別紙様式第2－1

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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 2009年4月10日	新医薬品等の区分 該当なし		総合機構処理欄
一般的名称	別紙のとおり		研究報告の公表状況	第82回日本細菌学会総会 (2009年3月12日～14日)		公表国 日本	
販売名(企業名)	別紙のとおり						

研究報告の概要

問題点：retrospectiveな解析により、マダニ媒介性の新興感染症である「アナプラズマ症」のヒト感染症例が日本で初めて確認された。

「アナプラズマ症」は、1994年に米国で初めて確認されたマダニ媒介性の新興感染症で、その病原体はリケッチア目に分類される*Anaplasma phagocytophilum*である。本菌は、ヒトの顆粒球に特異的に感染して、発熱を伴ったリケッチア症様の疾患を引き起こす。我が国では、これまで「アナプラズマ症」のヒト感染症例は確認されていなかった。今回、*A. phagocytophilum*の感染が疑われる発熱性疾患患者を見出したので報告する。2002年～2003年に高知県で発生した発熱性疾患患者において、「日本紅斑熱」が疑われた18名の患者の血餅からDNAを抽出し、*A. phagocytophilum*に特異的な*p44/msp2*外膜蛋白遺伝子群を標的としたNested PCRを行った。その結果、2名の患者から*p44/msp2*遺伝子群のPCR産物が検出された。その後、得られた増幅産物をTAクローニングし、無差別にそれぞれ27個と40個の組換え体を選出して、塩基配列を決定し系統樹解析を行った。その結果、得られた*p44/msp2*クローンはそれぞれの患者に特異的なクラスターを形成することが判った。また、2名の患者のうちの1名は、「日本紅斑熱」起因細菌である*Rickettsia japonica*の16SrDNAもPCRにより増幅されたことから、この1名は、*A. phagocytophilum*と*R. japonica*の混合感染であることが判明した。以上、今回のretrospectiveな解析により、日本国内にも*A. phagocytophilum*感染による「アナプラズマ症」の存在が強く示唆された。よって、今後は、大規模な患者探索が望まれる。

使用上の注意記載状況・その他参考事項等

記載なし

報告企業の意見	今後の対応
別紙のとおり	今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。

MedDRA/J ver.12.0

別紙

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤乾燥ペプシン処理人免疫グロブリン、⑥乾燥スルホ化人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン*、⑧乾燥濃縮人活性化プロテインC、⑨乾燥濃縮人血液凝固第Ⅷ因子、⑩乾燥濃縮人血液凝固第Ⅸ因子、⑪乾燥抗破傷風人免疫グロブリン、⑫抗HBs人免疫グロブリン、⑬トロンビン、⑭フィブリノゲン加第XⅢ因子、⑮乾燥濃縮人アンチトロンビンⅢ、⑯ヒスタミン加人免疫グロブリン製剤、⑰人血清アルブミン*、⑱人血清アルブミン*、⑲乾燥ペプシン処理人免役グロブリン*、⑳乾燥人血液凝固第Ⅸ因子複合体*、㉑乾燥濃縮人アンチトロンビンⅢ
販売名(企業名)	①献血アルブミン20"化血研"、②献血アルブミン25"化血研"、③人血清アルブミン"化血研"*、④"化血研"ガンマーグロブリン、⑤献血静注グロブリン"化血研"、⑥献血ベニロン－Ｉ、⑦ベニロン*、⑧注射用アナクトC2、500単位、⑨コンファクトF、⑩ノバクトM、⑪テタノセーラ、⑫ヘパトセーラ、⑬トロンビン"化血研"、⑭ボルヒール、⑮アンスロビンP、⑯ヒスタグロビン、⑰アルブミン20%化血研*、⑱アルブミン5%化血研*、⑲静注グロブリン*、⑳ノバクトF*、㉑アンスロビンP1500注射用
報告企業の意見	アナプラズマ症はマダニにより媒介される発熱性疾患で、その病原体は顆粒球に特異的に感染する0.2～2μmの大きさの球状もしくは楕円状の偏性寄生性のグラム陰性桿菌である。1994年、米国で発熱性疾患患者の好中球の中にエーリキア様細菌の感染が認められ、ヒト顆粒球エーリキア症病原体〔Human Granulocytic Ehrlichiosis (HGE) agent〕と呼ばれるようになった。その後、1996年にはその病原体が分離報告され、さらに2001年にはEhrlichia属からAnaplasma属へと配置換えされて、*Anaplasma phagocytophilum*という学名が付された。それに伴って、昨今ではその病名もヒト顆粒球アナプラズマ症［Human Granulocytic Anaplasmosis (HGA)］と呼ばれている。*A. phagocytophilum*は、ヒトの他、ウマやヒツジなどにも感染し、アナプラズマ症を引き起こすことから「人獣共通感染症」病原体としても知られている。(http://idsc.nih.go.jp/iasr/27/312/dj312d.html) *A. phagocytophilum*によるアナプラズマ症の発生は欧米が中心であるが、2006年に日本においても*A. phagocytophilum*がマダニから検出されたことが初めて報告された。 弊所で製造している全ての血漿分画製剤の製造工程には、約0.2μmの「無菌ろ過工程」および、*A. phagocytophilum*よりも小さいウイルスの除去を目的とした平均孔径19nm以下の「ウイルス除去膜ろ過工程」が導入されているので、仮に製造原料に*A. phagocytophilum*が混入していたとしても、これらの工程により除去されるものと考えられる。更に、これまでに本剤によるアナプラズマ症感染の報告例は無い。 以上の点から、本剤はアナプラズマ症感染に対して一定の安全性を確保していると考えるが、今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。

