医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2008. 11. 20	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社)		Lessa F, Leparc GF, Benson K, Sanderson R, Van Beneden CA, Shewmaker PL, Jensen B, Arduino MJ, Kuehnert MJ. Transfusion. 2008 Oct;48(10):2177-83.	米国

研究報告の概要 121

○ルーチンの細菌培養スクリーニングの実施にもかかわらず、細菌に汚染されたプール血小板の輸血が原因となったC群連鎖球菌感染死亡症例

背景:慢性骨髄単球性白血病の高齢男性が、全血8本から製造したプール血小板(PLT)の輸血後48時間以内に呼吸困難を発現し死亡した。当該受血者の血液及びバッグに残存したプールPLTの培養でC群連鎖球菌(GCS)が生育したため、感染源と検査が偽陰性となった原因を調査した。

試験デザインおよび方法:関連した8本の赤血球(RBC)の培養を行い、また、関連供血者の検体を入手した。16SのrRNAとパルスフィールドゲル電気泳動(PFGE)により分離株を特定した。血液センターのスクリーニング方法についても調査した。

結果:死亡した男性とRBC8本のうち1本から培養されたベータ溶血性GCSが一致した。供血から20日後に採取した当該供血者の咽頭スワブはGCS陽性であり、*Streptococcus dysgalactiae* subsp. *equisimilis*と同定された。受血者、RBC、残存PLTと供血者咽頭スワブの分離菌はPFGEで区別できなかった。供血者は供血前後の症状や感染について否定した。血液センターのPLT細菌スクリーニングは、検出限界が1バッグ当たり15 CFUの市販の細菌検出システム(BacT/ALERT, bioMérieux)を使用して行われていた。

結論:PLTのGCS汚染原因として、無症候の供血者の関与が示唆された。現在の検査法は、すべての細菌汚染を検出するのに十分ではなく、特に培養量が制限されるプールPLTでは難しい。PLTの細菌汚染検出の向上が求められる。

報告企業の意見	今後の対応
ルーチンの細菌培養スクリーニングを実施したプール血小板の輸血を受けた患者が、呼吸困難を発症、死亡し、患者血液、製剤及び無症候の供血者からC群連鎖球菌が検出されたとの報告である。	日本赤十字社では、輸血による細菌感染予防対策として、すべての輸血用血液製剤を対象に保存前白血球除去及び初流血除去を導入している。さらに、輸血情報リーフレット等により細菌感染やウイルス感染について医療機関へ情報提供し注意を喚起しているほか、細菌感染が疑われる場合の対応を周知している。今後も細菌やウイルスの検出や不活化する方策について情報の収集に努める。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

TRANSFUSION COMPLICATION

Fatal group C streptococcal infection due to transfusion of a bacterially contaminated pooled platelet unit despite routine bacterial culture screening

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BACKGROUND: An elderly man with chronic myelomonocytic leukemia developed respiratory distress and died less than 48 hours after transfusion of a pool of eight whole blood–derived platelets (PLTs). Blood cultures from the recipient and cultures of remnants from the pooled PLT bag grew group C streptococci (GCS). An investigation was conducted to identify both the infection's source and the reasons for the false-negative screening result.

STUDY DESIGN AND METHODS: Red blood cell (RBC) units (cocomponent from the eight donations) were traced, quarantined, and cultured. Specimens from the implicated donor were obtained. Isolates were identified and typed by 16S rRNA and pulsed-field gel electrophoresis (PFGE). The blood center screening method was reviewed.

RESULTS: β-Hemolytic GCS, cultured from 1 of 8 RBC units, linked the fatal case to a single donor. The donor's throat swab collected 20 days after donation was positive for the presence of GCS, identified as *Streptococcus dysgalactiae* subsp. *equisimilis*. Isolates from the recipient, RBC unit, residual PLTs, and donor's throat swab were indistinguishable by PFGE. The donor denied any symptoms of infection before or after donation. PLT bacterial screening at the blood center was performed using a commercially available bacterial detection system (BacT/ALERT, bioMérieux) with a threshold of 15 colony-forming units per bag.

