		Pediatric patients (n = 51) Transfusions (n = 487)	
Characteristic	Any AE	ATRs	Any AE	ATRs	Any AE	ATRs
Patients with 1 or >AE	15 (3.5)	8 (1.9)	6 (1.8)	2 (0.6)	9 (17.6)	6 (11.8)
Transfusions with 1 or >AE	19 (1.0)	10 (0.5)	6 (0.4)	2 (0.1)	13 (2.7)	8 (1.6)
Signs/symptoms per transfusion†						
Fever	5 (0.3)	1 (<0.1)	2 (0.1)	1 (<0.1)	3 (0.6)	0
Chills	7 (0.4)	2 (0.1)	4 (0.3)	2 (0.1)	3 (0.6)	0
Itching	5 (0.3)	4 (0.2)	1 (<0.1)	0	4 (0.8)	4 (0.8)
Urticaria	7 (0.4)	6 (0.3)	1 (<0.1)	0	6 (1.2)	6 (1.2)
Dyspnea	1 (<0.1)	0	1 (<0.1)	0	0	0
Anxiety	4 (0.2)	0	2 (0.1)	0	2 (0.4)	0
Other	6 (0.3)	2 (0.1)	1 (<0.1)	0	5 (1.0)	2 (0.4)
Signs/symptoms per patient†						
Fever	4 (0.9)	1 (0.2)	2 (0.6)	1 (0.3)	2 (3.9)	0
Chills	5 (1.2)	2 (0.5)	4 (1.2)	2 (0.6)	1 (2.0)	0
Itching	5 (1.2)	4 (0.9)	1 (0.3)	0	4 (7.8)	4 (7.8)
Urticaria	5 (1.2)	4 (0.9)	1 (0.3)	0	4 (7.8)	4 (7.8)
Dyspnea	1 (0.2)	0	1 (0.3)	0	0	0
Anxiety	4 (0.9)	0	2 (0.6)	0	2 (3.9)	0
Other	6 (1.4)	2 (0.5)	1 (0.3)	0	5 (9.8)	2 (3.9)

* Data are reported as number (%). No AEs were reported for infant patients; thus, these patients and transfusions are not included in this table.
† Number of signs/symptoms can exceed number of AEs due to multiple observed signs/symptoms per AE.
ATR = causal relationship that an AE was possibly related, probably related, or related to INTERCEPT-CPA transfusion.

AEs and ATRs after PLT transfusion

The incidences of AEs and ATRs were evaluated on a per-transfusion as well as per-patient basis (Table 3). On a per-transfusion basis, 19 transfusions (95% CI, 1.0%-1.5%) were associated with an AE. Of these AEs, 10 (95% CI, 0.5%-0.9%) were classified as ATRs possibly, probably, or related to INTERCEPT-CPA transfusion. No SAEs, no cases of TT-sepsis, no cases of TRALI, and no deaths due to INTERCEPT-CPA transfusions were reported. On a per-patient basis, 15 patients (95% CI, 3.5%-5.7%) who received at least one transfusion of INTERCEPT-CPAs experienced an AE after PLT transfusions (Table 3). Only 8 patients (95% CI, 1.9%-3.6%) experienced an ATR attributed to INTERCEPT-CPA transfusion (Table 3).

Overall patient population: characteristics of clinical signs and symptoms associated with PLT transfusion

Of all AEs, on a per-transfusion basis, the most frequently observed symptoms/signs (0.3%-0.4% of 1950 transfusions) were fever, chills, itching, and urticaria (Table 3). Anxiety (0.2%) was the second most frequently reported symptom/sign. Only one incident of dyspnea was reported. Additional symptoms in the category of "other" included tachycardia, facial flushing, body pain, and cough, but with an individual incidence of 0.1% or less of transfusions. Most of the ATRs were described principally as Grade 1 urticaria (0.3%) and itching (0.2%) with all other symptoms/signs observed at a rate of 0.1% or less of transfusions.

On a per-patient basis, the most frequently observed symptoms/signs (1.2% of 427 patients) were chills, itching, and urticaria (Table 3). Fever and anxiety (0.9%) were the second most frequently observed symptoms/signs. One patient (0.2%) experienced a single episode of dyspnea. Additional symptoms in the category of "other" included tachycardia, facial flushing, body pain, and cough, each with an individual incidence of 0.5% or less on a per-patient basis. Most of ATRs were described as Grade 1 itching (0.9%), urticaria (0.9%), and chills (0.5%) with all others observed at a rate of 0.2% or less per patient.

Characteristics of AEs and ATRs in pediatric patients

Pediatric patients experienced a higher incidence of AEs than adult patients (Table 3). On a per-transfusion basis, 13 AEs (2.7%) and 8 ATRs (1.6%) occurred in pediatric patients compared to 6 AEs (0.4%) and 2 ATRs (0.1%) in adult patients. On a per-patient basis, 9 pediatric patients (17.6%) experienced at least 1 AE compared to 6 adult patients (1.8%). Similarly, 6 pediatric patients (11.8%) experienced at least 1 ATR compared to 2 adult patients (0.6%).

For all AEs reported in pediatric patients, the symptoms/signs were predominantly Grade 1 in severity consisting of fever, chills, itching, urticaria, anxiety, tachycardia, and facial flushing (Table 3). For pediatric patients experiencing ATRs, the symptoms/signs included itching, urticaria, tachycardia, and facial flushing, none of which were reported in adult patients. No AEs were associated with the 91 INTERCEPT-CPA transfusions administered to 41 infants who required PLT support.

Characteristics of AEs and ATRs associated with transfusion of split components

Of the 1950 transfusions, 540 INTERCEPT-CPAs were obtained from a split PLT component. The rates of AEs and ATRs on a per-transfusion basis for split components were 0.4 and 0%, respectively, compared to 1.2 and 0.7% for whole components. Of the 19 AEs reported, only 2 AEs (one in a 77-year-old male patient and one in a 16-year-old male patient) were associated with transfusion of a split INTERCEPT-CPAs (Table 4).

TABLE 4. Effect of split components on the frequency of AEs and ATRs*

Component	Transfusions	AEs	ATRs
Split INTERCEPT-CPAs	540	2 (0.4)	0 (0)
Whole INTERCEPT-CPAs	1410	17 (1.2)	10 (0.7)
Total	1950	19 (1.0)	10 (0.5)

* Data are reported as number (%).

Incidence of TT-CHIKV

A substantial proportion of transfusions were administered to hematology-oncology patients treated with potentially immune-suppressive therapy. There were no cases of TT-CHIKV reported in this survey based on the test results using an investigational assay for viral nucleic acid or posttransfusion clinical observation for signs and symptoms of CHIKV infection.

DISCUSSION

CHIKV resulted in an epidemic on La Réunion Island in which approximately 41% of the population was infected. Serologic and epidemiologic surveillance studies estimated the prevalence of asymptomatic infection at 15% of total CHIKV infections.[1] Efforts to identify infected blood donors with either serologic assays or CHIKV specific nucleic acid amplification assays have shown considerable variability and suboptimal sensitivity.[15] The mean risk of contamination of a blood donation throughout the epidemic was estimated at 132 per 100,000 donations, and at the peak of the epidemic, the risk was estimated at 1,500 per 100,000 donations.[1] At the time of the current study, optimal methods to detect infected donors with low viral titers were not available, and a NAT with sensitivities of 40 to 350 copies/mL was only developed later.[16] In the period of this study, collection of CHIKV-contaminated PLTs from asymptomatic donors was plausible. During the epidemic before use of pathogen inactivation, two cases of TT-CHIKV were suspected, but neither case could be conclusively proven.[10] At least one blood-borne transmission due to a needle-stick has been documented.[4]

This study accomplished multiple objectives. Foremost, it provided hemovigilance data to evaluate the effectiveness of the INTERCEPT Blood System to prevent PLT TT-CHIKV during an epidemic. These data are especially relevant given the specific association of CHIKV with

PLTs,[17,18] which could lead to low detection sensitivity for serum-based tests. In addition to evaluating the efficacy to prevent TT-CHIKV, this study provided an opportunity to extend the safety profile of INTERCEPT PLTs transfused to a broad patient population. Finally, this study permitted an evaluation of the operational logistics of the INTERCEPT PLT system implemented under emergency conditions.

Data provided by EFS-La Réunion for the years 2004 and 2005 with conventional PLT components suspended in 100% plasma indicated an ATR incidence of 2.2 and 5.4% of PLT transfusions among heavily transfused pediatric oncology-hematology patients, respectively.[19] In comparison, this study demonstrated a lower incidence (1.6%) of ATRs per PLT transfusion. These results are consistent with reported ATR frequencies reported for INTERCEPT PLT components in routine use from multiple European centers,[12,14] but lower than the frequencies reported for treated PLT components in the EuroSprite (6%) and the SPRINT clinical trials (3%).[20,21] The higher incidence of ATRs observed in the clinical trials may have been due to differences in patient populations, which in the clinical trials consisted largely of heavily transfused hematology-oncology patients undergoing hematopoietic stem cell transplantation. Similar to previous studies, all of the ATRs observed in the current survey were of mild severity, and none were indicative of clinical CHIKV. It is relevant to note that the size of this study was insufficient to characterize the incidence of septic transfusion reactions, although none were reported.

The clinical symptoms of CHIKV infection include fever, severe polyarthralgia, myalgia, dermatitis, hemorrhage, meningoencephalitis, respiratory failure, cardiovascular decompensation, and fulminant hepatitis with a mortality rate of one in 1000 during the La Réunion epidemic.[22] Thus, review of primary medical records should have been sufficiently sensitive to detect TT-CHIKV. No TT-CHIKV cases were detected in the patient population monitored in this study after implementation of the INTERCEPT Blood System for PLTs.

The retrospective surveillance described in this report provided an opportunity to evaluate the sensitivity of the AFSSAPS active hemovigilance system[11] to detect transfusion-related AEs. We did not detect any additional transfusion-related AEs in our independent review of primary medical records compared to the AFSSAPS/eFIT database for transfusion-related incidents. This limited experience is consistent with the sensitivity of the AFSSAPS hemovigilance system in detecting transfusion-related AEs.

This study included a substantial number of pediatric patients, some of whom were infants. None of the prior studies with INTERCEPT PLT components included a substantial infant patient population. Interestingly, pediatric patients had the highest rate of AEs and ATRs after transfusion of INTERCEPT-CPAs. This finding may not be surprising because the proportion of hematology-oncology patients and the levels of PLT component exposure were higher among pediatric patients. On the other hand, no AEs or ATRs were observed in infants who received INTERCEPT-CPA transfusions largely for nonmalignant medical disorders, but this population was of limited size and less intensively transfused.

The study also provided experience with the implementation and operational logistics of the INTERCEPT system in a remote, small regional blood center. EFS-Ile de La Réunion performs approximately 100 to 150 apheresis PLT collections per month.[23] Complete conversion to pathogen inactivation of PLT components was achieved in 2 weeks. In routine operation, no additional personnel were required after implementation of the INTERCEPT system.

This is the first study to demonstrate the utility of pathogen inactivation as a proactive approach to prevent a potentially TT infection during an epidemic. The technology facilitated the availability of PLT components that otherwise were in limited supply. This experience is relevant given the observation of imported cases of CHIKV infection in metropolitan France, Germany, the United Kingdom, Belgium, Norway, the Czech Republic, Canada, and the United States[3,24,25] and the autochthonous outbreak of CHIK infection in the Emilia-Romagna region of Italy.[26] The success of EFS-La Réunion in implementing the INTERCEPT Blood System demonstrates the utility of pathogen inactivation to support the availability of labile blood components during an epidemic.

ACKNOWLEDGMENTS

The authors are grateful for the support of Chantal Waller in providing guidance and training for the conduct of the hemovigilance study of pathogen-inactivated PLT components. Lynette Sawyer performed critical studies to validate inactivation of CHIKV in PLT and plasma components. The authors thank Carole Ripaud for assistance in facilitating communications between the investigators of EFS La Réunion and Cerus Corporation and Jessica Hanover for editorial assistance. We also acknowledge the efforts of Arnaud Besnard in facilitating this study.

CONFLICT OF INTEREST

Three authors (DS, LL, and LC) were affiliated with and held stock or stock options in Cerus Corporation during the conduct of this study. MJ was a consultant to Cerus Corporation, and CC received a research grant from Cerus Europe BV for conduct of this study. JPC received research support and serves on Scientific Advisory Boards for Cerus Corporation.

REFERENCES

1. Brouard C, Bernillon P, Quatresous I, Pillonel J, Assal A, De Valk H, Desenclos JC; workgroup "Quantitative Estimation of the Risk of Blood Donation Contamination by Infectious Agents." Estimated risk of *Chikungunya viremic* blood donation during an epidemic on Reunion Island in the Indian Ocean, 2005 to 2007. Transfusion 2008;48:1333-41.
2. Schuffenecker I, Iteman I, Michault A, Murri S, Frangeul L, Vaney MC, Lavenir R, Pardigon N, Reynes JM, Pettinelli F, Biscornet L, Diancourt L, Michel S, Duquerroy S, Guigon G, Frenkiel MP, Bréhin AC, Cubito N, Desprès P, Kunst F, Rey FA, Zeller H, Brisse S. Genome microevolution of Chikungunya viruses causing the Indian Ocean outbreak. PLoS Med 2006;3:1-16.
3. Krastinova E, Quatresous I, Tarantola A. Imported cases of Chikungunya in metropolitan France: update to June 2006. Euro Surveill 2006;11:E060821-060824.
4. Cordel H, Quatresous I, Paquet C, Couturier E. Imported cases of Chikungunya in metropolitan France, April 2005-February 2006. Euro Surveill 2006;11:E060420-060423.
5. Rasongles P, Isola H, Kientz D, Cazenave JP, Sawyer L, Corash L. Rapid implementation of photochemical pathogen inactivation (INTERCEPT) for preparation of platelet components during an epidemic of Chikungunya virus. Vox Sang 2006;91(Suppl 3):32.
6. Sawyer L, Sampson-Johannes A, Dubensky T, Kinsey J, Tsetsarkin K, Vanlandingham DL, Higgs S. Inactivation of chikungunya virus in platelets and plasma using the INTERCEPT Blood System™. Vox Sang 2007;93(Suppl 1):170.
7. Lin L, Dikeman R, Molini B, Lukehart SA, Lane R, Dupuis K, Metzel P, Corash L. Photochemical treatment of platelet concentrates with amotosalen and UVA inactivates a broad spectrum of pathogenic bacteria. Transfusion 2004;44: 1496-504.
8. Lin L, Hanson CV, Alter HJ, Jauvin V, Bernard KA, Murthy KK, Metzel P, Corash L. Inactivation of viruses in platelet concentrates by photochemical treatment with amotosalen and long-wavelength ultraviolet light. Transfusion 2005;45: 580-90.
9. Castro E, Girones N, Bueno JL, Carrion J, Lin L, Fresno M. The efficacy of photochemical treatment with amotosalen HCl and ultraviolet A (INTERCEPT) for inactivation of *Trypanosoma cruzi* in pooled buffy-coat platelets. Transfusion 2007;47:434-41.
10. Angelini MF, Fressy P, Rasongles P. Epidémie de chikungunya à la Réunion et transfusion sanguine. Le Bulletin de l' Hemovigilance de l'Afssaps 2006;13:9-10.
11. Andreu G, Morel P, Forestier F, Rebibo D, Janvier G, Herve P. Hemovigilance network in France: organization and analysis of immediate transfusion incident reports from 1994-1998. Transfusion 2002;42:1356-64.
12. Osselaer JC, Messe N, Hervig T, Bueno J, Castro E, Espinosa A, Accorsi P, Junge K, Jacquet M, Flament J, Corash L. A prospective observational cohort safety study of 5106 platelet transfusions using components prepared with photochemical pathogen inactivation treatment. Transfusion 2008;48:1061-71.
13. Bernard GR, Artigas A, Brigham KL. The American-European Consensus Conference on ARDS: definitions, mechanisms, relevant outcomes, and clinical trial coordination. Am J Respir Crit Care Med 1994;149:818-42.
14. Osselaer JC, Cazenave JP, Lambermont M, Garraud O, Hidajat M, Barbolla L, Tardivel R, Defoin L, Waller C, Mendel I, Raidot JP, Kandel G, De Meuter R, Fabrigli P, Dehenau D, Arroyo JL, Padrón F, Gouezec H, Corral M, Jacquet M, Sundin D, Lin L, Corash L. An active haemovigilance programme characterizing the safety profile of 7437 platelet transfusions prepared with amotosalen photochemical treatment. Vox Sang 2008;94:315-23.
15. Mantke OD, Niedrig M, ENIVD. Laboratory capacity for detection of chikungunya virus infections in Europe. Euro Surveill 2007;12. Available from: http://www.eurosurveillance.org/ew/2007/070913.asp#2
16. Laurent P, Le Roux K, Grivard P, Bertil G, Naze F, Picard M, Staikowsky F, Barau G, Schuffenecker I, Michault A. Development of a sensitive real-time reverse transcriptase PCR assay with an internal control to detect and quantify chikungunya virus. Clin Chem 2007;53:1408-14.
17. Chernesky MA, Larke RP. Contrasting effects of rabbit and human platelets on chikungunya virus infectivity. Can J Microbiol 1977;23:1237-44.
18. Larke RP, Wheelock EF. Stabilization of chikungunya virus infectivity by human blood platelets. J Infect Dis 1970;122: 523-31.
19. Angelini-Tibert MF, Rasongles P. Intolerance des CPA traites par INTERCEPT—Amotosalen en onco-pediatrie. Paris: Etablissement Francais du Sang; 2007.
20. van Rhenen D J, Gulliksson H, Cazenave JP, Pamphilon D, Ljungman P, Klüter H, Vermeij H, Kappers-Klunne M, de Greef G, Laforet M, Lioure B, Davis K, Marblie S, Mayaudon V, Flament J, Conlan M, Lin L, Metzel P, Buchholz D, Corash L; euroSPRITE trial. Transfusion of pooled buffy coat platelet components prepared with photochemical pathogen inactivation treatment: the euroSPRITE trial. Blood 2003;101:2426-33.
21. 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 4th, Davis K, Lin JS, Metzel P, Corash L, Koutsoukos A, Lin L, Buchholz DH, Conlan MG. Therapeutic efficacy and safety of platelets treated with a photochemical process for pathogen inactivation: the SPRINT trial. Blood 2004;104:1534-41.
22. Renault P, Solet JL, Sissoko D. A major epidemic of chikungunya virus infection on Reunion Island, France, 2005-2006. Am J Trop Med Hyg 2007;77:727-31.
23. Angelini-Tibert MF, Currie C, Slaedts M, Corash LM, Rasongles P. Safety of platelet components prepared with

photochemical treatment (INTERCEPT) transfused to pediatric oncology-hematology patients. Transfusion 2007; 43:22A.
24. Depoortere E, Coulombier D. Chikungunya risk assessment for Europe: recommendations for action. Euro Surveill 2006;11. Available from: http://www.eurosurveillance.org/ew/2006/060511.asp#2
25. Warner E, Garcia-Diaz J, Balsamo G, Shranatan S, Bergmann A, Blauwet L, Sohail M, Baddour L, Reed C, Baggett H, Campbell G, Smith T, Powers A, Hayes N, Noga A, Lehman J. Chikungunya fever diagnosed among international travelers—United States, 2005-2006. MMWR Morb Mortal Wkly Rep 2006;55:1040-2.
26. Rezza G, Nicoletti L, Angelini R, Romi R, Finarelli AC, Panning M, Cordioli P, Fortuna C, Boros S, Magurano F, Silvi G, Angelini P, Dottori M, Ciufolini MG, Majori GC, Cassone A; CHIKV study group. Infection with chikungunya virus in Italy: an outbreak in a temperate region. Lancet 2007;370:1840-6.

