Hemovigilance monitoring of platelet septic reactions with effective bacterial protection systems

Richard J. Benjamin,1 Thomas Braschler,2 Tina Weingand,3 and Laurence M. Corash1

BACKGROUND: Delayed, large-volume bacterial culture and amotosalen/ultraviolet-A light pathogen reduction are effective at reducing the risk of bacterial proliferation in platelet concentrates (PCs). Hemovigilance programs continue to receive reports of suspected septic transfusion reactions, most with low imputability. Here, we compile national hemovigilance data to determine the relative efficacy of these interventions.

STUDY DESIGN AND METHODS: Annual reports from the United Kingdom, France, Switzerland, and Belgium were reviewed between 2005 and 2016 to assess the risk of bacterial contamination and septic reactions.

RESULTS: Approximately 1.65 million delayed, large-volume bacterial culture-screened PCs in the United Kingdom and 2.3 million amotosalen/ultraviolet-A–treated PCs worldwide were issued with no reported septic fatalities. One definite, one possible, and 12 undetermined/indeterminate septic reactions and eight contaminated “near misses” were reported with delayed, large-volume bacterial cultures between 2011 and 2016, for a lower false-negative culture rate than that in the previous 5 years (5.4 vs. 16.3 per million; odds ratio, 3.0; 95% confidence interval, 1.4-6.5). Together, the Belgian, Swiss, and French hemovigilance programs documented zero probable or definite/certain septic reactions with 609,290 amotosalen/ultraviolet-A–treated PCs (<1.6 per million). The rates were significantly lower than those reported with concurrently transfused, nonpathogen-reduced PCs in Belgium (<4.4 vs. 35.6 per million; odds ratio, 8.1; 95% confidence interval, 1.1-353.3) and with historic septic reaction rates in Switzerland (<6.0 vs. 82.9 per million; odds ratio, 13.9; 95% confidence interval, 2.1-589.2), and the rates tended to be lower than those from concurrently transfused, nonpathogen-reduced PCs in France (<4.7 vs. 19.0 per million; odds ratio, 4.1; 95% confidence interval, 0.7-164.3).

CONCLUSION: Pathogen reduction and bacterial culture both reduced the incidence of septic reactions, although under-reporting and strict imputability criteria resulted in an underestimation of risk.
HEMOVIGILANCE FOR TRANSFUSION SEPSIS

Volume 57, December 2017  TRANSFUSION  2947

treatment system (riboflavin/UV; Terumo BCT) is widely marketed; however, HV data on clinical outcomes are limited.6-9 The INTERCEPT blood system (amotosalen/UVA light; Cerus Corporation) is approved by the US Food and Drug Administration. Clinical outcomes data are available from randomized controlled trials and observational studies10-12 and from national HV programs in France, Switzerland, and Belgium. PR using amotosalen/UVA-light and delayed large-volume bacterial culture (DLVBC) screening with the BacT/ALERT system share extensive clinical HV experience published by regulatory authorities as well as in vitro technology assessments that document their efficacy.1,13-18

Confirmed documentation of a septic transfusion reaction (STR) is challenging when effective processes are implemented, and high-imputability cases are rare, because other causes may be more likely. HV programs generally define definite STRs as those in which a transfusion reaction is associated with the same bacterial strain in recipient blood cultures and the residual PCs. However, causes other than failure of the bacterial-detection or PR system should be considered, including improper performance of culture screening or PR; late contamination during post-manufacture storage (i.e., caused by “pin holes” or bag leaks)19,20; or false-positive bag cultures due to retrograde flushing of the intravenous line by contaminated blood from the recipient.21 With both PR and DLVBC interventions, the latter causes need to be excluded before it can be concluded that the bacterial protection system has failed.

In this report, we review suspected STRs reported by the UK Serious Hazards of Transfusion (UK SHOT) HV system22 after DLVBC screening was implemented by the National Health System Blood and Transplant (NHSBT), which issued 85.5%, and the Northern Ireland Blood Transfusion Service (NIBTS) which issued 2.9% of all PCs in the United Kingdom in 201623; cases reported to the Swiss, Belgian, and French HV systems; and spontaneous reports to Cerus Corporation after amotosalen/UVA-light implementation. Case reports are provided that highlight the difficulty in interpreting HV data and the level of investigation increasingly required when determining imputability.

MATERIALS AND METHODS

HV data for platelet bacterial contamination and transfusion-related sepsis from the United Kingdom, France, Belgium, and Switzerland were compiled for various years between 2005 and 2016 relative to the introduction of DLVBC or amotosalen/UVA-light PR. Comparisons of HV data from national systems suffer from limitations, which include dissimilar donor populations and underlying rates of bacterial contamination; various arm-disinfection techniques; the use of whole-blood versus apheresis PCs; as well as variable and incomplete patient assessments, different definitions of outcomes, and variations in the rigor of reporting.1 The imputability definitions described in the HV reports for STR vary and are listed in Table 1,22-26 Cerus Corporation uses Centers for Disease Control and Prevention National Healthcare Safety Network criteria for imputability assessments.25 None of the HV programs provide a detailed description of those criteria or how they are used. The French, Belgian, and Swiss HV programs report probable and definite/certain cases together, preventing comparisons within each subgroup. Given these limitations, formal comparisons of outcomes from different HV programs are considered to have less value than comparisons of varying approaches used within the same country.

DLVBC screening was instituted by the NHSBT in England and North Wales in February 2011, and in the NIBTS for all apheresis and buffy-coat PCs, allowing for a 7-day shelf-life after delayed release into inventory on Day 3 after collection.18 At NHSBT screening is performed on a 16-mL to 20-mL sample taken from each PC 36 hours after donation. At NIBTS a 16-mL sample is taken 48 hours after collection from the mother bag. For both services, an 8-mL inoculum per bottle is incubated under both aerobic and anaerobic conditions on the BacT/ALERT system until the end of the shelf-life.16 The UK SHOT reports covering England, Wales, Scotland, and Northern Ireland from 2006 to 201623 were reviewed. HV data for the period from 2011 to 2016 with DLVBC were compared with the data from the previous period (2006-2010), when culture screening was limited to quality-control and investigational studies.