CONCLUSION: An asymptomatic donor was implicated as the source of GCS-contaminated PLTs. Current screening methods for PLTs are not sufficient to detect all bacterial contamination. Pooled PLTs are a particular challenge because the small volume of individual units places limits on culturing strategies. Improved detection of bacterial contamination of PLTs is needed.

Bacterial infection due to transfusion of contaminated platelet (PLT) components is an important patient safety concern.[1,2] Before the adoption of a standard requiring blood collection and transfusion service members to limit and detect bacterial contamination in all PLT components by AABB in 2004,[3] the estimated rate of bacterial contamination of PLT products ranged from 1 in 2000 to 1 in 3000 PLT units,[4] although the frequency of recognized sepsis from these products is much lower. Not all bacterially contaminated PLT units will result in a clinically recognized septic reaction; thus, the estimated rate of transfusion-related sepsis (1 in 100,000 units) for pooled PLTs before 2004 is likely to represent a substantial underestimation of the

ABBREVIATIONS: GCS = group C streptococci; PFGE = pulsed-field gel electrophoresis; WB = whole blood.

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This investigation was supported by the Office of Workforce and Career Development, Centers for Disease Control and Prevention.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Department of Health and Human Services.

Received for publication February 26, 2008; revision received March 31, 2008, and accepted April 2, 2008.

doi: 10.1111/j.1537-2995.2008.01802.x

TRANSFUSION 2008;48:2177-2183.

problem.[5] Implementation of routine bacterial screening by blood centers represents an important advance toward ensuring the safety of PLT components. It does not, however, eliminate the risk of transfusion-related sepsis and death.[2,6,7] Current bacterial screening methods for PLTs have different levels of sensitivity, and none of them is likely to detect all pathogens.[8] Although culture is considered one of the best bacterial screening methods available, false-negative culture results can occur that lead to transfusion of bacterially contaminated blood components.[7]

Bacteria that contaminate blood products may originate from donor skin flora, from donor asymptomatic bacteremia, or from contamination during blood processing.[9-11] Most pathogens reported as causes of transfusion-related sepsis are organisms associated with skin contaminants,[2,3,7] suggesting that contamination is more likely to occur at the time of collection.

In this article, we report a PLT unit with a false-negative bacterial detection screening result. The event resulted in the death of the recipient by an unusual organism not previously associated with transfusion-related sepsis. An investigation was conducted to determine both the source of PLT contamination and the reasons for the false-negative screening result.

CASE REPORT

In April 2007, public health officials at the Florida Department of Health were notified of a fatal group C streptococcal infection after blood transfusion. The Centers for Disease Control and Prevention (CDC) was invited to assist in the investigation and the Food and Drug Administration (FDA) was notified of the potential transfusion-associated fatality. The patient was a 67-year-old man with refractory leukemia who received a pool of eight whole blood (WB)-derived PLTs. The patient was diagnosed with chronic myelomonocytic leukemia in April 2006. He never responded to chemotherapy treatments and required frequent transfusions. On April 16, 2007, when the patient presented to an outpatient infusion center to receive a PLT transfusion, his PLT count was 5×10^9 per L and he had no symptoms of infection. He received a pool of 8 (instead of the usual 8) WB-derived PLT units because of his previous history of poor response to PLT transfusions. No medication was given before transfusion. The PLT units were screened for bacteria using blood culture media (BacT/ALERT bottle, bioMérieux, Durham, NC), and no growth was observed after 5 days of incubation in the instrument.

At the end of the transfusion, the patient had chills for which a narcotic analgesic was administered. One hour after the transfusion was completed the patient became tachycardic, hypotensive, and hypoxic. The patient was transferred to the intensive care unit where his clinical status rapidly deteriorated, requiring ventilatory support and vasopressor agents. Sepsis was suspected, and broad-spectrum antibiotics were begun after blood cultures were collected. The following day, the patient's condition continued to worsen. He died less than 48 hours after the transfusion.