Universal adoption of pathogen inactivation of platelet components: impact on platelet and red blood cell component use

Jean Claude Osselaer, Chantal Doyen, Laurence Defoin, Cecile Debry, Maité Goffaux, Nathalie Messe, Maryse Van Hooydonk, Andre Bosly, Jin Sying Lin, Lily Lin, and Laurence Corash

BACKGROUND: Pathogen inactivation of platelet (PLT) components (INTERCEPT Blood System, Cerus Europe) was implemented into routine practice at a blood center supporting a tertiary care hospital. Utilization of platelet components (PCs) and red blood cell (RBC) components was analyzed for 3 years before and 3 years after introduction of pathogen inactivation to assess the impact of pathogen inactivation on component use.

STUDY DESIGN AND METHODS: This was a retrospective analysis of prospectively collected data. An electronic database used in routine blood bank hemovigilance to monitor production and use of blood components was analyzed to assess clinical outcomes.

RESULTS: Transfusion records were analyzed for 688 patients supported with conventional PCs and 795 patients supported with pathogen inactivation PCs. Additional analyses were conducted for intensively transfused hematology patients. Patient demographics (age category, sex, and diagnostic category) were not different in the two observation periods. For all patients, mean numbers of PC per patient were not different for conventional PCs and pathogen inactivation PCs (9.9 ± 19.5 vs. 10.1 ± 20.9, $p = 0.88$). Data for hematology patients (272 conventional PCs and 276 pathogen inactivation PCs) confirmed that days of PLT support were not different (31.6 ± 42.6 vs. 33.1 ± 47.9, $p = 0.70$) nor was total PLT dose (10^{11}) per patient (87.3 ± 115.4 vs. 88.1 ± 111.6, $p = 0.93$). RBC use, for all patients and hematology patients, was not different in the two observation periods, either during periods of PLT support or outside periods of PLT transfusion support.

CONCLUSION: Pathogen inactivation of PCs had no adverse impact on component use during a 3-year observation period of routine practice.

During the past four decades multiple new procedures and practices have been introduced to improve the safety and efficacy of platelet (PLT) transfusion therapy. Technology innovations for collection and preparation have included plateletpheresis, preparation of whole blood–derived buffy coat PLTs, additive solutions (ASs), process and filtration leukoreduction, initial blood draw diversion, and gamma irradiation.[1] Additional innovations to detect bacterial contamination,[2] change the PLT transfusion threshold,[3] reduce the incidence of alloimmunization,[4] and change the PLT transfusion dose[5] have been evaluated in clinical trials of varying size and scope, and many of these innovations have been introduced into routine clinical practice.[6,7] In 2003 pathogen inactivation preparation of PLT components was introduced into clinical practice in Europe.[8,9]

These innovative technologies have been evaluated in clinical trials; however, additional information regarding the impact of new technology on blood center operations and patient outcomes can be obtained from the experience in routine use. A recent international consensus conference on pathogen inactivation technology recommended that data be collected during routine use to monitor impact as these novel technologies were

ABBREVATIONS: BTC = Blood Transfusion Center; CUMG = Cliniques Universitaires Mont Godinne; PC(s) = platelet component(s).

From the Transfusion Center and the Hematology Service, Cliniques Universitaires de Mont Godinne, Université Catholique de Louvain, Yvoir, Belgium; and Cerus Corporation, Concord, California.

Address reprints requests to: Laurence Corash, MD, Cerus Corporation, 2411 Stanwell Drive, Concord, CA 94520; e-mail: lcorash@cerus.com.

Received for publication May 15, 2008; revision received January 14, 2009; and accepted January 19, 2009.

doi: 10.1111/j.1537-2995.2009.02151.x

TRANSFUSION 2009;49:1412-1422.

adopted.[10] In 2003 the Blood Transfusion Center (BTC), Cliniques Universitaires Mont Godinne (CUMG), initiated universal routine use of PLT components prepared with photochemical pathogen inactivation treatment (INTERCEPT Platelet System, Cerus Europe BV, Amersfoort, The Netherlands) for transfusion support of patients with thrombocytopenia. Three randomized controlled clinical trials were conducted with this pathogen inactivation technology in support of CE Mark registration,[11-13] but data on component utilization were collected only for conduct of clinical trials in which the experimental components were produced in limited quantities rather than in routine production.

The BTC Mont Godinne is the sole source of PLT components and the principal source of red blood cell (RBC) concentrates for a tertiary care medical center. The blood center collects data on the use of blood components under the auspices of a hemovigilance program. We conducted an analysis of the utilization of PLT and RBC components for 3 years before the adoption of pathogen inactivation technology for PLTs and for 3 years after the adoption of this technology to evaluate the impact of this new technology on component utilization under routine clinical practice conditions.

MATERIALS AND METHODS

Overall study design

The BTC Mont Godinne collects and supplies all blood components for a 400-bed teaching hospital of the Université Catholique de Louvain (Mont Godinne, Yvoir, Belgium). The BTC performs approximately 2000 plateletpheresis and 7000 whole-blood collections per year to support a diverse patient population with major subpopulations cared for by hematology-oncology and cardiovascular surgery specialists. The BTC issues all labile blood components for transfusion and maintains an electronic database to record the transfusion or destruction of all issued blood components. Longitudinal transfusion records are maintained for all recipients of these components either in hospital or in outpatient treatment clinics. Data for this study on utilization of blood components were obtained from electronic blood bank records and clinical laboratory records. The period from October 1, 2000, to September 30, 2003, constituted the control period when all PLT components were prepared without pathogen inactivation treatment, and the period from November 1, 2003, through October 30, 2006, constituted the test period when all PLT components were prepared with pathogen inactivation. One of the authors (JCO) has been the medical director of the BTC during the entire period covered by this study and supervised the hemovigilance program. Individual patient informed consent was not required to obtain the data collected as this study was conducted under the existing hemovigilance program for the BTC in compliance with Belgian Law to monitor the impact of new technologies on blood transfusion practice.[14] Patient privacy was protected under the hemovigilance program.

Collection and preparation of PLT components

Control period

During this 3-year period 85% of PLT components were collected on a cell separator (Amicus, Fenwal, Inc., Round Lake, IL) and 15% on another cell separator (Spectra, Gambro BCT, Boulder, CO) with process leukoreduction. For components collected on the Amicus device, T-Sol PLT AS (Fenwal, Inc., La Châtre, France) was used in a ratio of approximately 35% plasma and 65% T-sol. For components collected on the Spectra device, PLTs were suspended in 100% plasma. For both platforms, components were prepared as conventional PLT components in compliance with national regulatory requirements. After component preparation the PLT concentrations (10^9/L) and the volumes (mL), based on weight, of each component were measured and the total PLT dose per component was calculated (10^{11} PLTs). PLT components were treated with gamma irradiation as required for patient specific indications, and tested for cytomegalovirus (CMV) antibodies as required for patient specific indications. During this period, PLT components were stored for up to 5 days.

Test period

During this 3-year period all PLT components were collected on the Amicus cell separator (Fenwal, Inc.) with process leukoreduction and with the InterSol PLT AS (Cerus Europe BV) in a ratio of approximately 35% plasma and 65% Intersol. After collection, the PLT concentrations (10^9/L) and volumes (mL), based on weight, of each component were measured and the total PLT dose was calculated (10^{11} PLTs). These components were prepared with pathogen inactivation under the CE Mark registration and in accordance with national regulatory requirements. Within the first 24 hours after collection, all PLT components were treated with pathogen inactivation (150 µmol/L amotosalen HCl and 3 J/cm^2 UVA light, 320-400 nm) according to the manufacturer's directions for use (INTERCEPT, Cerus Europe BV). For quality control (QC), the final volume of treated PLT components was measured on approximately 30% of components and used to estimate the final PLT content of each component based on volume loss. Pathogen inactivation treatment was used in place of gamma irradiation and CMV serology testing to meet specific patient indication requirements, and it was used instead of bacteria detection to meet national regulatory requirements. With pathogen inactivation treatment, PLT components were stored for either 5 or 7 days in accordance with national regulatory requirements.

During both study periods, blood center operational logistics permitted issuance of PLT components within the

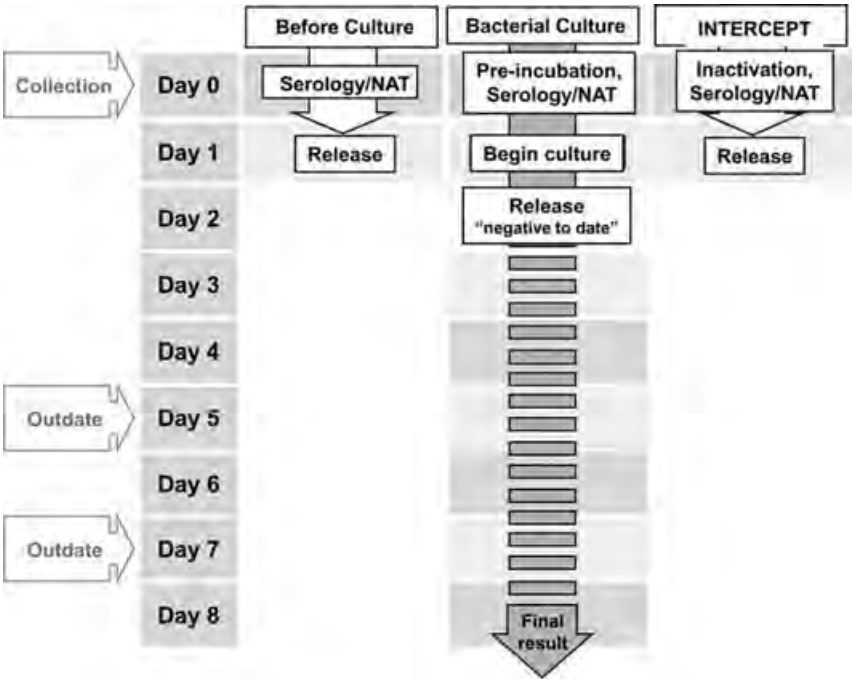

Fig. 1. Operational logistics for production and release of PLT components using three methods. Before culture = conventional testing without bacterial culture; bacterial culture = conventional testing with bacterial culture; and INTERCEPT pathogen inactivation with conventional testing.

same time frame after collection, testing, and pathogen inactivation treatment (Fig. 1). The preparation of RBC concentrates did not change in any substantial manner during the 6-year study observation period, and all RBC concentrates were leukoreduced by filtration during both periods. RBC concentrates were treated with gamma irradiation and tested for CMV antibody per specific patient requirements using the same methods in both study periods.

Transfusion of PLT and RBC components and data collection

During both study periods, primary care physicians ordered all blood components per standard of care. A proportion of patients had multiple PLT transfusions and multiple periods of PLT support during the 6-year observation period. A period of PLT support was defined as the interval between the first PLT transfusion and all subsequent PLT transfusions with less than 5 days between PLT transfusions. If an interval of more than 5 days occurred between PLT transfusions, then a new period of PLT support was considered initiated. The total duration of PLT support for each patient was defined as the number of days between the first and the last PLT transfusion within the same period of PLT support. The days of PLT support for multiple periods were summed to obtain a total duration of support for each patient during each observation period. These definitions were based on the assumption that if no PLT transfusions were required after a 5-day interval then transfusion-dependent thrombocytopenia was no longer present. These definitions have previously been used in randomized clinical trials to evaluate PLT transfusion therapeutic efficacy.[11-13]

The BTC Mont Godinne entered all blood components into an electronic database at time of collection and labeling (Blood Bank Management System, 4S Information Systems, Minthorne, UK; or CTS Serveur, INLOG, Limonest, France). When components were issued for transfusion a standard form accompanied each component and was returned to the blood center after transfusion or with unused components. No patients were excluded from this analysis. The BTC maintained a longitudinal transfusion record for each patient supported with blood components including unique patient identification data, clinical service location for transfusion, unique blood component identification number, date of transfusion, dose of PLT component transfused, and age of PLT component transfused. The BTC obtained patient clinical laboratory data from electronic laboratory records linked to unique patient identification records with protection of patient confidentiality under the hemovigilance program. Using these electronic data capture systems, greater than 99% of issued blood components were traceable within the system.

Data analysis and statistical methods

Data from the BTC were extracted into a computer database (SAS, SAS, Inc., Cary, NC). Values for all continuous variables were summarized as mean and standard deviation (SD), median, and range. Differences in mean values between the two observation periods were compared by two-sample t-test for continuous data and Fisher's exact test for categorical data. Differences in median values between the two observation periods were compared by the two-sample Wilcoxon test. All p values reported were two-sided, and statistical significance was declared at a p value of less than 0.05.

RESULTS

PLT collections, yields, and processing losses

During the control period, 5576 collections were performed with a mean yield of 6.28×10^{11} PLTs. The 95%

central interval for the distribution of transfused doses in the control period ranged from 2.1×10^{11} to 6.3×10^{11} PLTs. During the test period, 5997 collections were performed with a mean yield of 6.82×10^{11} PLTs. After routine implementation of the INTERCEPT technology, 12,002 products were treated with pathogen inactivation. Approximately 50% of collections were targeted as double-dose collections and were divided into two therapeutic PLT doses. The 95% central interval for the distribution of transfused doses ranged from 1.9 to 5.3×10^{11} PLTs during the test period. Eight pathogen inactivation procedures had technical failures (0.06%), of which 5 were due to operator error and 3 were due to disposable failures associated with incorrect placement of a clamp during storage leading to pinhole leaks. As part of the QC process, 4066 components during the test period were assayed for volume loss during pathogen inactivation processing. Mean volume loss without accounting for addition of amotosalen (15 mL) was $8.2 \pm 2.2\%$ and the distribution of volume losses exhibited a 25th percentile of 7.0% and a 75th percentile of 9.4%. Volume loss correlated with PLT loss. With consideration of the dilution due to addition of amotosalen (4.4%), the mean processing loss was 12.6%.

Patient populations and demographics

During the control period, 688 patients received one or more PLT components compared to 795 patients during the test period (Table 1). The increased number of patients in the test period reflected increased clinical activity as new clinical programs were implemented at the CUMG. Patient demographics with respect to age distribution, sex, and primary diagnostic category did not differ significantly in the two treatment periods (Table 1). During the two study periods approximately 90% of the population receiving PLT support also required one or more RBC concentrates, and the demographics for these patients did not change significantly during the two periods (Table 2).

TABLE 1. Demographics of all patients receiving PLT components in the two observation periods

Study period	Control	Test	p Value
Patients (n)	688	795	
Mean age, years	60.8	62.9	0.01
≤16 (%)	0.9	1.0	
17-64 (%)	49.7	45.3	
≥65 (%)	49.4	53.7	0.23*
Male (%)	62.8	62.3	0.87
Primary diagnosis (%)†			
Hematology	39.5	34.7	
Oncology	6.5	8.8	
CV surgery	31.7	34.7	
Other‡	22.2	21.8	0.12*

* Represents p value for the distribution.
† Patients were classified by primary diagnosis based on the clinical service providing medical care and ordering blood components.
‡ The designation of other refers to patients on general medical services and surgical services other than cardiovascular (CV) surgery.

Utilization of PLT and RBC components

For the complete patient population receiving one or more PLT components in either period, the mean number of PLT transfusion support periods and the distribution of support periods were not statistically different (Fig. 2, Table 3). The mean duration of PLT support, the mean number of PLT transfusions, the total PLT dose, and the

TABLE 2. Demographics of all patients receiving both PLT and RBC components in the two observation periods

Study period	Control	Test	p Value
Patients (n)	629	721	
Mean age, years (%)	61.3	62.9	0.06
≤16 (%)	0.8	0.8	
17-64 (%)	48.8	45.5	
≥65 (%)	50.4	53.7	0.48*
Male (%)	61.7	61.0	0.82
Primary diagnosis (%)†			
Hematology	40.9	36.9	
Oncology	6.0	7.9	
CV surgery	32.3	35.9	
Other‡	20.8	19.3	0.19*

* Represents p value for the distribution.
† Patients were classified by primary diagnosis based on the clinical service providing medical care and ordering blood components.
‡ The designation of other refers to patients on general medical services and surgical services other than cardiovascular (CV) surgery.

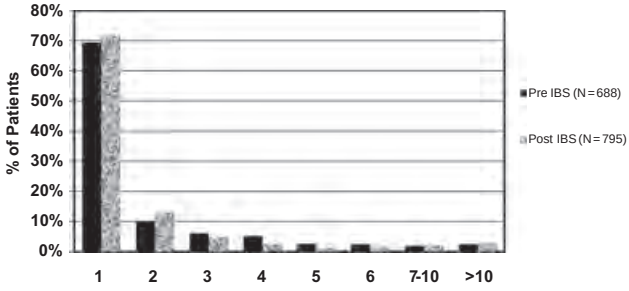

Fig. 2. The distribution of the periods of PLT transfusion support for all patients receiving PLT components. The frequency distribution of the periods of PLT support for patients during the period before INTERCEPT (pre-IBS, ■; n = 688) and the period after INTERCEPT (post-IBS, ■; n = 795) was determined. The proportion of patients is expressed on the ordinate and the number of periods of PLT transfusion support on the abscissa. There was no statistical difference in the distribution of support cycles between observation periods.

mean dose of PLTs per day of transfusion support were not different between the two treatment periods (Table 3). For patients receiving both PLT and RBC components (control, 629; test, 721), the mean numbers of RBC concentrates transfused per patient (Table 4) were not different in the two observation periods (control 16.5 ± 20.9 vs. test 16.5 ± 21.5, p = 0.98).