*現在製造を行っていない

P2-182　国内初の新興感染症「アナプラズマ症」について

○大橋 典男[1]，烏 日図[1]，高 娃[1]，川森 文彦[1,2]，高野 愛[3,4]，川端 寛樹[3,4]，安藤 秀二[5]，岸本 壽男[6]（静岡県大・食品栄養科学・微生物[1]，静岡県環衛研・微生物[2]，岐阜大学院・連合獣医[3]，国立感染研・細菌一[4]，国立感染研・ウー[5]）

「アナプラズマ症」は，1994 年に米国で初めて確認されたマダニ媒介性の新興感染症で，その病原体はリケッチア目に分類される *Anaplasma phagocytophilum* である。本菌は，ヒトの顆粒球に特異的に感染して，発熱を伴ったリケッチア症様の疾患を引き起こす。我が国では，これまで「アナプラズマ症」のヒト感染症例は確認されていなかった。今回，*A. phagocytophilum* の感染が疑われる発熱性疾患患者を見出したので報告する。2002 年～2003 年に高知県で発生した発熱性疾患患者において，「日本紅斑熱」が疑われた 18 名の患者の血餅から DNA を抽出し，*A. phagocytophilum* に特異的な *p44/msp2* 外膜蛋白遺伝子群を標的とした Nested PCR を行った。その結果，2 名の患者から *p44/msp2* 遺伝子群の PCR 産物が検出された。その後，得られた増幅産物を TA クローニングし，無差別にそれぞれ 27 個と 40 個の組換え体を選出して，塩基配列を決定し系統樹解析を行った。その結果，得られた *p44/msp2* クローンはそれぞれの患者に特異的なクラスターを形成することが判った。また，2 名の患者のうちの 1 名は，「日本紅斑熱」起因細菌である *Rickettsia japonica* の 16S rDNA も PCR により増幅されたことから，この 1 名は *A. phagocytophilum* と *R. japonica* の混合感染であることが判明した。以上，今回の retrospective な解析により，日本国内にも *A. phagocytophilum* 感染による「アナプラズマ症」の存在が強く示唆された。よって，今後は，大規模な患者探索が望まれる。

【非会員共同研究者：千屋誠造（高知衛研），福永和俊（高知衛研），船戸豊彦（室戸病院），浜宇津良治（中芸クリニック），塩尻正明（愛媛県立中央病院），中島秀樹（高知大）】

別紙様式第 2－1

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
				2009 年 2 月 6 日	該当なし	
一般的名称	別紙のとおり		研究報告の公表状況	第 56 回日本ウイルス学会学術集会（2008 年 10 月 27 日）	公表国	
販売名（企業名）	別紙のとおり				日本	