The patient's blood cultures were positive 1 day after the collection and showed Gram-positive cocci in pairs and in short chains, later identified as group C streptococci (GCS). Because the patient had onset of his illness soon after receiving PLTs, transfusion-related bacterial infection was suspected and an investigation was initiated.

MATERIAL AND METHODS

Culturing of blood components

The patient received PLTs derived from units of WB from eight different donors pooled by the hospital just before the time of transfusion. Seven of the 8 WB-derived PLT units were 3 days old, and 1 was 4 days old at the time of transfusion. Cultures of remnants from the pooled bag and of residual PLTs from each of the eight 50-mL individual-donor PLT bags were obtained. Cultures were performed at the hospital microbiology laboratory using the bacterial detection system (BacT/ALERT) for the recipient's blood and both chocolate agar plate and non-automated broth culture for residual PLTs and remnants of the pooled bag.

Cocomponents from each of the eight donations, including red blood cells (RBCs) and fresh-frozen plasma, were traced and quarantined. An 8-mL sample from each of the 8 RBC units was obtained and cultured by the blood center in the bacterial detection system.

Donor investigation and culturing

The implicated donor was interviewed, and specimens for culturing were collected including blood and swabs from throat, nose, antecubital skin, and perineal areas.

Isolate characterization

Isolates were submitted to the CDC for identification and typing. The isolates were characterized phenotypically using a conventional biochemical identification scheme and a rapid identification system (Rapid ID 32 Strep system, bioMérieux).[12-14] Comparative 16S rRNA gene sequencing[15] and pulsed-field gel electrophoresis (PFGE) analysis were performed as previously described.[16,17] PFGE patterns were analyzed with computer software (Bionumerics, Applied Maths, Inc., Austin, TX). A dendrogram was generated using unweighted pair group with arithmetic means and the Dice coefficient with a position tolerance of 1.25 percent and an optimization of 0.5 percent.

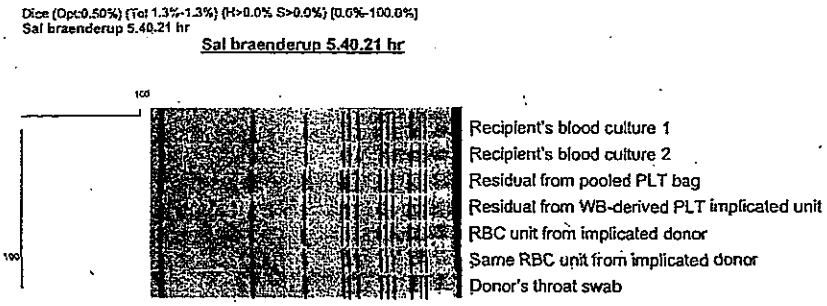

Fig. 1. PFGE and dendrogram of *S. dysgalactiae* subsp. *equisimilis* isolates recovered from the recipient's blood, a pooled PLT bag, an individual PLT unit, and the donor's RBC unit and throat swab, Florida, 2007.

Blood center screening method and validation test

Because the WB-derived PLT units used to make the pool were screened for bacterial contamination before being released, the screening method and the quality control (QC) test validation were reviewed.

RESULTS

Culturing of blood components

Cultures of the remnants from the pooled bag and of the residual PLTs from four of the eight individual PLT bags grew Gram-positive cocci later identified as GCS. The remaining RBC units, cocomponents of the eight WB-derived PLTs, were still available at the blood center and one of these RBC units also grew GCS. The presence of GCS in one RBC cocomponent, in addition to the WB-derived PTL units, allowed the event to be linked to a single donor.

Donor investigation and culturing

The implicated blood donor was a healthy 18-year-old girl with no history of illness in the 2 weeks before or since donation. She denied exposure to any sick people before donation and reported living with her parents, both of whom were apparently healthy. She had a history of four prior WB donations in the previous 19 months. In two of these four prior donations, WB-derived PLT was prepared, cultured negative, and transfused uneventfully. Culture of the donor's throat swab taken approximately 20 days after donation was positive for the presence of GCS. All other cultures from the donor failed to demonstrate GCS growth.