Because RBC concentrates may be transfused to correct anemia due to blood loss or marrow hypoproduction arising from many different causes, we also examined the use of RBC concentrates during periods of PLT transfusion support and outside periods of PLT transfusion support to determine if there were differences during transfusion-dependent thrombocytopenia and out side periods of transfusion-dependent thrombocytopenia. No significant differences were observed between study periods in the use of RBC components during support of thrombocytopenia and outside periods of PLT component transfusion support (Table 4).

TABLE 3. PLT transfusion support for all patients before and after adoption of pathogen inactivation treatment of PLT components

Parameter	Control*	Test*	p Value
Number of patients (n)	688	795	0.01
Periods of PLT support (n)	2.2 (3.2)	2.2 (4.4)	0.86
Days of PLT support (days)	14.2 (30.5)	13.1 (32.0)	0.49
Transfusions/patient (n)	9.9 (19.5)	10.1 (20.9)	0.88
Transfusions/day of support (n)	1.0 (0.5)	1.1 (0.6)	<0.01
Total dose/patient (10^{11})	41.5 (82.8)	36.7 (76.5)	0.24
Dose per day of support (10^{11})	4.2 (2.1)	4.0 (2.3)	0.20

* Data presented as mean (SD).

TABLE 4. RBC use by all patients during the observation periods, during periods of PLT support, and outside periods of PLT support before and after adoption of pathogen inactivation treatment of PLT components

Parameter	Control*	Test*	p Value
RBC use during the entire observation period			
Patients (n)†	688	795	
RBC units/patient (n)	15.1 (20.5)	15.0 (21.0)	0.90
RBC use during the entire observation period			
Patients (n)‡	629	721	
RBC units/patient (n)	16.5 (20.9)	16.5 (21.5)	0.90
RBC use during periods of PLT support			
Patients (n)§	545	634	
RBC units/patient (n)	10.4 (14.4)	10.7 (16.2)	0.71
RBC use outside periods of PLT support			
Patients (n)‖	504	581	
RBC units/patient (n)	9.4 (14.5)	8.8 (13.6)	0.47

* All values expressed as mean (SD).
† Patients transfused with PLT components during the observation period.
‡ Patients transfused with RBC components and PLT components during the entire observation period.
§ Patients transfused with RBC components during periods of PLT transfusion support.
‖ Patients transfused with RBC components outside periods of PLT transfusion support.

The broad patient population supported with PLT and RBC components was heterogeneous with respect to primary diagnoses and the level of PLT support required. To more specifically define the impact of pathogen inactivation on PLT component therapy, the subset of hematology patients was analyzed separately. This population required more intensive transfusion support with less acute blood loss due to surgical intervention and, thus, was a more homogeneous population in which to evaluate both PC and RBC use. The majority (more than 60%) of hematology patients had more than one period of PLT transfusion support (Fig. 3). The distributions of the periods of support were not statistically different between the two observation periods (Fig. 3).

Hematology patients were more intensively transfused with PLT components, had more periods of PLT support, and had more days of PLT support than the general patient population (Table 5). Comparison of the two observation periods demonstrated no significant differences in the mean number of PLT transfusion support periods, mean number of days of transfusion support, number of PLT transfusions, the dose of PLTs, nor the PLT dose per day of PLT support (Table 5). We examined the use of RBC components for hematology patients during the entire transfusion period as well as during and outside periods of PLT support (Table 6). In this analysis we included patients who received PLTs without RBC transfusion during PLT support and patients who received both PLT and RBC support. We observed no significant differences in use of RBC components by hematology patients during the entire observation period during periods of PLT support and outside periods of PLT support (Table 6).

Similarly, data were analyzed for a population of oncology patients followed during the 6-year observation period. These patients were less intensively transfused with PLT components than the hematology patients, but did have multiple periods of PLT support; however, the distribution of periods of support was not different (Fig. 4, Table 7). Significant differences in favor of INTERCEPT PC were detected in transfusions per patient, total PLT dose, and the dose per day of support. There were no significant differences in RBC concentrate use during the entire observation period for patients receiving PLT transfusions and for patients receiving PLT and RBC components (Table 8).

To examine the impact of transfusion practice and clinical responses to PLT and RBC transfusions, the mean

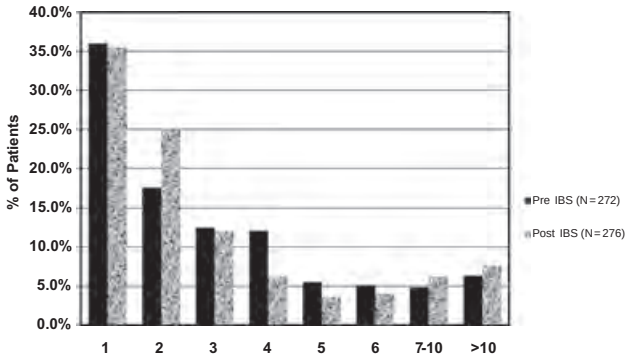

Fig. 3. The distribution of the periods of PLT transfusion support for hematology patients receiving PLT components. The frequency distribution of the periods of PLT support for patients for the period before INTERCEPT (pre-IBS, ■; n = 272) and the period after INTERCEPT (post-IBS, ▩; n = 276) was determined. The proportion of patients is expressed on the ordinate and the number of periods of PLT transfusion support on the abscissa. There was no statistical difference in the distribution of support cycles between observation periods.

and median lowest daily PLT counts were determined on a per-transfusion basis for the various patient populations during both observation periods (Table 9). We did not analyze data for the oncology patients due to the small number of transfusions; however, we did analyze data separately for cardiovascular surgery patients since they received PLT transfusions at higher PLT count levels. There was a very broad and asymmetric distribution of the daily lowest PLT count for all patient groups, and the median values were considerably different from the mean values. The proportion of PLT transfusions with recorded PLT counts in the medical record on the day of transfusion was similar in each period for each patient group (Table 9). While the mean values for the daily lowest PLT count were significantly higher ($p < 0.001$) in the test period for all patient groups, the median values were not statistically different ($p = 0.23$). Exploratory analyses using analysis of covariance (ANCOVA) models were performed on the transfused PLT dose with the nadir of PLT count included as a covariate in the models. Treatment group (test vs. control) and patient's primary diagnosis (hematology, oncology, cardiovascular surgery, and other) were also included in the models. Results from these exploratory analyses showed that, within each treatment group, the adjusted means (after the adjustment for the nadir of PLT count and/or the primary diagnosis) were nearly the same as the unadjusted sample means, and the observation of significant differences for the means and the nonsignificant difference for the median remain unchanged with and without the adjustment for the nadir of PLT count and/or the primary diagnosis.

TABLE 5. PLT transfusion support for hematology patients before and after adoption of pathogen inactivation treatment of PLT components

Parameter	Control*	Test*	p Value
Number of patients (n)	272	276	
Periods of PLT support (n)	3.7 (4.6)	4.1 (6.9)	0.40
Duration of support (days)	31.6 (42.6)	33.1 (47.9)	0.70
Transfusions/patient (n)	20.8 (27.1)	24.2 (30.5)	0.17
Transfusions/day of support (n)	0.8 (0.4)	0.8 (0.3)	0.13
Total dose/patient (10^{11})	87.3 (115.4)	88.1 (111.6)	0.93
Dose per day of support (10^{11})	3.2 (1.4)	3.0 (1.3)	0.12

* Data presented as mean (SD).

TABLE 6. RBC use by hematology patients during the observation periods, during periods of PLT support, and outside periods of PLT support before and after adoption of pathogen inactivation treatment of PLT components

Parameter	Control*	Test*	p Value
RBC use during the entire observation period			
Patients (n)†	272	276	
RBC units/patient (n)	24.5 (27.1)	26.4 (30.4)	0.43
RBC use during the entire observation period			
Patients (n)‡	257	266	
RBC units/patient (n)	25.9 (27.2)	27.4 (30.5)	0.55
RBC use during periods of PLT support			
Patients (n)§	222	244	
RBC units/patient (n)	16.4 (19.1)	17.6 (23.3)	0.54
RBC use outside periods of PLT support			
Patients (n)‖	237	235	
RBC units/patient (n)	12.7 (18.8)	12.7 (19.2)	1.00

* All values expressed as mean (SD).
† Patients transfused with PLT components during the observation period.
‡ Patients transfused with RBC components and PLT components during the entire observation period.
§ Patients transfused with RBC components during periods of PLT transfusion support.
‖ Patients transfused with RBC components outside periods of PLT transfusion support.

To further examine the impact of the adoption of pathogen inactivation treatment of PLT components on RBC use as a surrogate measure for bleeding, we determined the mean daily lowest hemoglobin (Hb) level per patient for those patients receiving both PLT and RBC concentrates (Table 10). No differences in the mean lowest daily Hb level were detected in any patient groups before and after implementation of pathogen inactivation for PLT components.

DISCUSSION

This study provides an assessment of the impact of pathogen inactivation on the utilization of PLT and RBC components in both broad and

TABLE 7. PLT transfusion support for oncology patients before and after adoption of pathogen inactivation treatment of PLT components

Parameter	Control*	Test*	p Value
Number of patients (n)	45	70	
Periods of PLT support (n)	2.0 (1.5)	1.8 (2.6)	0.72
Duration of support (days)	7.9 (11.2)	5.0 (11.0)	0.17
Transfusions/patient (n)	6.8 (9.1)	3.9 (6.1)	0.05
Transfusions/day of support (n)	1.0 (0.4)	1.0 (0.4)	0.91
Total dose/patient (10^{11})	27.5 (37.7)	14.5 (22.5)	0.02
Dose per day of support (10^{11})	4.3 (1.7)	3.7 (1.4)	0.04

* Data presented as mean (SD).

TABLE 8. RBC use by oncology patients during the two observation periods, before and after adoption of pathogen inactivation treatment of PLT components

Parameter	Control*	Test*	p Value
RBC use during the entire observation period			
Patients (n)†	45	70	
RBC units/patient (n)	8.6 (9.0)	8.0 (7.7)	0.73
RBC use during the entire observation period			
Patients (n)‡	38	57	
RBC units/patient (n)	10.2 (9.0)	9.9 (7.4)	0.86

* All values expressed as mean (SD).
† Patients transfused with PLT components during the observation period.
‡ Patients transfused with RBC components and PLT components during the entire observation period.

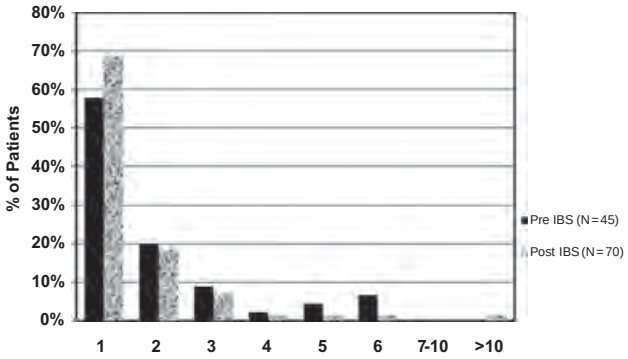

Fig. 4. The distribution of the periods of PLT transfusion support for oncology patients receiving PLT components. The frequency distribution of the periods of PLT support for patients before adoption of INTERCEPT (pre-IBS, ■; n = 45) and the period after INTERCEPT (post-IBS, ▩; n = 70) was determined. The proportion of patients is expressed on the ordinate and the number of periods of PLT transfusion support on the abscissa. There was no statistical difference in the distribution of support cycles between observation periods.

specialized patient populations. This study is unusual in its scope, in that it covers a relatively long period during which the new technology was used in routine practice, and it provides data from a comparative period before adoption of the new process. In addition to providing information on the impact of the innovation, the study provides information on the utilization of PLT and RBC components in a broad patient population and in specific patient populations with more intense transfusion requirements. To our knowledge, this is the first study to examine the impact of a new PLT preparation technology on the routine production and utilization of PLT components for an observation period as long as 6 years.

In Phase 1 and 2 clinical trials with autologous radiolabeled 5-day-old PLT components transfused to healthy subjects, we detected a 15% to 20% reduction in PLT viability.[15] Subsequently, a large Phase 3 clinical trial demonstrated increased utilization of PLT components treated with pathogen inactivation compared to conventional PCs.[12] This observation was due in part to difficulties with production of consistent PLT doses during the clinical trial and the stringent requirement to avoid off-protocol transfusions.[12] Analysis of patients in both treatment groups supported with components consistently containing more than 3.0×10^{11} PLTs, showed no difference in utilization of PLT components.[16] In light of these observations, we sought to evaluate PLT component utilization in the context of routine practice with production of PLT components over long periods.

In this study, the patient populations in the two observation periods were comparable with the exception of a 15% increase in the number of patients receiving PLT transfusions due to increased clinical activity at the study center. In both periods, approximately 90% of patients were transfused with PLT and RBC components providing the opportunity to examine the impact of the new PLT technology on use of RBCs. There was considerable variation in the duration of PLT support due to heterogeneity of the patient population, even among specific diagnostic groups, such as hematology patients. This heterogeneity reflected the diverse population of patients who received PLT support in clinical practice and provided a realistic measure of the impact of a new technology. In addition, the long time of observation in which patients experienced multiple periods of PLT support during multiple chemotherapy cycles contributed to the heterogeneity, but provided a comprehensive assessment of the impact of the new technology on component use.

A substantial number of more intensively transfused hematology patients were available in both periods to allow assessment of the impact of pathogen inactivation on PLT and RBC utilization among repeatedly transfused patients. During both observation periods, hematology patients received the majority of PLT components due to longer periods of transfusion-dependent thrombocytope-

TABLE 9. Daily lowest PLT count for patients transfused before and after adoption of pathogen inactivation treatment of PLT components

Parameter	Control	Test	p Value
All patients			
PLT transfusions (n)*	6812	7994	
PLT dose/transfusion†	4.2 (1.0)	3.6 (0.9)	<0.001
PLT count (10^9/L)‡	47.1 (71.4)	63.5 (95.3)	<0.001
Median PLT count (10^9/L)	23.0	22.0	0.39§
Cardiovascular surgery patients			
PLT transfusions (n)*	415	534	
PLT dose/transfusion†	4.3 (1.0)	3.8 (0.8)	<0.001
PLT count (10^9/L)‖	71.2 (51.4)	77.4 (45.1)	0.09
Median PLT count (10^9/L)	59.0	68.0	0.005§
All hematology patients			
PLT transfusions (n)*	5658	6686	
PLT dose/transfusion†	4.2 (1.0)	3.6 (0.9)	<0.001
PLT count (10^9/L)¶	44.1 (72.3)	60.6 (97.8)	<0.001
Median PLT count (10^9/L)	21.0	20.0	0.23§
*Intensively transfused hematology patients***			
PLT transfusions (n)*	5630	6663	
PLT dose/transfusion†	4.2 (1.0)	3.6 (0.9)	<0.001
PLT count (10^9/L)††	44.0 (72.0)	60.5 (97.7)	<0.001
Median PLT count (10^9/L)	21.0	20.0	0.25§

* The total number of PLT transfusions administered during each period with at least one PLT count on the day of transfusion.
† The mean (SD) for the PLT dose (10^{11}).
‡ The mean daily lowest PLT count (SD) for transfusions with a PLT count available in the medical record on day of transfusion. During the control and test periods there were 4690 (68.8%) and 5797 (72.5%) transfusions, respectively, with recorded PLT counts.
§ Based on two-sample Wilcoxon test.
‖ The mean daily lowest PLT count (SD) for transfusions with a PLT count available in the medical record on day of transfusion. During the control and test periods there were 320 (77.1%) and 370 (69.3%) transfusions, respectively, with recorded PLT counts.
¶ The mean daily lowest PLT count (SD) for transfusions with a PLT count available in the medical record on day of transfusion. During the control and test periods there were 4144 (73.2%) and 4914 (73.4%) transfusions, respectively, with recorded PLT counts.
** Intensively transfused hematology patients were defined as those patients who received two or more PLT transfusions in a treatment period.
†† The mean daily lowest PLT count (SD) for transfusions with a PLT count available in the medical record on day of transfusion. During the control and test periods there were 4120 (73.1%) and 4893 (73.4%) transfusions, respectively, with recorded PLT counts.

nia. While it is possible that changes in chemotherapy regimens over the 6-year observation period resulted in less intensive marrow suppression minimizing the impact of a change in PLT component use, several factors argue against this effect. First, the mean duration of PLT transfusion support and the number of periods of transfusion support were similar in the two observation periods, indicating that changes in primary disease treatment had minimal impact on the marrow suppression and the extent of transfusion-dependent thrombocytopenia. In addition, the similar use of RBC components during and outside periods of PLT support in both observation periods is consistent with a lack of change in hematopoietic function secondary to primary disease therapy.

Implementation of pathogen inactivation did result in mean PLT losses of 12.6%. This was partially compensated for by collection of larger PLT doses during the period after implementation of pathogen inactivation. This required an additional 10 minutes of donor collection time, but was well tolerated without impact on donor recruitment or retention. For the broad patient population, adoption of pathogen inactivation for PLT components had no significant impact on utilization of PLT components as measured by multiple indices including mean number of transfusions per patient, mean total dose of PLTs per patient, or mean PLT dose per day of support. The only significant difference detected was an increase in the mean number of PLT transfusions per day of support from 1.0 to 1.1 ($p < 0.01$) among the broad patient population. This difference may have arisen due to a small decrease in the mean duration of PLT support from 14.2 to 13.1 days ($p = 0.49$) after implementation of pathogen inactivation. The reason for the slight decrease in the mean duration of PLT support is unclear. Because the decrease in duration of PLT support was not significant, we concluded that the increase in mean number of PLT transfusions per day was likely not clinically relevant. This difference was not detected among hematology or oncology patients.

Because PLT transfusions are used to support patients with either quantitative or qualitative PLT deficits with potential for bleeding, we examined the use of RBC concentrates as an indirect measure of hemostasis. While RBC transfusions may be administered to correct anemia due to hypoproduction as well as bleeding, data on use of RBC support provide a partial measure of the impact of PLT transfusion on prevention of bleeding. For all patients supported with PLT components in either period, there was no impact on the requirement for RBC transfusion after introduction of pathogen inactivation of PLT components. This trend was consistent for patients supported with PLT and RBC components during periods of PLT support, when patients would be at greatest risk for bleeding, and outside periods of PLT support when RBC use would be most reflective of transfusion to support hypoproductive anemia.