研究報告の概要

問題点：日本国内の前立腺がん患者集団中に XMRV 感染の存在が示唆された。

XMRV（Xenotropic MuLV-related virus）は 2006 年に米国の前立腺がん患者で発見された新規 Gammaretrovirus である。感染している前立腺がん患者の 40%に RNaseL 遺伝子の一定の変異（QQ 変異）が報告されており、自然免疫の一端を担う RNaseL と XMRV 感染の関連が強く示唆されてきた。
日本国内の前立腺がん患者血清及び大阪府赤十字血液センターにおける感染症検査終了後の献血検体血清を用いて、ウェスタンブロット法で抗体の検出を、さらに、前立腺がん患者における抗体陽性血清について nested RT-PCR を行い XMRV 核酸検出を試みた。前立腺がん患者 30 名、献血者 120 名のスクリーニングを行ったところ、Gag に対する特異的抗体反応が前立腺がん患者 2 名、献血者 5 名の血清で認められた。Gag 抗体陽性の前立腺がん患者血清 1 検体よりウイルス核酸を検出した。
献血者及び前立腺がん患者の抗体反応性が HTLV で観察されたような HTLV-Gag-indeterminate pattern の類似現象である可能性を含め、更なる検討を続ける予定である。

使用上の注意記載状況・その他参考事項等

記載なし

報告企業の意見	今後の対応
別紙のとおり	今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。

MedDRA/J ver.11.1

(25)

別紙

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤乾燥ペプシン処理人免疫グロブリン、⑥乾燥スルホ化人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン*、⑧乾燥濃縮人活性化プロテインC、⑨乾燥濃縮人血液凝固第Ⅷ因子、⑩乾燥濃縮人血液凝固第Ⅸ因子、⑪乾燥抗破傷風人免疫グロブリン、⑫抗 HBs 人免疫グロブリン、⑬トロンビン、⑭フィブリノゲン加第ⅩⅢ因子、⑮乾燥濃縮人アンチトロンビンⅢ、⑯ヒスタミン加人免疫グロブリン製剤、⑰人血清アルブミン*、⑱人血清アルブミン*、⑲乾燥ペプシン処理人免役グロブリン*、⑳乾燥人血液凝固第Ⅸ因子複合体*、㉑乾燥濃縮人アンチトロンビンⅢ
販売名(企業名)	①献血アルブミン 20 "化血研"、②献血アルブミン 25 "化血研"、③人血清アルブミン "化血研" *、④ "化血研" ガンマーグロブリン、⑤献血静注グロブリン "化血研"、⑥献血ベニロン－Ⅰ、⑦ベニロン*、⑧注射用アナクトC2,500 単位、⑨コンファクトF、⑩ノバクトM、⑪テタノセーラ、⑫ヘパトセーラ、⑬トロンビン "化血研"、⑭ボルヒール、⑮アンスロビンP、⑯ヒスタグロビン、⑰アルブミン 20%化血研*、⑱アルブミン 5%化血研*、⑲静注グロブリン*、⑳ノバクトF*、㉑アンスロビンP 1500 注射用
報告企業の意見	XMLV が属するガンマレトロウイルス属はレトロウイルス科の１つで、多くの種ががん遺伝子を有し、肉腫や白血病を引き起こす。ガンマレトロウイルス属の代表的ウイルスには、マウス白血病ウイルス（MuLV）がある。ガンマレトロウイルス属ウイルスは、一本のプラス鎖 RNA を核酸として持ち、直径 80～100nm でエンベロープを有している。 本剤の製造工程には、冷エタノール分画工程、ウイルス除去膜ろ過工程あるいは加熱工程等の原理の異なるウイルス除去及び不活化工程が存在しているので、ウイルスクリアランスが期待される。各製造工程のウイルス除去・不活化効果は、「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン（医薬発第 1047 号、平成 11 年 8 月 30 日）」に従い、ウシウイルス性下痢ウイルス（BVDV）、仮性狂犬病ウイルス（PRV）、ブタパルボウイルス（PPV）、A 型肝炎ウイルス（HAV）または脳心筋炎ウイルス（EMCV）をモデルウイルスとして、ウイルスプロセスバリデーションを実施し、評価を行っている。今回報告した XMLV は、エンベロープの有無、核酸の種類等からモデルウイルスとしては BVDV が該当すると考えられるが、上記バリデーションの結果から、本剤の製造工程がこれらのウイルスの除去・不活化効果を有することを確認している。また、これまでに本剤による XMLV の感染の報告例は無い。 以上の点から、本剤は XMLV に対する安全性を確保していると考える。