Amotosalen/UVA-light treatment was introduced for all pooled buffy-coat or apheresis PCs in Switzerland in 2011 with a 5-day shelf-life, which was extended to a 7-day outdate in July 2013. HV data from 2011 to 2015 for high-imputability STRs (Grade 3 and 4) (Table 1) are published by Swissmedic, which is the Swiss regulatory agency for therapeutic products.24 Swissmedic HV reports from 2005 to 2010 allow a formal comparison of STR and bacterial contamination rates before and after the introduction of PR.

Amotosalen/UVA-light treatment was introduced in France for all buffy-coat or apheresis PCs distributed in the Alsace region of the Établissement Français du Sang in 2006 and subsequently in various overseas departments.27,28 PCs have a 5-day shelf-life. Bacterial culture screening of PCs has not been implemented in France.29 The French regulatory agency (Agence Nationale de Sécurité du Médicament et des Produits de Santé) publishes an annual HV analysis. A report is required for each component transfused,30 but only STRs with imputability grades of 2 and 3 (Table 1) are documented in the analysis. Results from 2006 through 2015 were compiled, and concurrently collected data were compared from areas that
TABLE 1. Imputability criteria for inclusion as an STR in national HV and Cerus safety reports

| United Kingdom\(^{22,23}\) | Switzerland\(^24\) | Belgium\(^{25}\) | France\(^{26}\) | Cerus (NSHN)\(^{26}\) |
|-------------------------------|-----------------|-----------------|-----------------|------------------|
| Confirmed STRs are defined as evidence of infection in the recipient post-transfusion, with no evidence of infection pre-transfusion and no evidence of an alternative source of infection, plus evidence of the same organism in the donor or evidence of contamination of the blood component | 0 = Not evaluable; 1 = excluded/unlikely (the reaction is definitely/more likely to be due to other causes); 2 = possible (the reaction could be explained by the transfusion as well as by other causes); 3 = probable (the reaction does not appear to be due to another cause); 4 = definite (in all probability, the reaction was caused by the transfusion) | N = not assessed; 0 = excluded and unlikely; 1 = possible, questionable (if the information available cannot establish whether the adverse reaction is attributed to the blood or blood component to other causes); 2 = probable (when there is clear evidence that the adverse reaction can be attributed to the blood or blood components); 3 = certain, proven (if there is convincing evidence that the adverse reaction must be attributed beyond reasonable doubt to the blood or blood component) | NE = not evaluable (insufficient data to assess imputability); 0 = excluded/probable (evidence is available that clearly suggests attributing the adverse event to causes other than transfusion, once the investigation is complete); 1 = possible (evidence is available that does not allow the clear attribution of the adverse event to the transfusion or to other causes, once the investigation is complete); 2 = probable (the available evidence clearly allows the attribution of the adverse event to the transfusion, once the investigation is complete); 3 = certain (evidence that cannot be questioned allows the certain attribution of the adverse event to the transfusion, once the investigation is complete) | Definite: ONE or more of the following:  
• Evidence of the pathogen in the transfused component  
• Evidence of the pathogen in the donor at the time of donation  
• Evidence of the pathogen in an additional component from the same donation  
• Evidence of the pathogen in an additional recipient of a component from the same donation; AND no other potential exposures to the pathogen could be identified in the recipient  
AND EITHER  
• Evidence that the recipient was not infected with the pathogen prior to transfusion  
OR  
• Evidence that the identified pathogen strains are related by molecular or extended phenotypic comparison testing with statistical confidence (p < 0.05)  
Probable: ONE or more of the following:  
• Evidence of the pathogen in the transfused component  
• Evidence of the pathogen in the donor at the time of donation  
• Evidence of the pathogen in an additional component from the same donation  
• Evidence of the pathogen in an additional recipient of a component from the same donation  
AND EITHER  
• Evidence that the recipient was not infected with this pathogen before transfusion  
OR  
• No other potential exposures to the pathogen could be identified in the recipient  
Possible: Case fails to meet definite, probable, doubtful, or ruled out imputability criteria |
did and did not use PR. Collection and skin-disinfection methods, initial sample diversion, and leukocyte-reduction practices were consistent within the country.

Amotosalen/UVA-light treatment of PCs was introduced in Belgium for approximately 40% of the PC supply in 2009 and for the entire country by November 2015. Conventional PCs were culture screened using 4-mL to 8-mL samples inoculated into aerobic culture bottles the day after collection. Amotosalen/UVA-treated PCs were stored with a 7-day shelf-life until 2010; then, storage was reduced to 5 days. Annual HV reports are published by the Federaal Agentschap voor Geneesmiddelen en Gezondheidsproducten. The data between 2009 and 2015 were compiled, and concurrently collected data were compared from areas using bacterial detection with those using PR.

Between 2003 and 2010, Cerus Corporation conducted active HV phase IV studies in Europe. A report describing transfusion-related adverse events was required for each transfusion, regardless of outcome. Cerus Corporation also receives spontaneous reports of adverse events that are suspected to involve amotosalen/UVA-treated platelets. These were investigated and reported to national regulatory agencies, as required by law. Case reports pertaining to Swiss and Belgian HV reports were summarized to provide a clinical context for those reports.

**Statistical analysis**

Data from HV studies are presented descriptively, and illustrative cases reports are provided, as defined by the individual HV programs. Comparison of STR rates was performed using odds ratios (ORs), and 95% confidence intervals (CIs) were determined using the Fisher exact test. For point estimates with zero values, the rates, ORs, and 95% CIs were determined assuming a minimum value of 1, and the rates are depicted as “less than” the calculated value.

**RESULTS**

In the United Kingdom, the SHOT data for 2006 through 2010, before the implementation of DLVBC screening, were summarized (Table 2): 13 contaminated PCs derived from seven collections were involved in 10 confirmed STRs, including three fatalities; in addition, eight near misses were reported with the discovery of contaminated PCs before transfusion. This included one collection in 2009 that was associated with a single near miss contaminated PC and four co-component PC transfusions in one adult and one neonate. Ten contaminated PCs from seven collections and five near misses were previously reported to the NHSBT by McDonald and colleagues. In total, 1,286,821 PCs were issued during these years, for an overall rate of proven PC contamination of 16.3 per million (Fig. 1) and an overall fatality rate of 2.3 per million.