CDC laboratory results

The β-hemolytic *Streptococcus* specimen isolated from recipient's blood, remnants from the pooled bag, RBC unit, and donor's throat swab were confirmed to possess the Lancefield group C antigen. The conventional biochemical test reactions and the rapid identification system results were identical for all isolates, and the isolates were identified as *Streptococcus dysgalactiae* subsp. *equisimilis*. The 16S rRNA sequences were identical for all strains. Comparative 16S rRNA sequence analysis with reference strain 16S sequences in the CDC *Streptococcus* database showed the highest similarity (99.86%) to *S. dysgalactiae* subsp. *equisimilis*. PFGE analysis revealed that all isolates were indistinguishable (Fig. 1).

Review of blood center screening method and validation test

The following methods describe the procedure for blood donation preparation and PLT culture screening performed at the blood center. Before blood collection, the antecubital area is scrubbed for 30 seconds using a single-use applicator with a solution of 2 percent (wt/vol) chlorhexidine gluconate and 70 percent (vol/vol) isopropyl alcohol (ChloraPrep, Enturia, Inc., Leawood, KS). Blood collection is then performed using a single-use blood collection kit (Fenwal, Chicago, IL).

After the separation of PLTs from PLT-rich plasma, units are rested at room temperature (e.g., 20-24°C) for 2 hours. The units have an integrally attached tubing segment 9 to 12 inches in length. After the resting period is completed, the attached tubing segment containing between 1.6 and 2.4 mL of PLT-rich plasma is stripped and refilled three times to ensure that the tubing is filled with PLT-rich plasma that is representative of the content of the bag. The segments are then sealed and labeled with the corresponding unit number, cut, and placed in an incubator at 37°C for 24 hours. This subsequent incubation is performed to accelerate the bacterial growth in the segments as demonstrated previously.[18] At the completion of the incubation time, the segments are welded to a sampling harness using a sterile connecting device (TSCD, Terumo Medical Corp., Sommerset, NJ). The content of up to six segments is drawn from the segments using the syringe in the harness (Fig. 2). The syringe content is then inoculated into a single aerobic blood culture bottle (BacT/ALERT), and the bottle is incubated for 5 days for bacterial growth. PLT units are released if no growth is detected after 12 hours of incubation in the culture bottle. A final interpretation on the culture bottle is made after 5 days of incubation at 37°C.

The test for the detection of bacterial contamination was validated by spiking studies using pellets with standardized concentration (EZ-CFU, MicroBiologics, St Cloud, MN) of *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 8739), and *Staphylococcus*

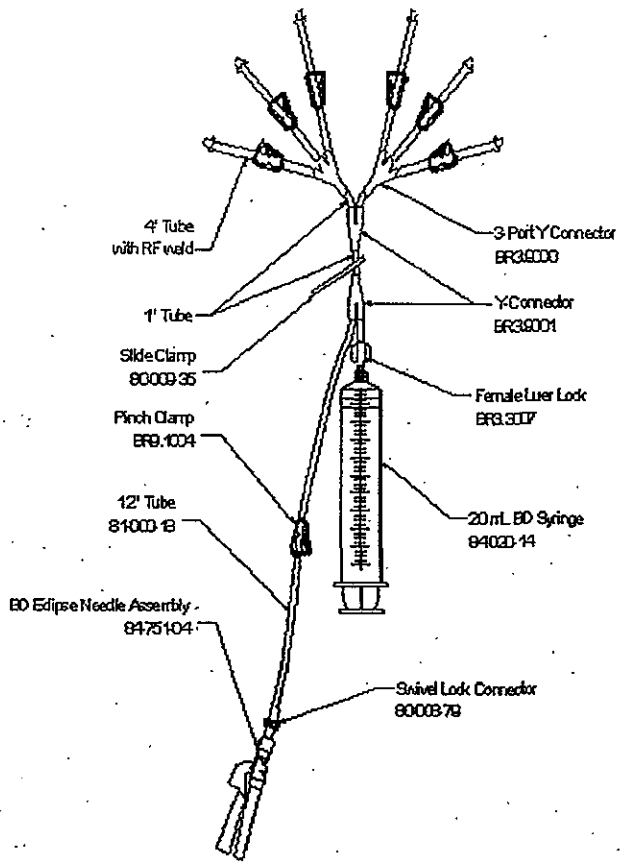

Fig. 2. Procedure for pooled PLT culture screening performed at the blood center. The syringe in the harness is used to draw the contents of up to six tubing segments containing PLT-rich plasma.