The broad patient population included a substantial proportion of cardiovascular surgery patients (approx. one-third of the population in both periods) with short-term PLT support and obligatory intraoperative blood loss. To examine the impact of pathogen inactivation in a

TABLE 10. Daily lowest Hb level for patients transfused before and after adoption of pathogen inactivation treatment of PLT components

Parameter	Control	Test	p Value
All patients			
Patients (n)[1]	626	713	
Hb (g/dL)[2]	8.5 (1.1)	8.5 (1.0)	0.82
Median Hb (g/dL)	8.6	8.5	
Cardiovascular surgery patients			
Patients (n)*	201	257	
Hb (g/dL)†	8.3 (1.0)	8.2 (1.0)	0.11
Median Hb (g/dL)	8.2	8.1	
All hematology patients			
Patients (n)*	257	266	
Hb (g/dL)†	9.0 (0.7)	8.9 (0.7)	0.12
Median Hb (g/dL)	9.0	8.9	
Intensively transfused hematology patients‡			
Patients (n)*	235	249	
Hb (g/dL)†	8.9 (0.6)	8.9 (0.6)	0.42
Median Hb (g/dL)	9.0	8.9	

* The number of patients transfused with both PLT and RBC concentrates and a Hb level recorded in the medical record on the day of PLT transfusion.
† The mean (SD) daily lowest Hb level (g/dL) per patient for each patient group.
‡ Intensively transfused hematology patients were defined as those patients who received two or more PLT transfusions in a treatment period.

more stable and intensively transfused population, we specifically analyzed data for hematology patients. This subpopulation comprised slightly more than one-third of the broad patient population in both observation periods, and the hematology patients had more periods of PLT support and a longer cumulative duration of PLT support (mean, 32-33 days) compared to that of the general population (mean, 13-14 days). The multiple periods of PLT support (approx. four as the average) included many patients repeatedly transfused during each 3-year observation period. Multiple indices of PLT component utilization including mean transfusions per patient, mean transfusions per day of PLT support, mean total PLT dose per patient, and mean dose per day of support were not statistically different between the observation periods. Among hematology patients, we observed a significantly lower PLT dose per day of PLT support (3.0×10^{11} vs. 3.2×10^{11} PLTs per day, $p = 0.05$) after introduction of pathogen inactivation. However, the data for this intensively transfused population supported during multiple periods of PLT transfusion over a 3-year observation period did not indicate any impact on PLT utilization after introduction of pathogen inactivation for PLT components (Table 5).

The hematology patient subpopulation provided a patient population in which to evaluate the impact of a change in PLT component preparation on the use of RBCs both during and outside periods of PLT support. This population was not substantially impacted by major surgical interventions, thus providing a more reliable estimate of the impact of a change in PLT component on both hemostasis, as measured by RBC transfusion requirements during periods of PLT support, and the impact on RBC production, as measured by RBC transfusion outside periods of PLT support. No significant differences in RBC use were detected after introduction of pathogen inactivation for PLT components. We interpret these observations to indicate that hemostasis and RBC production were not affected by a change in PLT component preparation.

We also examined the impact of pathogen inactivation on PLT use by oncology patients. However, this was a small patient population; and the data were limited. Consistent with the observations for hematology patients, we did not detect any highly significant impact on PC or RBC use due to the change in PLT component; however, these observations require confirmation in a larger population.

As an added means to examine the impact of a change in PLT component on transfusion practice and clinical outcome, we examined data on the daily lowest PLT count and daily lowest Hb level on days of PLT and RBC transfusions. We hypothesized that the lowest PLT count and the lowest Hb within 1 day on days of PLT and RBC transfusion, respectively, would provide a comparative indicator of the level of thrombocytopenia and anemia on the day of transfusion in the two observation periods. These data were analyzed on a per-transfusion basis as the laboratory indices were related to specific transfusion events. We analyzed these data for all patients as well as for specific subsets including cardiovascular surgery patients, hematology patients, and intensively transfused hematology patients (those with two or more periods of PLT transfusion support). During the 6-year period of this study at CUMG, the broadly accepted clinical threshold for PLT transfusion was a PLT count of 10×10^9 to 20×10^9/L and for RBC transfusion a Hb level of 9 to 10 g/dL. The BTC Mont Godinne does not require a specific pretransfusion PLT count or Hb level to issue a blood component. Primary care physicians ordered blood components based on individual patient assessments and their independent clinical judgment.

We used the PLT count data in two ways. First, the daily lowest PLT count served as a comparison of potential changes in degree of thrombocytopenia between the two observation periods. For all the patient groups analyzed, the mean lowest daily PLT count was associated with a large SD, and the difference in the mean and median values was a result of the heterogeneous distribution of PLT levels in the large patient population. Except for cardiovascular surgery patients, the median values were not significantly different between the groups and were 21×10^9 to 23×10^9/L, reasonably close to expected transfusion threshold values for patients with hypoproliferative thrombocytopenia. This trend was similar for more inten-

sively transfused hematology and oncology patients as well. Cumulatively, these data suggest that after implementation of pathogen inactivation, patients did not experience a lower PLT count nadir during PLT support.

We postulated that the mean lowest daily Hb level could serve as a surrogate measure for hemostasis provided that there was no indication for differential suppression of erythropoiesis by the new PLT component. We concluded that the similar use of RBC components outside periods of PLT support, especially for hematology patients, supported this hypothesis. The lack of difference in the lowest daily mean Hb levels on days of PLT transfusion is consistent with the conclusion that the new PLT components provided adequate hemostasis.

Recently, two multicenter hemovigilance studies monitoring the safety of 12,543 PLT components transfused to 2051 patients have shown that PLT components prepared with pathogen inactivation were well tolerated when used in routine practice.[17,18] In conjunction with these observations, the current study utilizing longitudinal data collected as part of a hemovigilance program demonstrates that PLT components prepared with pathogen inactivation can be implemented into routine practice without substantially impacting PLT or RBC component utilization over a substantial period of transfusion support.

CONFLICT OF INTEREST

J.C. Osselaer received research support for conduct of this study and serves on a speaker board for Cerus Corporation. J.S. Lin is a consultant to Cerus Corporation. L. Lin and L. Corash are employees of Cerus Corporation.

REFERENCES

1. Harris SB, Hillyer CD. Blood manufacturing: component preparation, storage, and transportation. In: Hillyer CD, Silberstein LE, Ness PM, Anderson KC, Roback JD, editors. Blood banking and transfusion medicine. 2nd ed. Philadelphia (PA): Churchill Livingstone; 2007. p. 183-204.
2. Blajchman MA, Beckers EA, Dickmeiss E, Lin L, Moore G, Muylle L. Bacterial detection of platelets: current problems and possible resolutions. Transfus Med Rev 2005;19:259-72.
3. Rebulla P, Finazzi G, Marangoni F, Avvisati G, Gugliotta L, Tognoni G, Barbui T, Mandelli F, Sirchia G. The threshold for prophylactic platelet transfusions in adults with acute myelogenous leukemia. N Engl J Med 1997;337:1870-5.
4. TRAP Study Group. Leukocyte reduction and ultraviolet B irradiation of platelets to prevent alloimmunization and refractoriness to platelet transfusions. N Engl J Med 1997; 337:1861-9.
5. Tinmouth AT, Freedman J. Prophylactic platelet transfusions: which dose is the best dose? A review of the literature. Transfus Med Rev 2003;17:181-93.
6. Slichter SJ. Background, rationale, and design of a clinical trial to assess the effects of platelet dose on bleeding risk in thrombocytopenic patients. J Clin Apher 2006; 21:78-84.
7. Slichter SJ. Evidence-based platelet transfusion guidelines. In: Gewirtz AM, Winter JN, Zuckerman K, editors. American Society of Hematology education program book. Washington, DC: American Society of Hematology; 2007. p. 172-8.
8. Osselaer J, Doyen C, Chatelain C, Debry C, Goffaux M, Messe N, Van Hooydonk M, Sonnet A, Bosly A, Lin JS, Corash L. Impact of photochemical treatment (INTERCEPT™) on platelet use in routine practice. Vox Sang 2006;91 Suppl 3:180.
9. Cazenave JP. Proceedings of a consensus conference: pathogen inactivation-making decisions about new technologies. Clinical experience with pathogen-inactivated platelets. Transfusion Med Reviews 2008;22:1-34.
10. Klein HG, Anderson D, Bernardi MJ, Cable R, Carey W, Hoch JS, Robitaille N, Sivilotti ML, Smaill F. Pathogen inactivation: making decisions about new technologies: report of a consensus conference. Transfusion 2007;47: 2338-47.
11. van Rhenen D J, Gulliksson H, Cazenave JP, Pamphilon D, Ljungman P, Klüter H, Vermeij H, Kappers-Klunne M, de Greef G, Laforet M, Lioure B, Davis K, Marblie S, Mayaudon V, Flament J, Conlan M, Lin L, Metzel P, Buchholz D, Corash L; euroSPRITE trial. Transfusion of pooled buffy coat platelet components prepared with photochemical pathogen inactivation treatment: the euroSPRITE trial. Blood 2003;101:2426-33.
12. 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 4th, Davis K, Lin JS, Metzel P, Corash L, Koutsoukos A, Lin L, Buchholz DH, Conlan MG. Therapeutic efficacy and safety of platelets treated with a photochemical process for pathogen inactivation: the SPRINT trial. Blood 2004;104:1534-41.
13. Janetzko K, Cazenave JP, Kluter H, Kientz D, Michel M, Beris P, Lioure B, Hastka J, Marblie S, Mayaudon V, Lin L, Lin JS, Conlan MG, Flament J. Therapeutic efficacy and safety of photochemically treated apheresis platelets processed with an optimized integrated set. Transfusion 2005; 45:1443-52.
14. Belgian Law of 8 December 1992 on privacy protection in relation to the processing of personal data as modified by the law of 11 December 1998 implementing Directive 95/46 EC and the law of 26 February 2003. Belgium: Belgian State Gazette; 2003.
15. Snyder E, Raife T, Lin L, Cimino G, Metzel P, Rheinschmidt M, Baril L, Davis K, Buchholz DH, Corash L, Conlan MG. Recovery and Lifespan of ^{111} indium radiolabeled platelets treated with pathogen inactivation using amotosalen HCl (S-59) and UVA light. Transfusion 2004;44:1732-440.
16. Murphy S, Snyder E, Cable R, Slichter SJ, Strauss RG,

McCullough J, Lin JS, Corash L, Conlan MG; SPRINT Study Group. Platelet dose consistency and its effect on the number of platelet transfusions for support of thrombocytopenia: an analysis of the SPRINT trial of platelets photochemically treated with amotosalen HCl and ultraviolet A light. Transfusion 2006;46:24-33.

17. Osselaer JC, Cazenave JP, Lambermont M, Garraud O, Hidajat M, Barbolla L, Tardivel R, Defoin L, Waller C, Mendel I, Raidot JP, Kandel G, De Meuter R, Fabrigli P, Dehenau D, Arroyo JL, Padrón F, Gouezec H, Corral M, Jacquet M, Sundin D, Lin L, Corash L. An active haemovigilance programme characterizing the safety profile of 7437 platelet transfusions prepared with amotosalen photochemical treatment. Vox Sang 2008;94:315-23.
18. Osselaer JC, Messe N, Hervig T, Bueno J, Castro E, Espinosa A, Accorsi P, Junge K, Jacquet M, Flament J, Corash L. A prospective observational cohort safety study of 5106 platelet transfusions using components prepared with photochemical pathogen inactivation treatment. Transfusion 2008;48:1061-71.

ORIGINAL ARTICLE

Use of additive solutions and pathogen inactivation treatment of platelet components in a regional blood center: impact on patient outcomes and component utilization during a 3-year period

Jean-Pierre Cazenave, Hervé Isola, Chantal Waller, Isabelle Mendel, Daniel Kientz, Michel Laforêt, Jean-Pierre Raidot, Gérard Kandel, Marie-Louise Wiesel, and Laurence Corash

BACKGROUND: The Etablissement Français du Sang Alsace (EFS Alsace) successively implemented universal use of platelet additive solutions (PASs) and pathogen inactivation (PI) for platelet components (PCs). To assess the impact of these changes, EFS Alsace evaluated PC use, red blood cell (RBC) component use, and transfusion-related adverse events after implementation of these new technologies.

STUDY DESIGN AND METHODS: EFS Alsace prospectively collects data on production, distribution, and response to transfusion of all blood components with greater than 99.5% data acquisition. Adverse events attributed to platelet (PLT) transfusions were collected through a mandatory, active hemovigilance program. A retrospective review of prospectively collected data was conducted covering three periods: 1) apheresis and whole blood–derived PCs in plasma, 2) apheresis and whole blood–derived PCs with PAS, and 3) PCs prepared with PI and PAS. Data on component utilization were analyzed for all patients receiving PCs in each period and for the subset of hematology-oncology patients to evaluate PC use in an intensely transfused population. Values for all continuous variables were summarized as mean and standard deviation, median, and range.

RESULTS: Approximately 2000 patients received PCs in each period. PLT and RBC use per patient was not increased after PI (analysis of variance, $F = 1.9$ and 2.9, respectively) and the incidence of acute transfusion reactions was significantly reduced ($p < 0.001$).

CONCLUSIONS: Universal use of PI was implemented without impacting component use, as indicated by total dose of PLTs per patient, and outcomes to transfusion were improved.

The Etablissement Français du Sang Alsace (EFS Alsace) is the sole provider of blood components for approximately 2 million inhabitants of the Alsace region of France and issues approximately 17,000 platelet components (PCs) per year. In 2005, the EFS Alsace implemented routine use of platelet additive solution (PAS) for production of PCs to reduce recipient exposure to allogeneic donor plasma and to increase salvage of plasma for production of therapeutic and fractionated plasma. The following year, 2006, the EFS Alsace implemented routine use of photochemical pathogen inactivation (PCT) to reduce the risk of transfusion-transmitted infection and adverse immune reactions associated with transfusion of PCs.

Each of these interventions has the potential to impact the therapeutic efficacy of PCs as well as impact utilization of other blood components and patient outcomes in response to transfusion therapy.[1,2] Component

ABBREVIATIONS: CSDP = concentrated single-donor platelet (program); EFS Alsace = Etablissement Français du Sang Alsace; PAS(s) = platelet additive solution(s); PC(s) = platelet component(s); PCT = photochemical pathogen inactivation; PI = pathogen inactivation; TA-GVHD = transfusion-associated graft-versus-host disease.

From the Etablissement Français du Sang (EFS)–Alsace, INSERM UMR-S949, and the Université de Strasbourg, Strasbourg, France; and Cerus Corp., Concord, California.

Address reprint requests to: Jean-Pierre Cazenave, Etablissement Français du Sang-Alsace, 10 rue Spielmann, BP 36, 67065 Strasbourg Cedex, France; e-mail: jeanpierre.cazenave@efs-alsace.fr.

Received for publication March 31, 2010; revision received July 9, 2010, and accepted July 12, 2010.

doi: 10.1111/j.1537-2995.2010.02873.x

TRANSFUSION **;**:**-**.

utilization and transfusion-related adverse events were selected as outcome variables because of clinical relevance to patient management. A decrease in therapeutic efficacy due to changes in PC treatment could result in the need for more transfusion support. A change in the incidence of adverse events associated with novel PCs would be highly relevant to both patients and treating physicians. To assess the impact of use of PASs and pathogen inactivation (PI) treatment, EFS Alsace conducted a retrospective review of PC use, red blood cell (RBC) component use, and transfusion-related adverse events during three different periods to evaluate the effect of each change in platelet (PLT) preparation. This review provided useful information regarding the progressive effects of each change for all patients supported with PCs during each observation period, and for specific patient populations, such as pediatric and hematology-oncology patients, which might be differentially affected by changes in the manufacture of PCs.

MATERIALS AND METHODS

Overall study design

The study covered three observation periods, each lasting from 9 to 13 months in duration. The variable length of each period was in part determined by the time required for switching between types of PCs and data for each period were utilized only once production consisted entirely of the new type of PC product. Period 2 was arbitrarily shorter due to the decision to implement PI as soon as possible. During the 13-month period from January 2003 to February 2004 (Period 1) all PCs derived either from whole blood collections or by apheresis collections were prepared in 100% allogeneic donor plasma. During the 9-month period from September 2005 to June 2006 (Period 2) all PCs were prepared in PAS (53%-68%) and residual donor plasma (32%-47%). During the 11-month period from September 2006 to August 2007 (Period 3) all PCs were prepared in PAS (53%-68%) and residual plasma (32%-47%) with PCT for PI. During each of these three periods, data were collected on the production, transfusion, and transfusion-related adverse events for all patients supported by the EFS Alsace. Data were collected in compliance with a national hemovigilance program with protection of patient confidentiality, and individual patient informed consent was not required.[3] Data on PC and RBC component use were analyzed for all patients receiving PCs during each observation period and for the subset of hematology-oncology patients. Hematology-oncology patients were selected for separate analysis with respect to blood component utilization because they are intensively transfused for prophylaxis of bleeding and provide a more stringent population in which to assess component utilization.

Production of PCs

The variables for preparation of PCs during each of the three periods are summarized in Table 1. Whole blood was collected into CPDA-1 anticoagulant from volunteer blood donors who met the EFS requirements for blood donation. Whole blood–derived buffy coat PCs were prepared as previously described.[4] Single-donor apheresis PCs were collected from qualified donors using an apheresis system (MCS+, Haemonetics, Braintree, MA). During the three periods, all PCs were leukoreduced by filtration ($<1 \times 10^6$ white blood cells [WBCs]/PC) to produce a therapeutic PC that was stored for up to 5 days with reciprocal agitation under temperature control (22-24°C) before transfusion.

Period 1

Whole blood donation had a mean volume of 462 ± 14 mL. Six whole blood–derived buffy coat concentrates were pooled and suspended in 100% donor plasma. Single-donor apheresis PCs were suspended in 100% donor plasma. During this period, two target doses for apheresis components were used, 3.5×10^{11} or 5.5×10^{11} PLTs per collection. Both whole blood–derived pooled buffy coat and apheresis PCs were irradiated with 2500 cGy, using a cesium source, according to established indications for prevention of transfusion-associated graft-versus-host disease (TA-GVHD).