With DLVBC screening from 2011 through 2016, 1,652,761 PCs were issued in the United Kingdom, and no septic fatalities were reported. One confirmed STR (nonfatal) and one possible STR were reported (Table 2). Twelve undetermined/indeterminate cases were described in which an STR could not be ruled out because of insufficient clinical information, although no further details were provided. In 450 suspected STR reports, the PCs could be excluded; and, in 152 cases, the PCs could be ruled out, although the basis of these determinations was not reported. Eight products derived from seven collections had visually detected clumps discovered before transfusion and were culture-positive for *Staphylococcus aureus* (7) and *Serratia marcescens* (1). Eight co-components of these contaminated PCs were identified as

| Year | No. of platelet units distributed | Excluded* | Ruled out† | Indeterminate/undetermined‡ | Definite sepsis | Possible sepsis | Near miss§ |
|------|---------------------------------|-----------|------------|-----------------------------|----------------|----------------|------------|
| 2006 | 259,654                         | 14        | 0          | 2                           | 0              | 0              | 0          |
| 2007 | 255,474                         | 3         | 1          | 0                           | 0              | 1              | 0          |
| 2008 | 258,419                         | 7         | 4          | 0                           | 0              | 1              | 0          |
| 2009 | 266,312                         | 13        | 3          | 4                           | 0              | 3              | 0          |
| 2010 | 246,962                         | 30        | 2          | 0                           | 0              | 3              | 0          |
| Total| 1,286,821                       | 67        | 6          | 13                          | 0              | 8              |            |
| 2011 | 260,278                         | 77        | 28         | 1                           | 0              | 0              | 0          |
| 2012 | 268,565                         | 70        | 27         | 2                           | 0              | 0              | 0          |
| 2013 | 268,630                         | 75        | 28         | 0                           | 0              | 0              | 0          |
| 2014 | 274,623                         | 56        | 36         | 1                           | 0              | 0              | 0          |
| 2015 | 273,695                         | 87        | 19         | 3                           | 1              | 1              | 0          |
| 2016 | 306,970                         | 85        | 14         | 5                           | 0              | 0              | 4          |
| Total| 1,652,761                       | 450       | 152        | 12                          | 1              | 1              | 8          |

* Suspected STRs were excluded by the lack of bacteria in the PC product and/or negative patient cultures.
† Ruled out suggests that there was no evidence that the transfusion was the cause of the infection.
‡ Indeterminants were cases in which an STR could not be ruled out because of the possibility of retrograde contamination of the PC after connection to an infusion set or by a lack of the PC bag available to culture.
§ Near misses occurred when the PC was noted to have an abnormal appearance before connection of the infusion set to the bag.
culture-negative and/or had been received in a transfusion without adverse events. The overall contamination rate from 2011 through 2016 is calculated at 5.4 per million issued PCs, which is significantly lower (OR, 3.0; 95% CI, 1.4-6.5) than in the prior 5-year period from 2006 to 2010. Three cases illustrative of the difficulty in determining imputability were described in more detail in the SHOT reports. One case in 2011 (Case S1, available as supporting information in the online version of this paper) was described as “undetermined,” with the finding of *Lactococcus lactis* ssp. in a PC bag after a reaction compatible with an STR, but no patient cultures or further evidence were available to determine causality. A second case (Case S2, available as supporting information in the online version of this paper; also reported by McDonald and co-workers18) in 2015 was considered a confirmed STR with documentation of the same strain of *S. aureus* from the patient’s blood culture, the residual PC bag, and a swab from the donor’s skin. A third case (Case S3, available as supporting information in the online version of this paper) was deemed a “possible STR”: a patient experienced symptoms compatible with an STR, and the hospital reported that *Streptococcus* spp. was identified from the patient’s blood culture 24 hours after transfusion. *Streptococcus agalactiae* and *Escherichia coli* were isolated from the returned PC pack. It was deemed impossible to confirm the PC as the source of the infection, and the case was interpreted as a “possible” STR and separately reported.18,23 Taken together, these case reports document that the SHOT HV program requires a demonstration of the same strain of bacteria from the patient, the residual PC bag, and the donor to impute a definite/confirmed STR after PC transfusion and that cases in which the clinical data do not include all of these requirements are classified with lower imputability, resulting in an underestimation of risk.

In France, between 2006 and 2015, there were 2,575,224 conventional apheresis and pooled whole-blood Buffy-coat PC transfusions, with 49 reported probable or certain STRs (19.0 per million) and nine fatalities (3.5 per million) (Table 3). No systematic bacterial culture screening was performed in France. During the same period, there were 214,293 amotosalen/UVA-treated PC transfusions, with no reported STRs (<4.7 per million; OR, 4.1; 95% CI, 0.70-164.3).29,31 Details of the STRs are not described in the HV reports.

In Switzerland, from 2005 to 2011, 13 STRs were reported for 156,773 apheresis and whole-blood Buffy-coat PC transfusions (82.9 per million), including three fatalities (19.1 per million). Two contaminated PCs were interdicted before transfusion in 2005, for an overall contamination rate of 95.7 per million transfusions (Fig. 1). From 2011 to 2015, 167,200 apheresis and Buffy-coat PCs treated with amotosalen/UVA were delivered by transfusion (Table 4) without reported probable or certain STRs (<6.0 per million) or fatalities, which constituted a significant reduction (OR, 13.9; 95% CI, 2.1-589.2). In 2015, a single illustrative case that was classified as a “possible” STR by Swissmedic was mentioned without further description in the annual HV report (Case S4, available as...
supporting information in the online version of this paper describes the case as reported to Cerus Corporation Safety Monitoring Program). This case highlights the difficulty in imputing causality despite identification of the same bacterial strains in patient blood cultures and residual PCs. A second case (Case S5, available as supporting information in the online version of this paper) reported to Cerus Corporation as a “possible” STR by the hospital physician but deemed “unrelated” to the transfusion by the blood center and not reported by Swissmedic in the annual HV report, is presented here as a possible illustrative example of successful pathogen inactivation of a heavily bacterially contaminated PC, with the PC showing Gram stain positivity with negative bacterial cultures after a relatively mild transfusion reaction.