aureus (ATCC 6538).[19] During these validation tests, the detection limit for bacterial contamination was shown to be approximately 15 colony-forming units (CFUs) per bag.

DISCUSSION

This is the first reported case of infection and death due to transfusion of GCS-contaminated PLTs. β-Hemolytic GCS are pathogenic to humans and other mammals.[20-23] Although lesser known than groups A and B streptococci, both group C and group G streptococci are part of skin, oral cavity, nasopharynx, gastrointestinal, and vaginal normal flora.[24,25] Invasive infections due to GCS have been increasingly recognized,[21,22] likely due to improvement in diagnostic laboratory techniques and improved reporting. The most common species of GCS isolated in human infections is *S. dysgalactiae* subsp. *equisimilis*.[22,23,26] Outbreaks of pharyngitis by GCS have been reported, especially among college students;[23,27] invasive infection by these microorganisms in otherwise healthy people is less common and includes skin and soft tissue infections (e.g., cellulitis, erysipelas), septic arthritis, abscesses, osteomyelitis, infective endocarditis, and bacteremia. Population-based surveillance for streptococcal infections in Denmark and Canada have shown that the incidence of invasive GCS infection ranges from 0.4 to 0.5 per 100,000 inhabitants per year, with a higher prevalence in persons older than 60 years of age or with underlying conditions.[28,29]

This fatal transfusion reaction associated with a false-negative screening test highlights the residual risk of sepsis and death from PLT units screened for bacterial contamination. Several factors could explain the reason for the negative culture result after 5 days of incubation in the blood culture bottle (BacT/ALERT): 1) the sampling process may have been inadequate and too little volume from individual WB-derived PLT bags was available in the tubing segments, resulting in no viable organisms in the culture bottle; 2) insufficient volume from the syringe may have been inoculated into a single aerobic culture bottle;[7,30] or 3) the bacterial load of PLT unit at the time of testing was below the detection limit of the blood center screening process (i.e., 15 CFUs/bag).

Although GCS also has been reported as a skin contaminant,[25,28] introduction through phlebotomy is less likely due to the aseptic processes used for venipuncture. Contamination of the PLT unit was probably due to bacteremia in the donor, although she had no clinical manifestations of skin or pharyngeal disease when evaluated. The implicated donor, an apparently healthy young girl, likely developed transient asymptomatic bacteremia due to the presence of GCS in her oral cavity at the time of donation. As a result of the investigation, this donor has been indefinitely deferred for blood donation.

GCS was also isolated from three other PLT units besides the implicated donor's unit. A probable explanation for this is that if the fifth PLT unit pooled in the bag by the hospital was from the implicated donor, this unit may have contaminated the port of the pooling bag, subsequently contaminating PLT Units 6, 7, and 8.

Persistence of bacterial growth in contaminated PLT components occurs due to the relatively warm storage temperature of PLT units. At 20 to 24°C, a small bacterial inoculum can grow quickly, resulting in a large number of organisms in the PLT unit by the time of transfusion. Because this rapid bacterial growth occurs under normal PLT storage conditions, older units (≥5-day storage) are more likely to have higher bacterial load than younger units (≤5-day storage). Because of this phenomenon, FDA mandates that the storage period of WB-derived PLT units cannot be longer than 5 days. More septic reactions including fatalities have been reported with older PLT units.[7] Interestingly, the fatal GCS case reported in this article was caused by a PLT unit transfused on Day 3 after collection, suggesting a very rapid bacterial growth during storage and hence a high bacterial load in this recently