Period 2

Whole blood donation had a mean volume of 460 ± 11 mL. Six buffy coat concentrates were pooled and suspended in approximately 35% donor plasma and 65% PAS (T-Sol, Fenwal, Inc., La Châtre, France). Single-donor apheresis PCs were collected from qualified donors using the MCS+ platform with the concentrated single-donor PLT (CSDP) program (Haemonetics) and suspended in approximately 45% donor plasma with 55%

TABLE 1. Variables for preparation of PCs

Variable	Period 1 (Plasma)	Period 2 (PAS)	Period 3 (PI)
Buffy coat PCs			
Whole blood (mL)	462 ± 14	460 ± 11	461 ± 11
Anticoagulant	CPDA-1	CPDA-1	CPDA-1
Pool size	6	6	6
Plasma target (%)	100	35	35
PAS (%)	0	65	65
Apheresis PCs			
Platform	MCS+	MCS+/CSDP	MCS+/CSDP
Target dose ($\times 10^{11}$)	3.5 or 5.5	4.5	4.5
Plasma target (%)	100	45	45
PAS (%)	0	55	55

T-Sol. During this second period, the collection target for apheresis products was set at 4.5×10^{11} PLTs per collection. Both whole blood–derived pooled buffy coat and apheresis PCs were irradiated with 2500 cGy according to established indications for prevention of TA-GVHD.

Period 3

Whole blood donation had a mean volume of 461 ± 11 mL. Six buffy coat concentrates were pooled and suspended in approximately 35% donor plasma and 65% PAS (Intersol, Fenwal, Inc.). Single-donor apheresis PCs were collected from qualified donors using the MCS+ platform and CSDP software (Haemonetics) and suspended in approximately 45% donor plasma with 55% Intersol. During the third period, the collection target for apheresis products was set at 4.5×10^{11} PLTs per collection. Both pooled whole blood–derived and apheresis PCs were processed (Fig. 1) within 24 hours of collection using amotosalen-HCl and UVA light photochemical PI treatment (INTERCEPT Blood System for Platelets, Cerus Europe BV, Amersfoort, the Netherlands) according to the manufacturer's directions for use.[5] In both cases, residual amotosalen and photoproducts were removed after 4 to 8 hours of incubation using a compound adsorption device. Apheresis PCs were released on Day 1 and pooled whole blood–derived PCs on Day 2 (Fig. 1). PI treatment was used in place of gamma irradiation for prevention of TA-GVHD. Residual amotosalen levels were measured in 1% of treated PCs for quality control.

Transfusion of PCs and hemovigilance monitoring

In all three study periods the PLT count and total PLT content were determined for each apheresis PC. In the first two periods, the PLT count and PLT content of pooled leukoreduced whole blood–derived buffy coat components were determined in a subset (3% for Period 1 and 17% for Period 2) of components for process control. In Period 3 PLT count and total PLT content was determined for each pooled leukoreduced whole blood–derived buffy coat component. PLT counts were measured by electrical impedance with calibration using an optical PLT counter (Sysmex XE 2100D, Roche Diagnostics, Meylan, France). Total PLT content of transfused components was determined based on PLT concentration and component volume expressed as total PLTs $\times 10^{11}$.

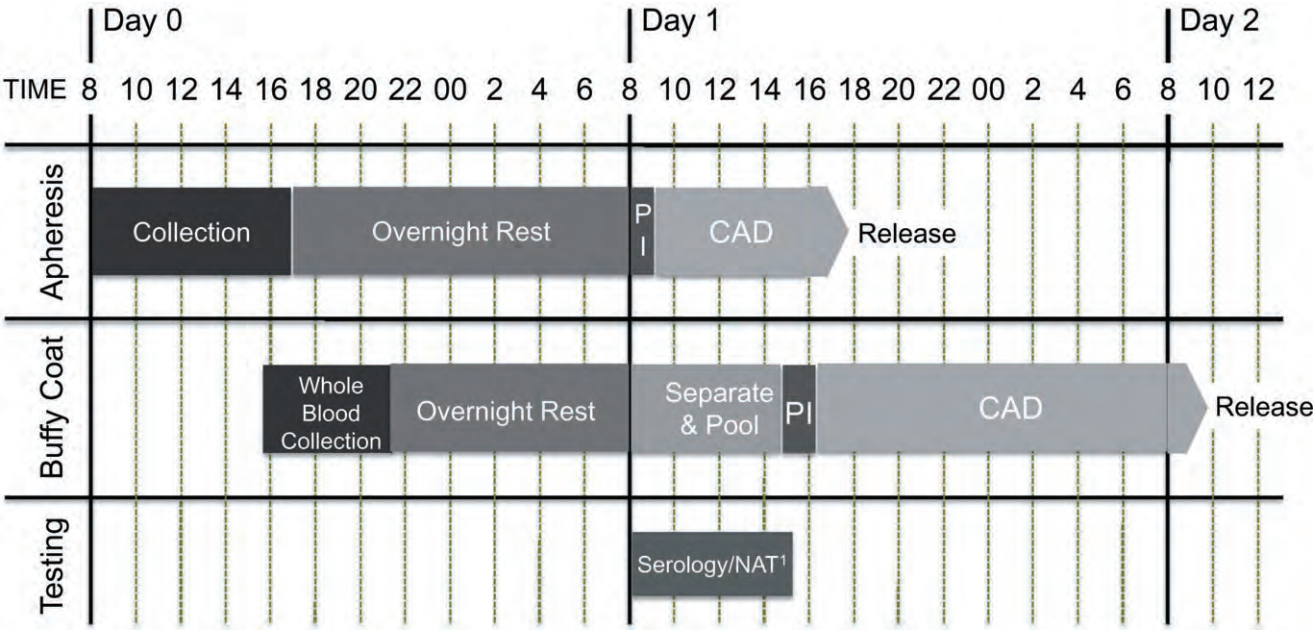

Fig. 1. PC processing workflow. The time in hours starting with the day of collection (Day 0) is indicated. Apheresis components are stored overnight on temperature-controlled shakers (22-24°C) followed by PI treatment and incubation in a compound absorption device (CAD) container from a minimum of 6 hours to a maximum of 16 hours before release for transfusion. Whole blood collections are stored overnight after collection. On Day 1 buffy coat concentrates are isolated, pooled, and treated with PI followed by CAD incubation and release on the beginning of Day 2. [1]Serology/NAT 8 a.m.–3 p.m. daily. PI and CAD = pathogen inactivation using the INTERCEPT Blood System; NAT = nucleic acid testing.

The EFS Alsace blood center maintains a database to monitor the production, issuance, transfusion, and utilization of all blood components. The blood center database contains information on patient demographics and clinical service location of transfusion. In each period, primary care physicians ordered PCs for transfusion according to French national guidelines published by the French Agency of Medical Safety of Health Products in 2003 and based on a recommended dose of 0.5×10^{11} to 0.7×10^{11} PLTs per 7 kg in adults and 0.5×10^{11} PLTs per 5 or 7 kg in pediatric patients for support of thrombocytopenia. The response to transfusion of blood components was monitored through the national active hemovigilance program under the direction of French Agency of Medical Safety of Health Products.[3] Under this program, all blood transfusions are monitored and potential transfusion adverse incidents are evaluated for severity and relationship to the transfused component in a transfusion incident report. Declaration is mandatory for each transfusion, whether or not a transfusion incident report has occurred. The severity of incidents is classified based on WHO criteria: Grade 1—absence of immediate or long-term vital threat, Grade 2—potential long-term morbidity, Grade 3—immediate vital threat, and Grade 4—death of the patient. The relation of the transfusion to the adverse effect is evaluated using a five-point scale basis: 0—excludes any causal relationship, 1—is doubtful, 2—is possible, 3—is likely, and 4—is unquestionable. The transfusion incident reports are submitted to regional hemovigilance network coordinators and entered into an electronic database.

Statistical analysis

Descriptive analyses were conducted for the demographic and clinical variables. Values for all continuous variables were summarized as mean and standard deviation (SD), median, and range. A one-way analysis of variance (ANOVA; single factor) was used to test the equality of PLT content per product, of the number of PCs transfused per patient, and of total dose of PLTs received by the patient between the three periods studied (Table 2). When the difference of ANOVA during the three periods was significant, a t test was used a priori to compare between Period 1 and 2, Period 1 and 3, and Period 2 and 3. All p values reported were two-sided, and significance was declared at a p value of less than 0.05 ($p < 0.05$). All statistical calculations were done with computer software (Excel, 2007 SP2 MSO, Microsoft Corp., Redmond, WA).

RESULTS

Demographics of patients transfused with PCs

In each of the three periods, 1678 to 2069 consecutive patients received one or more PCs (Table 3). Within each period, the largest proportion of PLT transfusions were administered on hematology-oncology care services (Table 3). Patients ranged in age from less than 1 to 106 years of age (Table 3).

TABLE 2. Production and utilization of PCs

Variable	Period 1*	Period 2†	Period 3‡
Number of components	10,629	9,151	13,241
PLT content of transfused components§			
Mean dose/unit ($\times 10^{11}$)	5.2	4.4	4.2
Median ($\times 10^{11}$)	5.4	4.7	4.4
Range ($\times 10^{11}$)	0.6-9.2	0.8-7.8	0.5-7.3
PC use/patient (n)‖			
Patients	2,050	1,678	2,069
Mean	5.2[b]	5.5[c]	6.4[b,c]
Median	2.0	2.0	2.0
Range	1-104	1-114	1-289
Total PLT dose/patient ($\times 10^{11}$)¶			
Mean	27.1	24.1	26.9
Median	10.9	9.4	9.1
Range	0.2-543	0.2-503	0.5-1,302

* PCs were prepared in plasma. During Period 1, PLT content of whole blood–derived PC was determined in 3% of components.
† PCs were prepared in plasma with PAS. During Period 2, PLT content of whole blood–derived PC was determined in 17% of components.
‡ PCs were prepared in plasma with PAS and PI treatment.
§ ANOVA, difference if $F \geq 3.0$. The difference is significant for the three periods ($F = 4396.9$). t test for two samples assuming equal variances: [a]P1 – P2, [b]P1 – P3, [c]P2 – P3. $p < 0.05$.
‖ ANOVA, difference if $F \geq 3.0$. The difference is significant for the three periods ($F = 6.8$). t test for two samples assuming equal variances: [a]P1 – P2, $p = 0.37$; [b]P1 – P3, $p = 0.0008$; [c]P2 – P3, $p = 0.01$.
¶ ANOVA. The difference is not significant for any comparisons: $F = 1.9$.

TABLE 3. Demographics of patients transfused with PCs

Demographic	Period 1*	Period 2†	Period 3‡
Number of patients	2050	1678	2069
Median age (years)	64	63	63
Age range (years)	3-97	<1-99	<1-106
Male (%)	59	60	62
Proportions (%) of patients transfused by clinical service			
Hematology-oncology	56.1	50.5	58.1
General medical	36.6	43.6	36.3
Cardiovascular surgery	7.3	5.9	5.7

* PCs were prepared in plasma.
† PCs were prepared in plasma and PAS.
‡ PCs were prepared in plasma and PAS with PI treatment.

Characteristics of PCs

During the three periods the PLT content per component changed as the methods of production changed (Table 2). With the introduction of PASs, production methods were adjusted to equalize the PLT content of apheresis and whole blood–derived PCs that were ordered interchangeably by primary care physicians. All PCs transfused demonstrated retention of swirling when issued by the blood center. PLT content per PC unit was significantly less in Period 2 compared to Period 1 ($p < 0.05$) and in Period 3 compared to Period 2 ($p < 0.05$) (Table 2). During Period 3, all the 13,241 components were tested for PLT content; the mean ± SD PLT content was $4.2 \times 10^{11} \pm 0.4 \times 10^{11}$ and the mean ± SD loss due to PI was 24 ± 4 mL containing $0.3 \times 10^{11} \pm 0.07 \times 10^{11}$ PLTs. For PCs treated with PI, residual amotosalen levels were measured in 1% of products (n = 201). The mean residual amotosalen concentration for whole blood and apheresis components was 0.24 ± 0.09 µmol/L (median, 0.22 µmol/L; maximum, 1.25 µmol/L).

Utilization of PCs

The utilization of PCs was determined per patient based on the number of components transfused and the total number of PLTs transfused per patient. The latter was of specific importance as the PLT content per component changed according to changes in the methods of production during the three periods (Table 2). For each period, the relative proportion of whole blood–derived and apheresis PCs remained constant, approximately 65%:35%, respectively. The mean number of PCs transfused per patient increased in Periods 2 and 3 compared to Period 1 (Table 2); however, the number of transfusion episodes and the total dose of PLTs transfused per patient were not different between the two periods (Table 2).

Utilization of RBC components

Utilization of RBCs was determined for patients who received one or more PCs during each observation period (Table 4). In each observation period, approximately 85% of patients transfused with PCs received at least one RBC unit. No significant difference in utilization of RBCs was detected between the three observation periods.

Acute transfusion reactions

Adverse events after transfusion of PCs were evaluated for relationship to transfusion and all events classified as doubtful, possible, likely, or unquestionable were defined as acute transfusion reactions. These events also included the observation of newly detected alloantibodies to RBC antigens with or without evidence of hemolytic transfusion reactions (Table 5). Cumulatively, for all three periods, 145 adverse events were reported, of which 46 were newly detected RBC alloantibodies without hemolysis secondary to concomitant transfusion of RBCs in 85% of the patients receiving PCs (Table 5). In Period 1, one death due to volume overload was reported. All other reactions were Grades 1 (61%) and 2 (33%) in severity. During Period 3 among the 18 reported reactions not due to RBC alloantibodies, eight were characterized by febrile reactions, three were characterized as allergic reactions, and one episode of transfusion-related acute lung injury (TRALI) associated with high-titer HLA antibodies from a multiparous donor was reported during Period 3 in association with transfusion of PI-treated apheresis PCs without exposure to any other blood components. Six transfusion reactions were not characterized further. No

TABLE 4. Utilization of RBC components

Variable	Period 1*	Period 2†	Period 3‡
Number of patients transfused with PCs	2,050	1,678	2,069
Number (%) of patients transfused with PCs and RBCs	1,715 (83.7)	1,355 (80.8)	1,749 (84.5)
Number of RBC units§	24,693	17,732	23,824
Mean number RBC units/patient	14.4	13.1	13.6

* PCs were prepared in plasma.
† PCs were prepared in plasma and PAS.
‡ PCs were prepared in plasma and PAS with PI treatment.
§ ANOVA. The difference between the three periods is not significant: F = 2.9.

TABLE 5. Adverse reactions after transfusion of PCs

Variable	Period 1*	Period 2†	Period 3‡
Number of patients transfused	2,050	1,678	2,069
Number of components transfused	10,629	9,151	13,241
Number of patients with adverse reactions	59	33	36
Number of adverse events§	67	41	37
Number of RBC alloantibodies detected	11	16	19
Number of RBC alloantibodies/1000 PCs	1.03	1.75	1.43
Number of transfusion reactions	56	25	18
Number of total reactions/1000 PCs	6.3	4.5	2.8
Number of PC-related reactions/1000 PCs‖	5.3[a,c]	2.7[a,b]	1.4[b,c]
Patients with PC reactions (%)¶	2.9[a,c]	2.0[a]	1.7[c]

* PCs were prepared in plasma.
† PCs were prepared in plasma and PAS.
‡ PCs were prepared in plasma and PAS with PI treatment.
§ Total transfusion reactions including alloantibodies detected to RBC antigens.
‖ Transfusion reactions excluding RBC alloantibodies not associated with symptomatic reactions. Chi-square test with $\alpha = 0.05$, difference if $p < 0.05$: [a]P1 – P2, p = 0.0053; [b]P2 – P3, p = 0.0214; [c]P1 – P3, $p = 7 \times 10^{-8}$.
¶ Symptomatic reactions to PCs, excluding RBC alloantibodies. Chi-square test with $\alpha = 0.05$, difference if $p < 0.05$: [a]P1 – P2, p = 0.0094; [b]P2 – P3, p = 0.0779; [c]P1 – P3, $p = 6.7 \times 10^{-8}$.

cases of transfusion-related sepsis were reported in any period. The incidence of transfusion-related adverse events per 1000 PCs transfused was significantly reduced with the implementation of PAS ($p = 0.005$) and further reduced with the implementation of PI ($p = 0.021$). Analysis of the incidence of transfusion-related adverse events per patient demonstrated significant reduction after implementation of PAS ($p = 0.009$) and further reduction with the implementation of PI ($p = 0.0779$).

Utilization of PCs by intensively transfused patients

To further characterize the impact of changes in PC production, the utilization of PCs and RBCs by intensively transfused patients with hematology-oncology disorders was examined. Evaluation was restricted to Periods 1 and 3 since use of PAS without PI was only an intermediate phase in the evolution of the PLT production process at EFS Alsace. During both Period 1 and Period 3 all PCs were leukoreduced ($<1 \times 10^6$ WBCs/PC), and the proportion of whole blood–derived buffy coat and apheresis PCs remained constant (62:38), respectively. Mean PLT content per PC was lower during Period 3 (4.2×10^{11}) compared to Period 1 (5.2×10^{11}) due to a decision to harmonize the PLT content of both types of products.

Approximately similar numbers of hematology-oncology patients were transfused during Periods 1 and 3 (Table 6). Although the number of PCs transfused per patient was increased during Period 3 compared to Period 1, the total dose of PLTs per patient was not increased (Table 7). The increase in the number of PCs was due to decreased PLT content per unit in Period 3 compared to Period 1.

DISCUSSION

In 2005, the EFS Alsace regional blood center initiated changes in the routine preparation of PCs with the introduction of PAS, which was followed by implementation of PI treatment in 2006. Each of these changes had the potential to impact the PLT content of components, the utilization of components, and patient outcomes. To assess these potential impacts, EFS Alsace conducted a retrospective analysis of three different periods, each of approximately 1 year duration. The comparison of each of these innovations with conventional PCs in 100% plasma provided a means to assess the impact in a controlled manner since the same blood center and processing staff prepared PCs in each of the observation periods. Although primary care physicians were aware of the changes in methods of PLT production, they continued to prescribe PCs based on similar standard of care guidelines in each of the observation periods. This provided approximately similar clinical conditions to monitor patient responses in each of the periods.