In Belgium, between 2009 and 2015, 252,809 conventional PCs screened with the BacT/ALERT culture system were distributed, and nine (35.6 per million) high-probability STRs were reported with zero fatalities (Table 5). During the same period, 227,797 amotosalen/UVA-treated PCs were distributed with no reported STRs, a statistically significant difference (<4.4 per million; OR, 8.1; 95% CI, 1.1-353.3).

Between 2003 and 2010, Cerus Corporation sponsored active HV studies that investigated 4067 patients who received 19,175 amotosalen/UVA-treated PCs. Three reports of suspected STRs were reported, all of which were evaluated as “unrelated” by the investigators (Table 6). Subsequently, Cerus Corporation received six spontaneous reports of suspected STRs from blood services worldwide. In two cases evaluated as “unrelated,” bacteria (S. hemolyticus, S. viridans) were isolated from the implicated PCs, patient cultures were negative or were not performed, and bacteria were ruled laboratory contaminants. In a third case, no evaluation of the residual component was performed, and evidence of contamination was lacking. Two cases from Switzerland are described above as Cases S4 and S5 available as supporting information in the online version of this paper.

### Table 3. Summary of hemovigilance reports of high-imputability (Grade 2 or 3) STRs from France

| Year | Conventional platelets | Amotosalen/UVA platelets |
|------|------------------------|--------------------------|
|      | No. of units transfused | Transfusion-transmitted sepsis (fatalities) | No. of units transfused | Transfusion-transmitted sepsis (fatalities) |
| France | | | |
| 2006 | 231,853 | 4 (0) | 6,420 | 0 (0) |
| 2007 | 232,708 | 9 (2) | 15,393 | 0 (0) |
| 2008 | 239,349 | 6 (1) | 15,544 | 0 (0) |
| 2009 | 241,634 | 9 (0) | 21,767 | 0 (0) |
| 2010 | 253,149 | 2 (1) | 22,632 | 0 (0) |
| 2011 | 267,785 | 2 (1) | 22,392 | 0 (0) |
| 2012 | 275,834 | 7 (2) | 24,849 | 0 (0) |
| 2013 | 281,288 | 4 (1) | 24,954 | 0 (0) |
| 2014 | 278,788 | 2 (0) | 26,676 | 0 (0) |
| 2015 | 272,836 | 4 (1) | 33,666 | 0 (0) |
| Total | 2,579,224 | 49 (9) | 214,293 | 0 (0) |

* This was a combined report for 2005/2006.† Estimated from the 2006 Swiss Red Cross Annual report, because the Swissmedic hemovigilance report did not report the actual value.‡ In addition, two near-miss incidences occurred in which contaminated platelet concentrates were interdicted before transfusion.

### Table 4. Summary of hemovigilance reports of high-imputability (Grade 3 or 4) STRs from Switzerland

| Year | Conventional platelets | Amotosalen/UVA platelets |
|------|------------------------|--------------------------|
|      | No. of units transfused | Transfusion-transmitted sepsis (fatalities) | No. of units transfused | Transfusion-transmitted sepsis (fatalities) |
| Switzerland | | | |
| 2005/2006* | 40,000† | 5 (2)‡ | — | — |
| 2007 | 22,937 | 2 (0) | — | — |
| 2008 | 27,669 | 2 (0) | — | — |
| 2009 | 29,654 | 3 (1) | — | — |
| 2010 | 29,900 | 1 (0) | — | — |
| 2011 | 6,613 | 0 (0) | 26,454 | 0 (0) |
| 2012 | — | — | 34,265 | 0 (0) |
| 2013 | — | — | 34,750 | 0 (0) |
| 2014 | — | — | 35,328 | 0 (0) |
| 2015 | — | — | 36,403 | 0 (0) |
| Total | 156,773 | 13 (3) | 167,200 | 0 (0) |
version of this paper. A final case of “possible STR” from Belgium was received in 2016 and is described as Case S6 (available as supporting information in the online version of this paper) as an additional illustration of the difficulty in ascribing the imputability of PC in suspected STR reactions when contamination of the PC at the time of transfusion cannot be excluded.

**DISCUSSION**

Bacterial culture screening and PR systems for PCs were developed and implemented based on in vitro assessments of analytical sensitivity or inactivation efficacy, respectively. It is only through large HV programs that their clinical impact can be measured. We describe the accumulating HV data in support of current highly effective bacterial culture and PR systems. Although alternate technologies for assuring bacterial safety are now in development or are available (e.g., rapid bacterial testing,2 cold-stored34 or frozen platelets,35 delayed bacterial culture,36,37 etc.), proof of their efficacy in routine practice must await widespread implementation and accumulated HV data. DLVBC using the BacT/ALERT system, as performed by the NHSBT and NIBTS, are the current best-in-class culture systems, because of the large, successful experience monitored by the UK SHOT program and subsequently partially published in a separate report.18 The findings support the in vitro data suggesting that the sensitivity of bacterial culture is increased by delayed sampling,18,38-40 use of large sample volumes (16-48 mL per PC collection), use of both aerobic and anaerobic cultures,41 and testing of all apheresis split products.18,37,42 Similarly, with best-in-class PR using amotosalen/UVA, substantial national HV reports evaluating clinical efficacy, head-to-head comparisons, and robust data on in vitro inactivation of a broad spectrum of bacterial species are available to confirm effectiveness.13-15,43

With DLVBC screening from 2011 to 2016, greater than 1.65 million PCs were delivered without any reported septic fatality.23 False-negative cultures reported by UK SHOT included a single definite STR case (subsequently described by McDonald and coworkers18) and eight contaminated products that were intercepted at the blood center or hospital blood bank, for an overall culture false-negative rate of 5.4 per million cultured PCs (Fig. 1), a significantly lower rate (p < 0.05) than that for the prior 5 years (2006 to 2010), when 21 PCs (16.3 per million) exhibited evidence of contamination. Interestingly, most near misses were *S. aureus*, suggesting that this species has a propensity to avoid detection by the BacT/ALERT system. The false-negative rate of DLVBC is lower than the 9.4 per million rate reported by the American Red Cross HV program using a less sensitive bacterial culture screening protocol with 4,063,371 distributed apheresis platelets44 and the smaller Belgian experience with less sensitive bacterial culture31 (Fig. 1) but is similar to the recently reported

---

### TABLE 5. Summary of hemovigilance reports of high-imputability (Grade 2 or 3) STRs from Belgium

| Year | Conventional platelets | Amotosalen/UVA platelets |
|------|------------------------|--------------------------|
|      | No. of units transfused | Transfusion-transmitted sepsis (fatalities) | No. of units transfused | Transfusion-transmitted sepsis (fatalities) |
| Belgium  |
| 2009  | 41,346 | 0 (0) | 27,564 | 0 (0) |
| 2010  | 41,597 | 2 (0) | 27,731 | 0 (0) |
| 2011  | 41,380 | 2 (0) | 27,586 | 0 (0) |
| 2012  | 41,668 | 2 (0) | 27,779 | 0 (0) |
| 2013  | 40,344 | 0 (0) | 29,456 | 0 (0) |
| 2014  | 38,221 | 3 (0) | 28,834 | 0 (0) |
| 2015  | 8,253  | 0 (0) | 58,847 | 0 (0) |
| Total | 252,809 | 9 (0) | 227,797 | 0 (0) |

### TABLE 6. Imputability of suspected STRs reported to Cerus Corporation’s safety program

| Source | Unlikely/unrelated | Possible | Probable | Definite |
|--------|--------------------|----------|----------|----------|
| Cerus HV* | 3                  | 0        | 0        | 0        |
| Switzerland | 3                  | 1        | 0        | 0        |
| Belgium    | 1                  | 1        | 0        | 0        |
| France     | 0                  | 0        | 0        | 0        |

* Three cases were reported during Cerus active HV studies,12 and 6 cases were subsequently spontaneously reported to Cerus safety program, including Case S4 (available as supporting information in the online version of this paper) mentioned in the Swiss HV report, Supporting Case S5 (available as supporting information in the online version of this paper) from Switzerland, and Case S6 (available as supporting information in the online version of this paper) from Belgium.
failure rate of 1 per 286,386 PCs (3.5 per million) using 3.8% minimal proportional sample volume bacterial culture screening.24 The Swiss, French, Belgian, and Cerus Corporation HV data characterize the effectiveness of amotosalen/UVA treatment to prevent STRs. Approximately 2.3 million PCs have been manufactured worldwide (Cerus Corporation; unpublished data) without a reported septic fatality. The rates of STR with PR PCs were significantly lower than with concurrently transfused, non-PR, non-DLVBC culture-screened PCs in Belgium (<4.4 vs. 35.6 per million; OR, 8.1; 95% CI, 1.1-353.3) and tended to be lower than the rates with non-PR, nonculture-screened PCs in France (<4.7 vs. 19.0 per million; OR, 4.1; 95% CI, 0.7-164.3). The comparison of concurrent STR rates for PCs not prepared with PR and amotosalen/UVA-treated PCs within each country suggests that the low reported septic rate with PR is not due to failure to report reactions or different classifications of imputability. In Switzerland, the 2005 to 2010 Swissmedic annual HV reports described the prior experience before the introduction of PR, when no bacterial culture screening was utilized.24 Bacterial contamination of PCs represented the highest risk related to blood components. The transfusion of bacterially contaminated PCs caused the death of two patients in 2005 and one in 2009. During the same period, 13 submitted reports of transfusion reactions were certainly or most probably caused by bacterially contaminated PCs. Six of those incidents were classified as life-threatening, and the other six were not severe reactions.24 The implementation of amotosalen/UVA treatment of PCs resulted in a significant decline in the STR rate from 82.9 per million (with 19.1 fatalities per million) transfused PCs to <6.0 per million STRs (OR, 4.1; 95% CI, 0.7-164.3).

Overall the French, Swiss, and Belgian national HV programs monitored a total of 609,290 amotosalen/UVA-treated PC transfusions without any reports of probable or definite/certain STRs or fatalities (<1.6 per million) (Tables 3–5). Together, the HV programs monitored a total of 2.98 million PC transfusions that were not pathogen reduced and recorded 71 high-probability septic reactions (23.8 per million) and 12 fatalities (4.0 per million). Interestingly, the rate was higher in Belgium (35.6 per million), where less sensitive bacterial culture screening and passive HV reporting was utilized, compared with France, where there was no bacterial culture screening and active HV reporting was used (19.0 per million) (Fig. 1).