This analysis was based on a comprehensive longitudinal database that tracked greater than 99% of PCs with respect to patient demographics and utilization by patients. In addition, the response to transfusion with respect to adverse outcomes was evaluated using an active hemovigilance program[3] for which data for greater than 99% of transfusions were reported in the EFS Alsace region. This study offered a unique opportunity to examine the impact of these changes in PLT production when implemented in routine use for support of a broad spectrum of patients. In addition, this study included a substantial population of intensively transfused hematology-oncology patients who may have been more sensitive to changes in the methods of PC production.

TABLE 6. Age demographics of hematology-oncology patients transfused with PCs

Demographics	Period 1*	Period 3†
Number of patients	671	699
Number (%) of infants-toddlers‡	10 (1.5)	5 (0.7)
Number (%) of children§	49 (7.3)	49 (7.0)
Number (%) of adults‖	612 (91.2)	645 (92.3)

* PCs were prepared in plasma.
† PCs were prepared in plasma and PAS with PI treatment.
‡ Infants and toddlers were 1 month to less than 3 years of age.
§ Children were 3 to 17 years of age.
‖ Adults were greater than 17 years of age.

TABLE 7. Utilization of PLT and RBC components by hematology-oncology patients

Variable	Period 1*	Period 3†	p value
Number of patients	671	699	
Number of PCs transfused			
Mean ± SD‡	8.7 ± 12	11 ± 20	0.01
Median‖	4.0	4.0	
Range‖	1-103	1-264	
Total PLT dose			
Mean ± SD‡	45.3 ± 62.8	46.1 ± 86.1	0.85
Median	21.6	18.4	
Range‖	0.8-536	1.5-1189	
Number of RBC units transfused			
Mean§	15.2 ± 16.5	13.6 ± 18.4	0.10
Median‖	9.0	8.0	
Range‖	0-93	0-272	

* PCs were prepared in plasma.
† PCs were prepared in plasma with PAS and PI treatment.
‡ Mean number of PCs transfused per patient.
‡ Mean total dose of PLTs (10^{11}) per patient.
§ Mean number of RBC units per patient.
‖ Data expressed per patient.

Patient demographics, in terms of age, sex, and primary care service for PC transfusion, were comparable between the three periods. Each of the observation periods included between 1678 and 2069 patients with transfusion of between 9151 and 13,241 PCs and 17,732 to 24,693 RBC units. Thus, this observational study encompasses a large experience, indeed much larger than that of most clinical trials in transfusion medicine that have been used to support implementation of major changes in transfusion practice, such as leukoreduction and adjustment of the transfusion threshold.[6,7] When adjustments were made for differences in PLT content of individual components, there were no significant differences in the total PLT dose required per patient, either for all patients or for intensively transfused hematology-oncology patients. Changes in the methods of PC production did not impact utilization of RBCs, indicative of preservation of effective hemostasis during periods of PLT transfusion support. This observation is of particular interest with respect to cardiovascular surgery patients and general medical patients who have not been studied in the randomized clinical trials of PCs treated with PI.[5,8,9] While the utilization of PCs and RBCs did not change in response to modifications in PLT processing, the incidence of adverse events imputed to transfusions of PCs decreased on both a per-transfusion and a per-patient basis. This observation is consistent with the significant reduction in acute transfusion reactions noted in a prior large randomized clinical trial.[9] Only a single case of TRALI was reported during each of these observation periods; this case was attributable to high-titer HLA antibodies in a multiparous apheresis PLT donor. No cases of transfusion-related sepsis were reported in any period.

The conclusions regarding efficacy and safety that may be drawn from this study are potentially limited due to the lack of a blinded, randomized trial design. However, the study has the advantage of comparative observation periods in a single region in which the staff preparing the PCs and the primary care physicians prescribing PCs and monitoring patient outcomes remained relatively stable. Moreover, the study was conducted in observation periods when each of the PC methods reflected routine production practices. Thus, the study involved assessment of the changes in PC production under realistic conditions. In addition, monitoring of patient clinical outcomes utilized objective measures, such as utilization of PCs and RBCs, and an active hemovigilance program with oversight by clinical monitors who were not associated with the blood center producing the blood components. On the basis of these factors, we believe that the data reported from this study indicate that PASs and PI treatment can be implemented into routine practice without impacting either PC or RBC utilization and with a reduction in acute transfusion reactions. The experience with respect to the safety profile and tolerability of PCs treated with PI in this study is consistent with that reported for several other large postmarketing hemovigilance studies.[10-12] It is also similar to the Belgian experience of universal routine use of Intercept-inactivated PCs for 3 years, that enables learning of how well the products function in broad populations.[13] In addition, the PI process was used in place of gamma irradiation for prevention of TA-GVHD and in place of cytomegalovirus serology resulting in the use of a single PLT inventory with elimination of these additional tests and procedures.

ACKNOWLEDGMENTS

We thank the clinicians of the Alsace region and in particular Prof. Raoul Herbrecht and Dr Bruno Lioure (Onco-Hematology Department, CHU de Hautepierre, Strasbourg, France) for their constant help and support.

CONFLICT OF INTEREST

JPC is a member of the European Scientific Advisory Board of Cerus Corporation. LC is an employee of Cerus Corporation. HI, CW, IM, DK, ML, JPR, GK, and MLW have no conflicts of interest.

REFERENCES

1. Alvarez-Larrán A, Del Río J, Ramírez C, Albo C, Peña F, Campos A, Cid J, Muncunill J, Sastre JL, Sanz C, Pereira A. Methylene blue-photoinactivated plasma vs. fresh-frozen plasma as replacement fluid for plasma exchange in thrombotic thrombocytopenic purpura. Vox Sang 2004;86: 246-51.
2. de la Rubia J, Arriaga F, Linares D, Larrea L, Carpio N, Marty ML, Sanz MA. Role of methylene blue-treated or fresh-frozen plasma in the response to plasma exchange in patients with thrombotic thrombocytopenic purpura. Br J Haematol 2001;114:721-3.
3. Andreu G, Morel P, Forestier F, Rebibo D, Janvier G, Herve P. Hemovigilance network in France: organization and analysis of immediate transfusion incident reports from 1994-1998. Transfusion 2002;42:1356-64.
4. Cazenave JP, Aleil B, Wiesel ML, Laforet M, Isola H. In vitro evaluation of pooled buffy coat platelets treated with photochemical pathogen inactivation using amotosalen. Vox Sang 2004;86:201-2.
5. Janetzko K, Cazenave JP, Kluter H, Kientz D, Michel M, Beris P, Lioure B, Hastka J, Marblie S, Mayaudon V, Lin L, Lin LS, Conlan MG, Flament J. Therapeutic efficacy and safety of photochemically treated apheresis platelets processed with an optimized integrated set. Transfusion 2005; 45:1443-52.
6. TRAP Study Group. Leukocyte reduction and ultraviolet B irradiation of platelets to prevent alloimmunization and refractoriness to platelet transfusions. N Engl J Med 1997; 337:1861-9.

7. Rebulla P, Finazzi G, Marangoni F, Avvisati G, Gugliotta L, Tognoni G, Barbui T, Mandelli F, Sirchia G. The threshold for prophylactic platelet transfusions in adults with acute myelogenous leukemia. N Engl J Med 1997;337:1870-5.
8. van Rhenen D, Gulliksson H, Cazenave JP, Pamphilon D, Ljungman P, Kluter H, Vermeij H, Kappers-Klunne M, de Greef G, Laforet M, Lioure B, Davis K, Marblie S, Mayaudon V, Flament J, Conlan M, Lin L, Metzel P, Buchholz D, Corash L. Transfusion of pooled buffy coat platelet components prepared with photochemical pathogen inactivation treatment: the euroSPRITE trial. Blood 2003;101:2426-33.
9. 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 JS, Metzel P, Corash L, Koutsoukos A, Lin L, Buchholz DH, Conlan MG. Therapeutic efficacy and safety of platelets treated with a photochemical process for pathogen inactivation: the SPRINT trial. Blood 2004;104:1534-41.
10. Osselaer JC, Cazenave JP, Lambermont M, Garraud O, Hidajat M, Barbolla L, Tardivel R, Defoin L, Waller C, Mendel I, Raidot JP, Kandel G, De Meuter R, Fabrigli P, Dehenau D, Arroyo JL, Padron F, Gouezec H, Corral M, Jacquet M, Sundin D, Lin L, Corash L. An active haemovigilance programme characterizing the safety profile of 7437 platelet transfusions prepared with amotosalen photochemical treatment. Vox Sang 2008;94:315-23.
11. Osselaer JC, Messe N, Hervig T, Bueno J, Castro E, Espinosa A, Accorsi P, Junge K, Jacquet M, Flament J, Corash L. A prospective observational cohort safety study of 5,106 platelet transfusions using components prepared with photochemical pathogen inactivation treatment. Transfusion 2008;48:1061-71.
12. Rasongles P, Angelini-Tibert MF, Simon P, Currie C, Isola H, Kientz D, Slaedts M, Jacquet M, Sundin D, Lin L, Corash L, Cazenave JP. Transfusion of platelet components prepared with photochemical pathogen inactivation treatment during a Chikungunya virus epidemic in Ile de La Réunion. Transfusion 2009;49:1083-91.
13. Osselaer JC, Doyen C, Defoin L, Debry C, Goffaux M, Messe N, Van Hooydonk M, Bosly A, Lin JS, Lin L, Corash L. Universal adoption of pathogen inactivation of platelet components: impact on platelet and red blood cell component use. Transfusion 2009;49:1412-22.

Therapeutic efficacy of platelet transfusion in patients with acute leukemia: an evaluation of methods

Torunn O. Apelseth, Øystein Bruserud, Tore Wentzel-Larsen, and Tor Hervig

BACKGROUND: Clinical effect of platelet (PLT) transfusion is monitored by measures of PLT viability (PLT recovery and survival) and functionality. In this study we evaluate and compare transfusion effect measures in patients with chemotherapy-induced thrombocytopenia due to treatment of acute leukemia.

STUDY DESIGN AND METHODS: Forty transfusions (28 conventional gamma-irradiated and 12 pathogen-inactivated photochemical-treated PLT concentrates [PCs]) were investigated. PC quality was analyzed immediately before transfusion. Samples were collected from thrombocytopenic patients at 1 and 24 hours for PLT increments and thromboelastography (TEG) with assessments of bleeding score and intertransfusion interval (ITI). Data were analyzed by Spearman's correlation. Patient and PC variables influencing the effect of transfusion were analyzed by use of a mixed-effects model.

RESULTS: PLT dose, storage time, and pathogen inactivation correlated with PLT recovery but not with PLT survival (including ITI), TEG, or clinical bleeding. Fever was negatively correlated with PLT survival but did not affect PLT recovery. After 1 and 24 hours, strong correlations were observed within measures of PLT viability and between PLT increment and the TEG value maximal amplitude (MA). Negative correlation was observed between late MA increment and clinical bleeding status after transfusion ($r = -0.494$, $p = 0.008$). PLT count increments did not correlate to clinical bleeding status.

CONCLUSIONS: PLT dose and quality of PCs are important for optimal immediate transfusion response, whereas duration of transfusion effect is influenced mainly by patient variables. The TEG value MA correlates with PLT count increments and bleeding, thus reflecting both PLT viability and functionality.

Allogeneic platelet (PLT) transfusions are used to prevent (prophylactic transfusions) and control (therapeutic transfusions) bleeding in patients receiving intensive chemotherapy.[1-5] But even though the risks of severe transfusion reactions and transmission of pathogens are minimized due to technologic progress and pathogen reduction methods, PLT transfusions are not free of complications.[6-8] It is therefore important to continue the search for an optimal transfusion policy in patients receiving PLT transfusions. Large randomized clinical trials are now performed aiming to define the optimal use of PLT transfusions, both by evaluation of transfusion strategies and by investigations of the influence of variables like PLT dose and transfusion triggers on the results of treatment.[3,5]

The tests used to assess the clinical efficacy of PLT transfusion reflect different approaches to minimize bleeding in patients. They influence routine transfusion practice as well as the results of clinical PLT transfusion studies. The clinical effect of PLT transfusion is monitored by measures of PLT viability and functionality. In studies of thrombocytopenic patients, PLT viability is usually

ABBREVIATIONS: API = absolute platelet increment; ITI = intertransfusion interval; MA = maximal amplitude; PC(s) = platelet concentrate(s); PCT = photochemical treated; TEG = thromboelastography.

From the Blood Bank, the Section for Hematology, Department of Medicine, and the Centre for Clinical Research, Haukeland University Hospital, Bergen; and the Gades Institute and the Institute of Medicine, University of Bergen, Bergen, Norway.

Address reprint request to: Torunn Oveland Apelseth, MD, Blood Bank, Haukeland University Hospital, N-5021 Bergen, Norway; e-mail: torunn.oveland.apelseth@helse-bergen.no.

This study was funded by a grant from "Helse Vest," the governmental administration of the health services in the western part of Norway.

Received for publication June 22, 2009; revision received October 12, 2009, and accepted October 18, 2009.

doi: 10.1111/j.1537-2995.2009.02540.x

TRANSFUSION 2010;50:766-775.

investigated by immediate PLT count increments, reflecting the PLT recovery together with late PLT count increment and intertransfusion interval (ITI) which reflects the PLT survival.[4,5,9-12] PLT functionality may be monitored by bleeding assessments[12,13] and point-of-care tests of hemostasis and PLT function like the thromboelastography (TEG) analysis.[14] While PLT count increments are fundamental in the prophylactic PLT transfusion strategy, the therapeutic transfusion strategy is based on bleeding assessments.

In our study we aim to evaluate and compare methods for measurement of clinical efficacy in acute leukemia patients receiving regular PLT transfusions due to severe chemotherapy-induced thrombocytopenia.

MATERIALS AND METHODS

Study design

This prospective observational study was approved by the local research ethics committee (Region III, University of Bergen, Bergen, Norway), and patients were recruited from the Section for Hematology, Department of Medicine, at Haukeland University Hospital from December 2006 until April 2007. During the study period, all patients diagnosed with acute leukemia expected to be in need of PLT transfusions due to severe chemotherapy-induced cytopenia were invited to participate in the study. With one exception, all patients accepted. After informed written consent was obtained, 10 patients with acute leukemia were included in the study, giving a total of 188 patient-days with chemotherapy-induced cytopenia. Of a total of 109 PLT transfusions, 40 PLT transfusions were selected to be characterized in detail based on the following criteria: daytime transfusions and data collection performed according to procedures. The study unit was defined as the PLT transfusion.

Preparation of PLT concentrates for transfusion

All PLT concentrates (PCs) fulfilled the European requirements.[15] Single-donor PCs (n = 17) were collected by single-needle apheresis procedure employing the elutriation principle to provide leukoreduced PLTs (Fenwal Amicus cell separator, Baxter Healthcare Corp., Deerfield, IL). Prestorage leukofiltered buffy coat PCs (n = 23) were produced by use of automated procedures (OrbiSac, CaridianBCT, Inc., Lakewood, CO). Twelve of the 40 single-donor and buffy-coat PCs were pathogen inactivated by photochemical treatment using amotosalen and UVA light (Intercept Blood System for Platelets, Cerus Corp., Concord, CA). Photochemical-treated (PCT) PCs were suspended in PASIII (Intersol, Fenwal, Inc., Lake Zurich, IL) and conventional PCs in PAS II (T-sol, Fenwal, Inc.) with 35% to 37% autologous plasma. Conventional PCs (n = 28) were gamma-irradiated immediately before transfusion (25 Gy; Gammacell 3000 Elan, Nordion International, Inc., Ottawa, Ontario, Canada). PCs were stored up to 168 hours at 22 ± 2°C under constant agitation in a flatbed PLT incubator (Helmer, Noblesville, IN). Bacterial testing was performed on all conventional PCs (BacT/ALERT, bioMerieux, Inc., Marcy l'Etoile, France). The preparation method of PCs transfused was not influenced by the investigators.

Patients

All patients were treated according to the same institutional guidelines with conventional intravenous cytarabine-based chemotherapy, and all developed severe chemotherapy-induced leukopenia with neutrophil counts below 0.5×10^9/L. PLT transfusions were requested by the patient's physician in accordance with international guidelines.[3,4,9] Prophylactic PLT transfusions were given to nonfebrile and clinically stable patients with peripheral blood PLT counts below 10×10^9/L and to febrile patients when PLT counts were below 20×10^9/L. In patients with increased risk of bleeding (e.g., before invasive procedures or recent hemorrhage) or ongoing bleeding, PLT transfusions were administered when peripheral blood PLT counts were below 20×10^9 to 50×10^9/L. Patients were examined for human leukocyte antibodies (FlowPRA Class I screening test, One Lambda, Inc., Canoga Park, CA) and human PLT antibodies.[16] Reticulated PLTs were examined regularly and used for prediction of hematopoietic reconstitution.[17]

Investigation procedure

A sample was drawn by sterile procedures from the PCs immediately before transfusion. The following analyses were performed for characterization of PC quality: 1) PLT dose, that is, number of CD61+ PLTs in PC and CD61+ PLT microparticles[18] (Cell-Dyn CD4000, Abbott Laboratories, Round Lake, IL); 2) metabolic variables, that is, pH at 22°C, pCO_2, HCO_3, pO_2, and concentrations of glucose and lactate (ABL 725, Radiometer Copenhagen, Denmark; Modular, Hitachi High-Technologies Corp., Tokyo, Japan); 3) PLT density (mean PLT component) concentration (Advia 120, Bayer HealthCare, Tarrytown, NY); and 4) lactate dehydrogenase (LDH; Modular, Hitachi High-Technologies Corp.).[18,19] Preparation method and storage time were registered for each PC.

Blood samples from the study patients were collected through a central venous catheter before (<8 hr) transfusion, 45 to 120 minutes after transfusion, and 18 to 24 hours after transfusion for examination of PLT counts (Cell-Dyn CD4000, Abbott Laboratories) and TEG (TEG hemostasis system 5000 series, Software Version 4.2, Haemoscope Corp., Niles, IL). For every study transfusion, the following

patient characteristics were evaluated: pretransfusion PLT count, bleeding, patient weight, fever at time of transfusion, infection (i.e., patient diagnosed with neutropenic fever, treatment with antibiotics, and/or serum level of C-reactive protein ≥100 mg/L the day of transfusion), and treatment with steroids or granulocyte-colony stimulating factor (G-CSF). In addition white blood cell counts, medication, ABO and HLA compatibility of transfusion, occurrence of transfusion complications, transfusion sequence number, and ITI were registered throughout study period. Event charts including transfusion patterns, reticulated PLT counts, PLT counts, and duration of neutropenia were made for all patients and treatment cycles to serve as basis for interpretation of results.