HV programs likely underestimate the prevalence of bacterial STRs, because stringent criteria are used to determine definite imputability, including evidence of patient infection and PC contamination and irrefutable evidence of a donor source, with confirmation of strain identity. Reports with incomplete investigations (e.g., because PCs are not available for culture) are considered undetermined or indeterminate. Some of these cases are probably due to bacterial contamination. Cases S3, S4 and S6 (available as supporting information in the online version of this paper) demonstrate the difficulty of imputing causality when retrograde contamination of PCs after connection to an infusion set cannot be excluded. Physicians also fail to recognize and/or to report sepsis, and patients may display no immediate symptoms resulting from PC contamination, often because of the concurrent receipt of antibiotic or anti-inflammatory medication. Hong and colleagues46 recently reported a single-institution study in which all PCs screened by initial culture for bacteria after manufacture, but not using DLVBC, were cultured again on issue from the hospital blood bank: 20 contaminated units were identified of 51,440 screened (388 per million). No transfusion reactions were reported to the blood bank, yet five patients (97.2 per million) had signs and symptoms on retrospective review that were compatible with an STR occurring 9 to 24 hours after transfusion, including one fatality.46,47 These findings underscore the need for heightened vigilance for STRs related to PC transfusion. Signs and symptoms of an STR can occur up to 24 hours after transfusion.46 Once sepsis is considered, a thorough investigation is necessary. This should involve two or more patient blood cultures to maximize the opportunity to detect bacteremia,48 and the residual PC container should be secured and open ports sealed to prevent contamination. A sample from the residual PCs should be Gram-stained to facilitate immediate treatment of the patient, and enumeration should be performed by plate count to assess bacterial concentration. Co-components should be retrieved and cultured, and recipients of co-components should be evaluated for sepsis. Bacterial culture determination times need to be recorded as an indication of concentration if this is not possible by a plate count. Finally, the potential source of contamination needs to be investigated, which will be organism dependent. Environmental contaminants, such as Pseudomonas aeruginosa, will warrant investigation of blood service and hospital facilities. Organisms like S. aureus or Klebsiella pneumoniae will require investigation of the donor, including skin, oral, or fecal cultures, as indicated by the strain of the bacteria involved.21 If the PC container is contaminated, then a thorough investigation will be necessary. The possibility that the culture or inactivation process was not performed or was not properly executed must be excluded. All efforts must be taken to rule out retrograde contamination by blood flowing from the patient, especially if the transfusion was performed through an intravenous catheter used for other medications. Contamination of the PC post-manufacturing should be excluded by examination for leaks in the bag. Irrefutable evidence of PC contamination includes a finding of contamination before the bag is connected to an infusion set (e.g., at the bedside) or evidence of PC contamination plus the same strain in a co-component, in the recipient of a co-component, or in cultures from the donor. Taken together, these findings...
highlight the difficulty in evaluating STR cases and emphasize the likelihood that HV systems will fail to capture all events as high-imputability cases.

Amotosalen/UVA PR and DLVBC have both been proven to increase the safety of the blood supply. It is unlikely that amotosalen/UVA PR will maintain its near-perfect record with regard to STRs, as documented by national HV programs, because imperfect reporting may have obscured cases. In vitro laboratory experiments under worst-case conditions demonstrate that PR can be overwhelmed by rapidly growing bacteria that exceed the system’s inactivation capacity. Bacterial spores are relatively resistant to inactivation and are a particular risk. Therefore, best-practice use of a PR system will require undertaking treatment within a defined period as defined by the manufacturer after collection to prevent potential growth beyond the capability of the system. The converse is true for bacterial screening, in which sampling is best performed as late as possible to maximize detection, allowing bacteria time to grow to sufficient numbers. Nevertheless, for the amotosalen/UVA treatment system, the manufacturer-defined specifications take into account efficacy within a defined treatment window, allowing for the possibility of bacterial growth between collection and PR treatment. Based on the cumulative HV surveillance data, the amotosalen/UVA system appears to be at least as (and potentially more) effective than the DLVBC system to prevent sepsis. In addition, amotosalen/UVA treatment provides protection against viruses and protozoa; it can replace other procedures, such as gamma irradiation; and treated platelets may be released into inventory 1 or 2 days earlier than with DLVBC, allowing for the transfusion of fresher platelets.

ACKNOWLEDGMENTS

We thank Drs Jose Coene and Veerle Campernolle for review of the Belgian data, Dr Susan Brailsford and Dr Carl McDonald of the NHSBT for comments on the article, and Jin-Sying Lin for statistical analysis. We also thank Nidhi Patel for microbiology support with Case S4 (available as supporting information in the online version of this paper).

CONFLICT OF INTEREST

RJB and LC are employees of Cerus Corporation. TB and TW declare no conflicts of interest.

REFERENCES

1. Benjamin RJ, McDonald CP. The international experience of bacterial screen testing of platelet components with an automated microbial detection system: a need for consensus testing and reporting guidelines. Transfus Med Rev 2014;28:61-71.
2. Stormer M, Vollmer T. Diagnostic methods for platelet bacteria screening: current status and developments. Transfus Med Hemother 2014;41:19-27.
3. Heaton WA, Good CE, Galloway-Haskins R, Yomtovian RA, Jacobs MR. Evaluation of a rapid colorimetric assay for detection of bacterial contamination in apheresis and pooled random-donor platelet units. Transfusion 2014;54:1634-41.
4. Brecher ME, Jacobs MR, Katz LM, et al. Survey of methods used to detect bacterial contamination of platelet products in the United States in 2011. Transfusion 2013;53:911-8.
5. Jacobs MR, Smith D, Heaton WA, Zantek ND, Good CE; PGD Study Group. Detection of bacterial contamination in prestorage culture-negative apheresis platelets on day of issue with the Pan Genera Detection test. Transfusion 2011;51:2573-82.
6. Allain JP, Owusu-Ofori AK, Assennato SM, Marschner S, Goodrich RP, Owusu-Ofori S. Effect of Plasmodium inactivation in whole blood on the incidence of blood transfusion-transmitted malaria in endemic regions: the African Investigation of the Mirasol System (AIMS) randomised controlled trial. Lancet 2016;387:1753-61.
7. Kaplan A, Lindgren B, Marschner S, et al. Evaluation of the post-transfusion platelet increment and safety of riboflavin-based pathogen reduction technology (PRT) treated platelet products stored in platelet additive solution for 5 days or less versus 6-7 days. Transfus Apher Sci 2016;54:248-52.
8. Mirasol Clinical Evaluation Study Group. A randomized controlled clinical trial evaluating the performance and safety of platelets treated with MIRASOL, pathogen reduction technology. Transfusion 2016;50:2362-75.
9. Rebulla P, Vaglio S, Beccaria F, et al. Clinical effectiveness of platelets in additive solution treated with two commercial pathogen-reduction technologies. Transfusion 2017;57:1171-83.
10. McCullough J, Vesole DH, Benjamin RJ, et al. Therapeutic efficacy and safety of platelets treated with a photochemical process for pathogen inactivation: the SPRINT Trial. Blood 2004;104:1534-41.
11. Knutson F, Osselaer J, Pierelli L, et al. A prospective, active haemovigilance study with combined cohort analysis of 19 175 transfusions of platelet components prepared with amotosalen-UVA photochemical treatment. Vox Sang 2015;109:343-52.
12. Corash L, Benjamin RJ. The role of hemovigilance and postmarketing studies when introducing innovation into transfusion medicine practice: the amotosalen-ultraviolet A pathogen reduction treatment model. Transfusion 2016;56Suppl 1:S29-38.
13. McDonald CP, Allen J, Pitt T, et al. Evaluation of the Mirasol pathogen inactivation system as an alternative to bacterial screening of platelet components. Vox Sang 2016;111(Suppl 1). Abstract 3A-S05-03.24.
14. Allen J, Pitt T, Aplin K, Ball J, et al. Evaluation of the Cerus INTERCEPT pathogen inactivation system as an alternative to bacterial screening of platelet components. Vox Sang 2016;111(Suppl 1). Abstract 3A-S05-04.25.
Rasonglè P, Angelini-Tibert MF, Simon P, et al. 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.

Eder AF, Kennedy JM, Dy BA, et al. Limiting and detecting bacterial contamination of platelets: an analysis with slow growth in contaminated units mandate an alternative approach to product safety. Transfusion 2016;56:1320-8.

Cap AP. Storage of platelets at 4°C prevents aggregate formation and preserves platelet functional responses. Transfusion 2016;56:1320-8.

Wagner SJ, Moroff G, Katz AJ, Friedman L. Comparison of bacteria growth in single and pooled platelet concentrates after deliberate inoculation and storage. Transfusion 1995;35:298-302.

Wagner SJ, Robinette D. Evaluation of an automated microbiologic blood culture device for detection of bacteria in platelet components. Transfusion 1998;38:674-9.

Benjamin RJ, Wagner SJ. The residual risk of sepsis: modeling the effect of concentration on bacterial detection in two-bottle culture systems and an estimation of false-negative culture rates. Transfusion 2007;47:1381-9.

Brecher ME, Means N, Jere CS, Heath D, Rothernberg S, Stutzman LC. Evaluation of an automated culture system for detecting bacterial contamination of platelets: an analysis with 15 contaminating organisms. Transfusion 2001;41:477-82.

Eder AF, Kennedy JM, Dy BA, et al. Limiting and detecting bacterial contamination of apheresis platelets: inlet-line diversion and increased culture volume improve component safety. Transfusion 2009;49:1554-63.
43. Lin L, Dikerman R, Molini B, et al. Photochemical treatment of platelet concentrates with amotosalen and long-wavelength ultraviolet light inactivates a broad spectrum of pathogenic bacteria. Transfusion 2004;44:1496-504.

44. Benjamin RJ, Dy B, Perez J, Eder AF, Wagner SJ. Bacterial culture of apheresis platelets: a mathematical model of the residual rate of contamination based on unconfirmed positive results. Vox Sang 2014;106:23-30.

45. Kamel H, Townsend M, Bravo M, Vassallo RR. Improved yield of minimal proportional sample volume platelet bacterial culture. Transfusion 2017;57:2413-9.

46. Hong H, Xiao W, Lazarus HM, God CE, Maitta RW, Jacobs MR. Detection of septic transfusion reactions to platelet transfusions by active and passive surveillance. Blood 2016;127:496-502.

47. Benjamin RJ. Transfusion-related sepsis: a silent epidemic. Blood 2016;127:380-1.

48. Lamy B, Darge S, Arendrup MC, Parienti JJ, Tattevin P. How to optimize the use of blood cultures for the diagnosis of bloodstream infections? A state-of-the-art. Front Microbiol 2016;7:697.

49. Wagner SJ, Benjamin RJ, Hapip CA, et al. Investigation of bacterial inactivation in apheresis platelets with 24 or 30 hours between inoculation and inactivation. Vox Sang 2016;111:226-34.

50. Benjamin RJ, Wagner SJ. Bacterial pathogen reduction requires validation under conditions of intended use. Transfusion 2015;55:2060-3.

51. Schmidt M, Hourfar MK, Sireis W, et al. Evaluation of the effectiveness of a pathogen inactivation technology against clinically relevant transfusion-transmitted bacterial strains. Transfusion 2015;55:2104-12.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher’s website.

Case S1: United Kingdom. In a case ruled as undetermined in 2011,23 bacteria were isolated from the implicated PC: A child received an apheresis PC; the patient’s blood pressure, pulse and temperature decreased; and breathlessness, nausea/vomiting, and a rash developed. The patient was not on and did not receive antibiotics, and no blood cultures were taken. The empty PC was returned to the blood service with one leaking port, washed with saline, and Lactococcus lactis ssp. lactis was isolated. No confirmatory tests could be performed. The patient received transfusion of a component with no adverse reaction. The case was considered “undetermined” due to a lack of sufficient information to rule out environmental contamination.

Case S2: United Kingdom. In 2015, a 6-day-old, whole blood-derived, pooled PC was transfused to a neutropenic woman age approximately 70 years with acute myeloid leukemia.23 Fifteen minutes into the transfusion, the patient became agitated, experienced rigors and tachycardia, and her temperature spiked to 38.7°C then rose overnight to 40°C. The transfusion was stopped, and the patient was given hydrocortisone, chlorphenamine, and antibiotics. She recovered and was discharged. The bacterial culture sample obtained during manufacture was negative at Day 7, and investigation revealed no procedural errors. The same molecular strain of S. aureus was isolated from patient blood cultures, residual PC pack cultures, and donor skin swabs. This case was interpreted as a confirmed STR and was reported separately by McDonald and colleagues.18

Case S3: United Kingdom. In 2015, a 50-year-old female outpatient received transfusion of a 7-day-old, whole blood-derived, pooled PC for aplastic anemia.23 The patient routinely received pretransfusion prophylaxis with hydrocortisone and chlorphenamine. Halfway through the transfusion, the patient developed rigors and angioedema with stable blood pressure. The patient was admitted, treated with antibiotics and steroids, and recovered. Bacterial culture of the PC during manufacture was negative, and no procedural errors were detected. The hospital reported that Streptococci spp. was identified from the patient blood culture 24 hours after transfusion. S. agalactiae and E. coli were isolated from the returned PC pack. It was not possible to confirm the PC as the source of the infection, and the case was interpreted as a “possible” STR and reported separately by McDonald and coworkers.18