Methods for documentation of transfusion effect

Posttransfusion absolute PLT increment (API) is affected by quality and number of PLTs transfused, as well as the dilution of PLTs in the patient's blood volume. According to European recommendations, we defined that an acceptable immediate API (at 1 hr after transfusion) occurred when PLT transfusion raised the PLT count above the transfusion threshold, that is, greater than 10×10^9/L.[9] To adjust for differences in PLT dose and blood volume of the patient, the corrected count increment (CCI) was calculated by use of the formula[9,20]

$$\mathrm{CCI} = \frac{\mathrm{API} \times \text{body surface area } (\mathrm{m}^2) \times 10^{11}}{\text{Number of PLTs transfused}}.$$

According to international guidelines, a successful transfusion was defined as a CCI of more than 7.5 for immediate clinical effect (PLT recovery) and a CCI of more than 4.5 for late clinical effect (PLT survival).[4,20] Body surface area was estimated by the formula of DuBois and DuBois.[21]

ITI was defined as hours from onset of study transfusion to the onset of the subsequent transfusion. When the interval between transfusions was more than 240 hours, which is longer than the expected life span of transfused PLTs,[22,23] we assumed that autologous PLT recovery had occurred, and transfusions were excluded from analysis.

Bleeding assessments consisting of physical examination and patient interview were performed each morning by trained nurses working at the hematologic ward.[12] For additional clinical information, the medical records of the patients were consulted. The bleeding assessments were reviewed independently by two adjudicators and graded in accordance with the WHO hemostatic assessments.[12] If disagreement occurred, the patient data were evaluated by a third adjudicator and consensus was achieved. WHO Grade of 2 or more was defined as primary bleeding endpoint according to recent PLT studies.[12,24-27] Accordingly therapeutic transfusions were defined as transfusions given to patients with WHO Grade 2 or more bleedings before transfusion. The proportion of days with WHO Grade 2 or more bleeding during neutropenia was calculated.[13] To evaluate WHO bleeding assessments used for daily monitoring of PLT transfusion effect, we calculated the difference in WHO bleeding status before and after each transfusion.

The blood coagulation process in patients before and after transfusion was investigated by TEG in citrated kaolin samples with heparinase cups to avoid contamination of sample by heparin from the central venous catheter. The following variables were investigated: 1) R, reaction time, the time to the beginning of clot formation, reflecting the level of coagulation factors; 2) angle (α), the buildup and cross-linking of fibrin, dependent on sufficient amounts of fibrinogen, thrombin, and PLTs; and 3) MA, the maximum amplitude, a measurement of maximum strength or stiffness of the developed clot, reflecting the amount and functional capacity of PLTs including their interaction with fibrin.[14] Reference levels were provided by the manufacturer.

Statistical analysis

Descriptive statistics were performed by use of computer software (SPSS 15.0 for Windows, SPSS, Chicago, IL) and results are presented as mean ± standard deviation (SD) if not otherwise stated. When analyzing PC characteristics not influenced by individual patient effects, two-sample t tests were performed (SPSS, Version 15.0). All patient data were analyzed, adjusted for individual patient effects. Analyses of dichotomous data were performed with generalized estimating equations (package gee, R Version 2.8.0, http://www.R-project.org, The R Foundation for Statistical Computing, Vienna, Austria). Correlation analysis, not adjusted for individual patient effect, was performed by Spearman's correlation (SPSS, Version 15.0) due to partly categorical data. Confidence intervals (CIs) were calculated as bootstrap BC_a CIs (package boot, R Version 2.8.1, The R Foundation for Statistical Computing).[28] Differences were considered significant when p values were less than 0.05.

To separate the effects of PC and patient variables on transfusion outcome measures, mixed-effects model (package nlme, R Version 2.8.0, The R Foundation for Statistical Computing) was performed.[29,30] If random parts of the mixed-effects model were unstable, generalized least squares were used. In this analysis we investigated the relationship between posttransfusion values of PLT counts, posttransfusion TEG values, and ITI and the following variables: fever, transfusion sequence number, patient weight, bleeding of WHO Grade 2 or more, PLT dose, storage time, preparation technique, and ABO identity. Analyses of PLT counts and TEG values were adjusted for time and pretransfusion values. The selection of variables investigated was based on previously published

articles,[23,31-36] and the number of variables was adapted to the total number of study transfusions. Mixed-effects analysis could not be performed for bleeding status due to the categorical nature of the variable.

RESULTS

Patients and PCs

Ten patients, four males and six females, diagnosed with acute myelogenous leukemia (eight patients) or acute lymphoblastic leukemia (two patients), were included in the study. Transfusion requirements were followed by the investigators from the administration of chemotherapy (five remission induction and eight consolidation treatment cycles) to hematopoietic reconstitution (i.e., $\geq 0.5 \times 10^9$/L neutrophil granulocytes in blood samples and no need for PLT transfusion), patient withdrawal (two patients), or death of patient (one patient, due to infection). All patients (ages 21-62 years) had a history of previous pregnancies and/or previous PLT transfusions, but no patient with previous splenectomy or disseminated intravascular coagulation was included in the study. Patients with HLA antibodies were transfused with HLA Class I–matched PCs (three patients) according to international guidelines.[9] One patient was diagnosed with weak positive HPA antibody at the time of inclusion and transfused according to routine practice without HPA-matched PCs. No patient developed HLA or HPA antibodies during the study. All transfusions were ABO compatible, by means of recipient having no antibodies incompatible with the red blood cell type of donor. One patient was diagnosed with neutropenic septicemia. These transfusions (four) were grouped and analyzed together with the transfusions given to patients with infection. In 11 transfusions, patients received treatment with hematopoietic growth factors. Three of these transfusions were given to acute lymphoblastic leukemia patients treated according to the Hyper-CVAD regimen and eight transfusions to acute myelogenous leukemia patients due to severe bacterial infections. The mean PLT dose given was 2.79 (range, 1.46-4.42) $\times 10^{11}$ per unit, and mean storage time of the PC was 86 (range, 21-167) hours. The metabolic status of the PC was as follows (mean ± SD): pH (22°C) 7.22 ± 0.14, pCO_2 2.98 ± 0.47 kPa, pO_2 17.3 ± 2.1 kPa, glucose concentration 5.18 ± 1.55 mmol/L, and lactate concentration 7.35 ± 3.64 mmol/L. The LDH concentration was 135 (range, 21-522) U/L, and the mean level of PLT microparticles was 26 (range, 6.7-50.6) $\times 10^9$/L.

Effect of PLT transfusion

PLT viability

PLT viability was evaluated by PLT count increments and calculation of ITI. PLT transfusion increased peripheral blood PLT counts after both 1 hour (mean increase, 12.5×10^9/L; 95% CI, 10.0×10^9-14.9×10^9/L) and 18 to 24 hours (mean increase, 7.2×10^9/L; 95% CI, 4.7×10^9-9.7×10^9/L). Significant correlations were observed between pre- and posttransfusion patient PLT counts (after 1 hr, $r = 0.569$, $p < 0.001$; after 24 hr, $r = 0.457$, $p = 0.005$).

Forty-six percent of PLT transfusions raised the patients' immediate PLT counts above the transfusion threshold, that is, more than 10×10^9/L, and were thus defined as acceptable according to the European recommendations.[9] When evaluating PLT viability by use of CCIs, 46% of the study transfusions were classified as successful after 1 hour, whereas 53% of transfusions fulfilled predefined criteria after 24 hours. Eight out of 10 patients received more than one transfusion per treatment cycle, and all of them experienced both successful transfusions and transfusion failures (Fig. 1). The results of comparative analysis of patient and PC characteristics in successful transfusions and transfusion failures are reported in Table 1. When evaluated by immediate response, the successful PLT transfusions were differentiated from transfusion failures by superior PLT quality (Table 1). In contrast, successful PLT transfusions evaluated by late response showed favorable patient characteristics. Mean ITI was 41 (range, 7-121) hours, and there was no significantly higher ITI in PLT transfusions characterized as successful by CCIs after either 1 or 24 hours.

PLT functionality

Eight patients experienced minor bleedings (petechiae and minor nosebleed), giving a total of 34 days (18.0%) with WHO Grade 1 bleedings during the total study period (188 patient-days). Only four patients experienced WHO Grade 2 or 3 bleeds, giving proportions of 7.4 and 2.1% of total patient-days, respectively. The time to first bleeding (WHO Grade ≥ 2) varied between 1 and 15 days and the individual proportion of days with Grade 2 or 3 bleeds varied from 6.3% to 66.7% for the affected patients (Fig. 1). No WHO Grade 4 bleed was observed during the study period. The bleeding event chart did not indicate any association between time to the first bleed and individual number of bleeds; rather, it did illustrate the individual differences in bleeding pattern among patients with the similar diagnosis and PLT counts (Fig. 1). In spite of high frequency of PLT transfusions, bleedings still occurred, and patients with the similar morning PLT counts did not experience the same number, timing, and severity of bleeding. Significant correlations were observed between 1) WHO bleeding status before and after transfusion ($r = 0.639$, $p < 0.001$) and 2) WHO bleeding status after transfusion and ITI ($r = -0.380$, $p = 0.020$). No significant correlation was observed between pretransfusion PLT counts and bleeding assessment before and 24 hours after transfusion, neither was any correlation observed

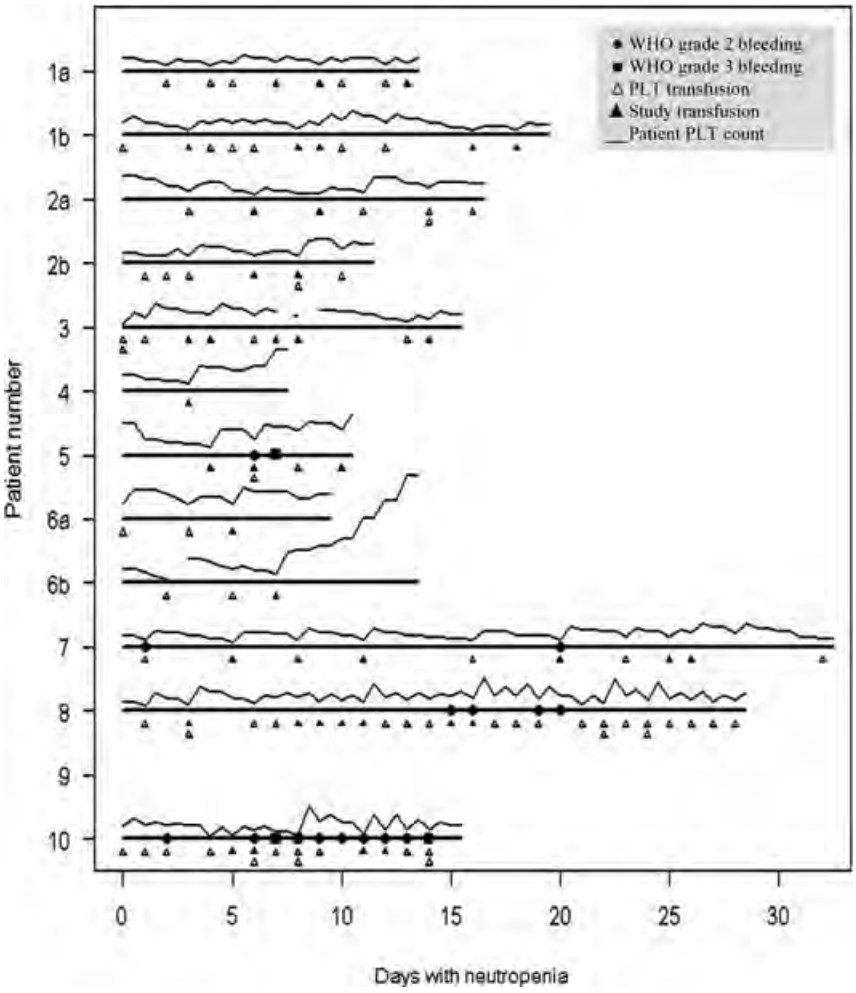

Fig. 1. The bleeding event chart displays WHO Grade 2 or higher bleedings, PLT transfusions, and morning PLT count during neutropenia for each patient and treatment cycle. During a total of 188 patient days, a total of 52 bleedings were reported: 34 minor bleeds (WHO Grade 1) and 18 clinically significant bleeds (14 WHO Grade 2 and four WHO Grade 3). The bleeding event chart illustrates the individual differences in bleeding pattern among patients with the similar diagnosis and PLT counts. During the study period two patient withdrawals (Patients 2b and 9) and one death (Patient 10) occurred.

between PLT count increments and change in clinical bleeding status after transfusion. When calculating the difference in WHO bleeding status before and after transfusion, no effect of PLT transfusion was observed either for the total of transfusions (mean ± SD, –0.03 ± 0.73) nor for the seven therapeutic transfusions when analyzed separately (mean ± SD, 0.14 ± 1.01).

The overall results of investigations by the TEG analyzer are reported in Table 2. PLT transfusion had an immediate but transient effect on initial clot formation that was reflected in the R and angle variables, whereas the effect on PLT clot strength and stability, the MA value, persisted until the next day. No effect was observed on PLT dose or the number of PLT microparticles on the shortening of R, increased angle, or MA increment after PLT transfusion when evaluated by correlation analysis.

How do the methods monitoring clinical effect of PLT transfusion intercorrelate?

Correlation analyses of the investigated methods for documentation of PLT transfusion were performed, and the results are presented in Table 3. Strong correlations were observed between the methods based on PLT counts (API, CCI, ITI, and MA) when used for evaluation of transfusion effects after both 1 hour and 24 hours. A negative correlation was observed between the increase in MA after 24 hours and the calculated difference in bleeding status after transfusion. Correspondingly, a negative correlation was observed between MA increment after 24 hours and clinical bleeding status after transfusion ($r = -0.494$, $p = 0.008$). No correlation was observed between PLT count increments and calculated difference in bleeding status or clinical bleeding status before or after transfusion.

Variables influencing effect of PLT transfusion

The effect of PLT transfusion varies between individual transfusions within patient and treatment cycle, but also between patients. The analysis of variables influencing outcome of PLT transfusion was therefore adjusted for individual patient effect. We found that the selected patient variables (transfusion sequence number, bleeding ≥ WHO Grade 2, fever, and patient weight) and PC variables (storage time, PLT dose, preparation technique, and ABO identity) influenced the different methods for documentations of transfusion effect differently (Table 4).

Elevation of PLT dose by 1×10^{11} per unit led to a mean increase in posttransfusion PLT count by 3.2×10^9/L, whereas prolonged storage time (by 1 hr) or pathogen inactivation by photochemical treatment reduced the posttransfusion PLT count by 0.04×10^9 and 3.9×10^9/L, respectively. Correspondingly prolonged storage time or pathogen inactivation reduced CCI by $0.02 \times m^2$/L and 1.7 PLTs $\times m^2$/L, respectively. Additional investigations showed reduced PLT dose and quality in pathogen inactivated PCs (Table 5). Patients being febrile

TABLE 1. Characteristics of successful transfusions versus transfusion failures*

Variable	CCI after 1 hr		CCI after 24 hr	
	CCI > 7.5, n = 17 (46%)	CCI ≤ 7.5, n = 20	CCI > 4.5, n = 19 (53%)	CCI ≤ 4.5, n = 17
Patient variables				
Fever	7 (41)	3 (15)	3 (16)	8 (47)†
Infection	12 (71)	11 (55)	8 (42)	14 (82)†
G-CSF	4 (24)	9 (45)	5 (26)	10 (59)†
PLT variables				
ABO-identical transfusion	12 (71)	13 (65)	12 (63)	12 (71)
Storage time (hr)	60 ± 30	106 ± 45‡	78 ± 39	90 ± 46
PLT dose ($\times 10^{11}$ per unit)	2.78 ± 0.63	2.81 ± 0.65	2.65 ± 0.57	3.07 ± 0.62‡
PLT concentration ($\times 10^9$/L)	929 ± 220	884 ± 147	888 ± 157	970 ± 203
Volume (mL)	301 ± 33	307 ± 42	298 ± 37	318 ± 33
pH at 22°C (mmHg)	7.30 ± 0.12	7.17 ± 0.12‡	7.23 ± 0.15	7.22 ± 0.14
pCO_2 (kPa)	3.21 ± 0.43	2.81 ± 0.37‡	3.08 ± 0.38	2.97 ± 0.48
HCO_3 (mmol/L)	7.3 ± 0.9	5.0 ± 1.5‡	6.4 ± 1.6	5.7 ± 1.6
pO_2 (kPa)	17.0 ± 2.1	17.5 ± 1.9	17.1 ± 2.0	17.3 ± 2.2
Glucose (mmol/L)	6.1 ± 0.9	4.5 ± 1.5‡	5.2 ± 1.7	5.4 ± 1.4
Lactate (mmol/L)	5.2 ± 3.0	8.9 ± 3.5‡	6.5 ± 3.4	8.4 ± 3.9
PLT density (mean PLT component) (g/dL)	19.8 ± 0.9	19.2 ± 0.8‡	19.6 ± 0.8	19.4 ± 1.0
PLT microparticles ($\times 10^9$/L)	22 ± 12	30 ± 10‡	25 ± 12	25 ± 10
LDH (U/L)	85 ± 66	162 ± 109‡	103 ± 64	159 ± 149

* Results are presented as number (%) or mean ± SD.
† $p < 0.05$ for difference between successful and nonsuccessful transfusions (test for dichotomous data adjusted for individual patient effect [gee, R]).
‡ $p < 0.05$ for difference between successful and nonsuccessful transfusions (t test for independent samples, SPSS 15.0).

TABLE 2. Investigations by TEG analyzer*

Time	R (min)	Angle (degree)	MA (mm)
Before transfusion	8.2 ± 1.7	47 ± 11	43 ± 11
1 hr after transfusion	6.8 ± 1.6†	58 ± 8†	52 ± 9†
24 hr after transfusion	8.7 ± 1.8	50 ± 8	49 ± 10†

* Test for continuous data adjusted for patient effect (package nlme, R). Results are presented as mean ± SD, n = 33.
† $p < 0.05$ for difference between posttransfusion and pretransfusion value.

at time of transfusion experienced a mean reduction of the ITI by 39% compared to nonfebrile patients (Table 4). The mean ITI was 27 ± 8 hours in febrile transfusions and 46 ± 30 hours in nonfebrile transfusions.