Case S4: Switzerland. In 2015, an 89-year-old male inpatient received a PC before planned surgery and suffered an acute reaction 35 minutes after initiating transfusion of an amotosalen/UVA-treated apheresis PC. Symptoms included fever (39.5°C), hypertension (158/69 mm Hg), tachycardia (148 beats per minute), shivering, and dyspnea. The patient was treated with antihistamines and steroids, and his symptoms resolved within 6 hours without antibiotics. Blood cultures were taken during the event, and the residual PC bag was sampled 6 hours later and cultured. The patient had a history of high-dose steroid and intravenous immunoglobulin treatment for idiopathic thrombocytopenia and was stable and afebrile before transfusion. Microbiology results received 24 hours later revealed a negative Gram stain of the residual PC but positive cultures for K. pneumoniae from the PC bag and patient blood cultures, with identical antibiotic-resistance profiles. Antibiotics were initiated, and the patient recovered. The investigator reported the case as a probable STR. An assessment by Cerus Corporation raised the possibility of retrograde contamination from the infusion set to the bag during transport to the microbiology department after the component was connected to the infusion set. The event was ruled as “possibly related” to the transfusion.
based on the following findings: The Gram stain on the residual PC was negative 6 hours after the reaction. Gram stain has a sensitivity of greater than $10^5$ colony-forming units per milliliter, and a negative result suggests a low concentration of bacteria. The patient had a pretreatment platelet count of $33 \times 10^9/L$ and a post-transfusion count of $62 \times 10^9/L$. It was considered unlikely that a contaminated PC would result in a robust platelet count increment. The patient was asymptomatic 6 hours after the initial reaction without antibiotic therapy despite documented sepsis, consistent with a pre-existing subclinical infection. There was a 6-hour delay between connection of the infusion set to the PC and culture sampling, allowing growth in the PC after the integrity of the bag was compromised. The time to positive culture of the residual PC was 10.6 hours, and that of the patient’s blood specimen was 9.1 hours, indicating a rapidly growing bacterial strain. Culture of a PC co-component (amotosalen/UVA-treated separately) and an untreated, concurrent plasma component from the same collection were negative for bacteria. Multiple experiments have documented robust inactivation at greater $5.6 \log_{10}$ of $K. pneumoniae$ with amotosalen/UVA treatment, although breakthroughs have been documented at higher concentrations. Amotosalen/UVA treatment was performed 6 hours after PC collection, rendering it unlikely that bacterial growth had exceeded the inactivation capacity. Investigation of the implicated patient and residual component $K. pneumoniae$ strains revealed a modest growth rate under optimal conditions in PC and robust inactivation of greater than $10^8 \log_{10}$ by amotosalen/UVA treatment. Donor and phlebotomist investigations, including throat and rectal swabs and urine and blood cultures, were negative for the implicated strain. Investigation of all donation, manufacturing, and PR processes demonstrated conformance with procedures. 

**Case S5: Switzerland.** The Swiss Red Cross reported an 18-year-old male who received an amotosalen/UVA-treated PC in 2015 associated with transient fever, tachycardia, and dyspnea; and the reporter suspected bacterial sepsis. The patient’s medical history included an infected central venous catheter and bacterial sepsis with repeatedly positive blood cultures. No concomitant medications were reported. The platelet count was $14 \times 10^9/L$ before and $7 \times 10^9/L$ after transfusion. No allergic reaction and normal blood pressure were noted during the reaction. The patient was given hydrocortisone and antihistamine, and he recovered. A bacterial culture from the residual PC was negative; however, Gram-positive cocci were seen by the microbiologists in a smear from the residual PC bag. The patient received transfusion of a second PC co-component without any adverse symptoms. The treating physician evaluated the reaction as a febrile nonhemolytic transfusion reaction classified as Grade 2 by the Swiss reporting system (“possibly related to the transfusion”) because of the positive Gram stain. The blood center reporter assessed the case as “not related” to the transfusion. The possibility that a positive Gram stain with negative culture might represent successful pathogen inactivation of a heavily contaminated PC was not considered at the time. The finding of a mild nonhemolytic transfusion reaction would be consistent with a reaction to bacterial metabolic products and lipopolysaccharide contamination. 

**Case S6: Belgium.** A spontaneous report was received from Belgium in 2016 of a 26-year-old male patient with acute myelogenous leukemia who received an amotosalen/UVA-treated PC. The patient was admitted 9 days earlier for elective placement of a Hickman catheter. Approximately 15 minutes after the start of the PC transfusion, the patient experienced chills and tachycardia, and the transfusion was discontinued. The patient’s body temperature was 37.8°C during the reaction. Blood pressure was 134/68 mm Hg before and 136/86 mm Hg during the reaction. The heart rate was 98 per minute before and 109 per minute after the reaction. The platelet count before transfusion was $14 \times 10^9/L$, and it was $18 \times 10^9/L$ after transfusion. The patient had no evidence of a prior infection but was under antibiotic treatment. A sample of the patient’s blood taken through the catheter 12 hours previously because of fever was culture negative. A second sample taken during the reaction from a dual-lumen catheter was positive for $S. haemolyticus$ from the white lumen but negative for the blue lumen. Residual PC samples taken for culture during the STR were positive for $S. haemolyticus$ and had the same antibiotic-resistance profile as the catheter sample. The patient was treated with antibiotics and recovered. Cultures of the $S. hemolyticus$ strain were not available for further evaluation, and an attempt to re-culture the refrigerated PC bag 14 days later failed. Investigation of the donors and the manufacturing procedures was unremarkable. Amotosalen/UVA treatment had been performed 20 hours after whole-blood collection, within the manufacturers’ specifications. A subsequent evaluation by Cerus Corporation demonstrated robust inactivation of two other strains of $S. hemolyticus$ in PCs containing greater than $10^6$ colony-forming units per milliliter. The hospital physician assessed the event as “probably” related to the PC transfusion. Cerus Corporation assessment noted the robust inactivation capacity for $S. hemolyticus$ strains and the likelihood of a line infection with retrograde contamination from the patient. Lacking further information, such as a Gram stain of the residual PC or the bacterial isolates for further testing, the reaction was considered “possibly” related.