Whereas the selected patient or PC variables showed significant influence on the measurements of PLT viability (PLT count increments and ITI), no significant influence was observed on PLT functionality measured by TEG. Mixed-effects analysis could not be performed for bleeding status.

Correlation analysis of variables influencing effect of PLT transfusion

Spearman's correlation analysis was performed to separate between variables influencing immediate and late clinical outcome of PLT transfusion. The analysis included bleeding outcomes and was not adjusted for individual patient effect.

The immediate clinical effect of PLT transfusion evaluated by measurements of PLT viability (PLT count increments) showed strong correlations to PLT dose, storage time, and pathogen inactivation by PCT (Table 6). When evaluated after 24 hours, correlations were observed between PLT count increments and fever, transfusion sequence number, and pathogen inactivation. No significant correlation was observed between ITIs and the patient or PC variables investigated. Similarly, no significant correlation was observed between the PLT functionality measure bleeding outcome and the investigated patient and PC variables. No correlation was observed for the TEG values R and angle, while a negative correlation was observed between MA increment after 24 hours and patient weight (Table 6). In summary, the results of correlation analysis show that PLT-related factors mainly influence the immediate clinical effect of PLT transfusions, whereas patient-related factors influence late clinical effect.

DISCUSSION

In this study we aimed to evaluate and compare methods used for documentation of clinical efficacy of PLT transfusions in patients receiving regular PLT transfusions due to severe chemotherapy-induced thrombocytopenia. Our results illustrate the advantages and disadvantages of the different methods when used for documentation of clinical effect of regular PLT transfusions. The challenges identified are discussed below.

By statistical analysis of 40 PLT transfusions we observed that PLT viability was affected by both patient- and PC-related variables. The variables identified in our

TABLE 3. Correlation of methods for documentation of transfusion effect*

Transfusion outcome measure	CCI	Transfusion interval (hr)	24-hr posttransfusion difference in WHO bleeding status	Posttransfusion difference in TEG values		
				R (min)	Angle (degree)	MA (mm)
API						
After 1 hr	0.918†	0.308	0.251	–0.406‡	0.403‡	0.507†
After 24 hr	0.952†	0.549†	–0.037	0.109	0.155	0.460‡
CCI						
After 1 hr		0.275	0.212	–0.330	0.410‡	0.440‡
After 24 hr		0.518†	–0.064	0.052	0.173	0.427‡
Transfusion interval (hr)						
After 1 hr			0.302	–0.458‡	0.362‡	0.035
After 24 hr			0.302	0.091	0.306	0.263
24 hr posttransfusion difference in WHO bleeding grade						
After 1 hr				–0.003	0.042	0.157
After 24 hr				–0.147	0.015	–0.382‡
Posttransfusion difference in TEG values						
R (min)						
After 1 hr					–0.562†	–0.100
After 24 hr					–0.638†	0.015
Angle (degree)						
After 1 hr						0.465†
After 24 hr						0.352

* Results shown as correlation coefficient (Spearman's correlation, SPSS 15.0), n = 40.
† Correlation is significant at the 0.01 level.
‡ Correlation is significant at the 0.05 level.

TABLE 4. Patient and PC variables that significantly influence clinical effect of PLT transfusion*

Transfusion outcome measure	Mean difference	95% CI	p value
API ($\times 10^9$/L), n = 40			
Increased API:			
PLT dose (by 1×10^{11} per unit)	3.2	0.3 to 6.1	0.033
Decreased API:			
Storage time (per hr)	–0.04	–0.07 to –0.00	0.032
Pathogen inactivation by PCT (yes)	–3.9	–7.4 to –0.5	0.027
CCI (PLTs $\times$ m^2/L), n = 40			
Decreased CCI			
Storage time (per hr)	–0.02	–0.05 to –0.00	0.041
Pathogen inactivation by PCT (yes)	–1.7	–2.7 to –0.6	0.003
Transfusion interval, n = 37			
% Reduction			
Fever (yes)	39	14 to 57	0.008

* Analyzed by mixed-effects model (package nlme, R).

study are in accordance with previous publications.[23,31-36] Evaluated by measures of PLT viability, PLT recovery was influenced by PC variables only, whereas PLT survival was influenced mainly by patient variables. Being a retrospective variable, the importance of calculated ITI in daily monitoring of thrombocytopenic patients is limited. It is also dependent on the individual transfusion trigger chosen for each patient, which makes comparisons between patients difficult.

PLT dose may influence both API and the calculated CCIs. As shown in our study, higher PLT dose may be observed in transfusions with lower CCIs. As discussed in a previous publication, this observation may be explained by the formula for calculation of CCI, which favors PLT transfusions with lower PLT dose.[33] A higher number of bleeds defined as WHO Grade 2 or higher and higher number of transfusions has previously been described in patients being transfused with low-dose PCs.[12] The majority of our study transfusions (28 of 40) would have been defined as low-dose transfusions according to this publication, and a low range of PLT dose may therefore explain why no correlation was observed between PLT dose and bleeding in our study.

Outcome measures based on bleeding are complex because the clinical impact of bleedings depend both on severity and on location. A calculation of difference in WHO bleeding status before and after each transfusion implies treating bleeding as a continuous variable, which it is not. The use of bleeding assessments in the evaluation of clinical effect of PLT transfusion therefore requires a different approach. Webert and colleagues[27] have shown that minor bleedings (WHO Grade 1) predict clinical significant bleedings (WHO Grade 2, 3, or 4) and that cumulative incidence functions for bleeding (Grade 1, 2, 3, or 4) increases with time. Correspondingly, we observed that the WHO bleeding status before and after transfusion was correlated. Webert and colleagues, however, found that the risk factors for mild bleeding included decreased PLT count, an association that was not confirmed by our observations. In our 40 transfusions, we

TABLE 5. PLT viability and transfusion characteristics presented according to preparation techniques

Transfusion outcome measure and variables	Conventional gamma-irradiated PCs, n = 28	Pathogen inactivated (PCT) PCs, n = 12	p value
PLT viability*			
CI after 1 hr	15 ± 8	7 ± 4	0.003
CCI after 1 hr	9.2 ± 4.1	5.3 ± 2.7	0.005
CI after 24 hr	9 ± 8	2 ± 6	0.196
CCI after 24 hr	5.8 ± 4.6	1.8 ± 4.4	0.890
Transfusion interval (hr)	43 ± 29	36 ± 24	0.360
PLT variables†			
Storage time (hr)	86 ± 46	86 ± 40	0.978
PLT dose ($\times 10^9$ per unit)	2.96 ± 0.63	2.41 ± 0.46	0.009
pH at 22°C (mmHg)	7.26 ± 0.13	7.13 ± 0.10	0.004
pCO_2 (kPa)	3.11 ± 0.39	2.66 ± 0.50	0.004
HCO_3 (mmol/L)	6.6 ± 1.3	4.3 ± 1.2	<0.001
pO_2 (kPa)	16.6 ± 1.8	19.0 ± 1.7	<0.001
LDH (U/L)	96 ± 67	224 ± 148	0.014

* Test for continuous data adjusted for patient effect (package nlme, R).
† t test for two independent samples (SPSS).

TABLE 6. Patient and PC variables that significantly correlated to clinical effect of transfusion*

Transfusion outcome measure	Correlation coefficient	95% CI	p value
API after 1 hr ($\times 10^9$/L)			
PLT dose	0.477	0.194 to 0.678	0.003
Storage time	–0.329	–0.609 to –0.001	0.047
Pathogen inactivation	–0.519	–0.712 to 0.251	0.001
CCI after 1 hr (PLTs × m^2/L)			
Storage time	–0.420	–0.651 to –0.110	0.010
Pathogen inactivation	–0.438	–0.661 to –0.154	0.007
API after 24 hr ($\times 10^9$/L)			
Fever	–0.386	–0.631 to 0.074	0.020
Transfusion sequence number	–0.392	–0.654 to –0.048	0.018
Pathogen inactivation	–0.406	–0.652 to –0.050	0.014
CCI after 24 hr (PLTs × m^2/L)			
Fever	–0.444	–0.664 to –0.154	0.007
Transfusion sequence number	–0.422	–0.682 to –0.084	0.010
Pathogen inactivation	–0.364	–0.638 to 0.009	0.029
MA increment after 24 hr (mm)			
Patient weight	–0.439	–0.709 to 0.019	0.019

* Analyzed by Spearman's correlation (SPSS 15.0) and bootstrap BC_a CI calculation (R).

observed no significant correlation between pretransfusion PLT counts and bleeding assessment before and 24 hours after transfusion, neither was any correlation observed between PLT count increments and change in clinical bleeding status after transfusion. This observation corresponds to the results of Friedmann and coworkers[36] who found no relationship between the lowest PLT count of the day and the risk of hemorrhage when investigating 2942 patients over a period of 10 years. These differences in conclusions may be explained by inconsistent reporting of bleeds (i.e., problems with separation of new and ongoing bleeds) or individual differences in grading and interpretation of bleeds by adjudicators, yet the discrepancy strongly indicates that the present bleeding assessment scheme does not provide the information needed to serve as documentation of clinical effect of PLT transfusion. In accordance with Heddle and colleagues[12] we therefore recommend a reevaluation of the present bleeding assessment scheme.

As individual patient and PC variables influence the methods for documentation and clinical evaluation of transfusion differently, a direct comparison between transfusion outcome measures was difficult to perform. By correlation analyses, however, we found no consistent correlation between measures of PLT viability and functionality. The strongest correlations were observed within measurements of PLT viability and between PLT count increments and MA, which reflects PLT number as well as functionality of PLTs. A negative correlation was, however, observed between clinical bleeding status and late MA increment, indicating that a high MA increment may be associated with less clinical bleeding. A previous publication reports that nonactivated thromboelastography was sufficiently sensitive to monitor changes after PLT transfusion in patients with severe to mild thrombocytopenia.[37] Our observations confirm that the effect of PLT transfusion can be visualized by TEG analysis, but by showing an association to clinical bleeding status they also indicate

that TEG may be a potentially useful surrogate measure of PLT functionality.

Based on our observations we conclude that further investigations and standardization of methods are needed for better documentation of PLT transfusion outcome and identification of patients at risk of bleeding. Our observations also indicate that both PLT dose and high quality of PC are important for achieving an optimal immediate response to PLT transfusions whereas duration of transfusion effect is influenced mainly by patient variables.

ACKNOWLEDGMENTS

We appreciate the patience and helpfulness shown by the nurses at the Section of Hematology, Department of Medicine, Haukeland University Hospital, during the collection of patient samples and daily bleeding assessments included in this work. We also thank Solfrid Bjørsvik and Linda Skordal at the Blood Bank, Haukeland University Hospital, for technical help and assistance with the thromboelastograph analysis and Kristin Paulsen and Aina Kvinnsland at the Institute of Medicine, University of Bergen, for technical assistance in measurements of reticulated platelets.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to the manuscript submitted to **TRANSFUSION**.

REFERENCES

1. Gaydos LA, Freireich EJ, Mantel N. The quantitative relation between platelet count and hemorrhage in patients with acute leukemia. N Engl J Med 1962;266:905-9.
2. Slichter SJ, Harker LA. Thrombocytopenia: mechanisms and management of defects in platelet production. Clin Haematol 1978;7:523-39.
3. Stanworth SJ, Hyde C, Heddle N, Rebulla P, Brunskill S, Murphy MF. Prophylactic platelet transfusion for haemorrhage after chemotherapy and stem cell transplantation. Cochrane Database Syst Rev 2004;(4):CD004269.
4. 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: clinical practice guidelines of the American Society of Clinical Oncology. J Clin Oncol 2001;19:1519-38.
5. Blajchman MA, Slichter SJ, Heddle NM, Murphy MF. New strategies for the optimal use of platelet transfusions. Hematology Am Soc Hematol Educ Program 2008;2008: 198-204.
6. Blajchman MA, Goldman M. Bacterial contamination of platelet concentrates: incidence, significance, and prevention. Semin Hematol 2001;38(4 Suppl 11):20-6.
7. Heddle NM, Blajchman MA, Meyer RM, Lipton JH, Walker IR, Sher GD, Constantini LA, Patterson B, Roberts RS, Thorpe KE, Levine MN. A randomized controlled trial comparing the frequency of acute reactions to plasma-removed platelets and prestorage WBC-reduced platelets. Transfusion 2002;42:556-66.
8. Snyder E, McCullough J, Slichter SJ, Strauss RG, Lopez Plaza I, Lin JS, Corash L, Conlan MG. Clinical safety of platelets photochemically treated with amotosalen HCl and ultraviolet A light for pathogen inactivation: the SPRINT trial. Transfusion 2005;45:1864-75.
9. Guidelines for the use of platelet transfusions. Br J Haematol 2003;122:10-23.
10. Cid J, Ramiro L, Escoda L, Llorente A. Efficacy of transfusion of platelet concentrates obtained by manual pooling or by semiautomated pooling of buffy-coats: a retrospective analysis of count increment, corrected count increment and transfusion interval. Vox Sang 2009;96:29-33.
11. Rebulla P. A mini-review on platelet refractoriness. Haematologica 2005;90:247-53.
12. Heddle NM, Cook RJ, Tinmouth A, Kouroukis CT, Hervig T, Klapper E, Brandwein JM, Szczepiorkowski ZM, AuBuchon JP, Barty RL, Lee KA. A randomized controlled trial comparing standard- and low-dose strategies for transfusion of platelets (SToP) to patients with thrombocytopenia. Blood 2009;113:1564-73.
13. Cook RJ, Heddle NM, Rebulla P, Sigouin CS, Webert KE. Methods for the analysis of bleeding outcomes in randomized trials of PLT transfusion triggers. Transfusion 2004;44: 1135-42.
14. Dickinson KJ, Troxler M, Homer-Vanniasinkam S. The surgical application of point-of-care haemostasis and platelet function testing. Br J Surg 2008;95:1317-30.
15. European Committe on Blood Transfusion. Guide to preparation, use and quality assurance of blood components. Strasbourg: Council of Europe; 2008. p. 149-60.
16. Bertrand G, Jallu V, Gouet M, Kjaer KM, Lambin P, Husebekk A, Kaplan C. Quantification of human platelet antigen-1a antibodies with the monoclonal antibody immobilization of platelet antigens procedure. Transfusion 2005;45:1319-23.
17. Ryningen A, Apelseth T, Hausken T, Bruserud O. Reticulated platelets are increased in chronic myeloproliferative disorders, pure erythrocytosis, reactive thrombocytosis and prior to hematopoietic reconstitution after intensive chemotherapy. Platelets 2006;17:296-302.
18. Apelseth TO, Hervig T. In vitro evaluation of platelet concentrates during storage: platelet counts and markers of platelet destruction. Transfus Apher Sci 2007;37:261-8.
19. Apelseth TO, Bruserud O, Wentzel-Larsen T, Bakken AM, Bjorsvik S, Hervig T. In vitro evaluation of metabolic changes and residual platelet responsiveness in photochemical treated and gamma-irradiated single-donor platelet concentrates during long-term storage. Transfusion 2007;47:653-65.

20. McFarland JG. Platelet and granulocyte antigens and antibodies. In: Roback JD, editor. Technical manual. Bethesda: American Association of Blood Banks; 2008. p. 525-40.
21. DuBois D, DuBois EF. A formula to estimate the approximate surface area if height and weight be known. Arch Intern Med 1916;17:863-71.
22. Hanson SR, Slichter SJ. Platelet kinetics in patients with bone marrow hypoplasia: evidence for a fixed platelet requirement. Blood 1985;66:1105-9.
23. Slichter SJ, Davis K, Enright H, Braine H, Gernsheimer T, Kao KJ, Kickler T, Lee E, McFarland J, McCullough J, Rodey G, Schiffer CA, Woodson R. Factors affecting posttransfusion platelet increments, platelet refractoriness, and platelet transfusion intervals in thrombocytopenic patients. Blood 2005;105:4106-14.
24. Slichter SJ. Relationship between platelet count and bleeding risk in thrombocytopenic patients. Transfus Med Rev 2004;18:153-67.
25. 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 4th, Davis K, Lin JS, Metzel P, Corash L, Koutsoukos A, Lin L, Buchholz DH, Conlan MG. Therapeutic efficacy and safety of platelets treated with a photochemical process for pathogen inactivation: the SPRINT Trial. Blood 2004;104:1534-41.
26. 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 randomized controlled trial with a sequential Bayesian design. Transfusion 2004;44:1711-9.
27. Webert K, Cook RJ, Sigouin CS, Rebulla P, Heddle NM. The risk of bleeding in thrombocytopenic patients with acute myeloid leukemia. Haematologica 2006;91:1530-7.
28. Efron B, Tibshirani RJ. An introduction to the bootstrap. New York: Chapman& Hall; 1993.
29. Dalgaard P. Introductory statistics with R. 1st ed. New York: Springer; 2002. p. 1-267.
30. Pinheiro JC. Mixed-effects models in S and S-plus. 1st ed. New York: Springer; 2000. p. 1-270.
31. Bishop JF, Matthews JP, McGrath K, Yuen K, Wolf MM, Szer J. Factors influencing 20-hour increments after platelet transfusion. Transfusion 1991;31:392-6.
32. Heim D, Passweg J, Gregor M, Buser A, Theocharides A, Arber C, Meyer-Monard S, Halter J, Tichelli A, Gratwohl A. Patient and product factors affecting platelet transfusion results. Transfusion 2008;48:681-7.
33. 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;39:586-92.
34. Kerkhoffs JL, Eikenboom JC, Schipperus MS, van-Wordragen-Vlaswinkel RJ, Brand R, Harvey MS, de-Vries RR, Barge R, van-Rhenen DJ, Brand A. A multicenter randomized study of the efficacy of transfusions with platelets stored in platelet additive solution II versus plasma. Blood 2006;108:3210-5.
35. Fabris F, Soini B, Sartori R, Randi ML, Luzzatto G, Girolami A. Clinical and laboratory factors that affect the posttransfusion platelet increment. Transfus Sci 2000;23:63-8.
36. Friedmann AM, Sengul H, Lehmann H, Schwartz C, Goodman S. Do basic laboratory tests or clinical observations predict bleeding in thrombocytopenic oncology patients? A reevaluation of prophylactic platelet transfusions. Transfus Med Rev 2002;16:34-45.
37. Salaj P, Marinov I, Markova M, Pohlreich D, Cetkovský P, Hrachovinová I. Thrombelastography monitoring of platelet substitution therapy and rFVIIa administration in haemato-oncological patients with severe thrombocytopenia. Prague Med Rep 2004;105:311-7.