*現在製造を行っていない

JNT 2008-010

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献血者ならびに前立腺がん患者における新規ヒトレトロウイルス XMRV に対する血清学的解析

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【目的と意義】
XMRV は 2006 年に米国の前立腺がん患者で発見された新規 Gammaretrovirus である。感染している前立腺がん患者の 40%に RNaseL 遺伝子の一定の変異 (QQ 変異) が報告されており、自然免疫の一端を担う RNaseL と XMRV 感染の関連が強く示唆されてきた。本研究では、日本の前立腺がん患者および献血者における XMRV 感染の有無を把握し、血液事業に対する影響を評価するとともに、前立腺がんと XMRV 感染の関連を追跡することを目的とする。
【材料と方法】
XMRV プラスミドクローン VP62 を 293T 細胞にトランスフェクションし、培養上清中に放出されたウイルスを不活化したのち、スクリーニング用抗原とした。ELISA 法でのバックグラウンドが高かったため、スクリーニングはウエスタンブロット法で行った。被検体は（1）インフォームドコンセントを得た前立腺がん患者血清、および（2）大阪府赤十字血液センターにおける感染症検査終了後の献血検体血清を用いた。さらに、前立腺がん患者における抗体陽性血清について nested RT-PCR を行い XMRV 核酸検出を試みた。
【結果】
これまでに、前立腺がん患者 30 名、献血者 120 名のスクリーニングを行ったところ、Gag に対する特異的抗体反応が前立腺がん患者２名、献血者５名の血清で認められた。Gag 抗体陽性の前立腺がん患者血清１検体よりウイルス核酸を検出した。
【考察】
日本国内の前立腺がん患者集団中に XMRV ウイルス感染の存在が示唆された。用いたウイルス抗原の Env に対する反応性が見られなかった原因として Env 量が極めて少ない、もしくは用いたクローンの Env とは交差反応しない可能性があり、現在これらの判定を進めている。献血者および前立腺がん患者の抗体反応性が HTLV で観察されたような HTLV-Gag-indeterminate pattern の類似現象である可能性を含め、更なる検討を続ける予定である。

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	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.	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）				米国		

研究報告の概要		使用上の注意記載状況・その他参考事項等
研究報告の概要	○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。	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

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。

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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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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,[2,5,7] 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.

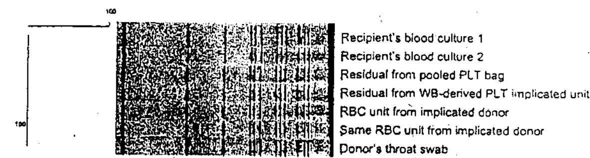

Fig. 1. PFGE and dendrogram of *S. dysgalactiae* subsp. *equisimilis* isolates recovered from the recipient's blood, a pooled PLT bag, an individual PLT unit, and the donor's RBC unit and throat swab, Florida, 2007.

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*

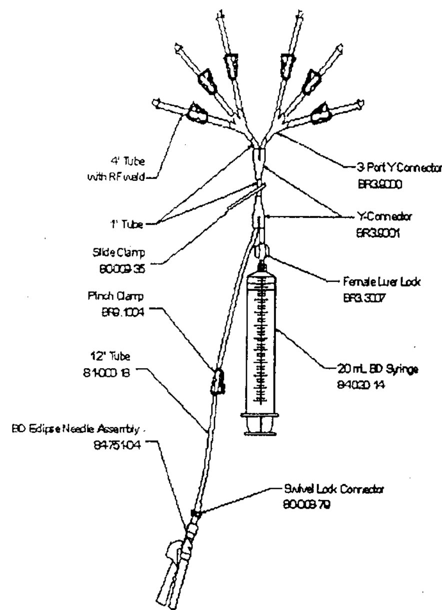

Fig. 2. Procedure for pooled PLT culture screening performed at the blood center. The syringe in the harness is used to draw the contents of up to six tubing segments containing PLT-rich plasma.

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

collected unit; this phenomenon has previously been noted in association with Gram-negative organisms.[3]

Detection of bacterial contamination in pooled WB-derived PLTs remains a challenge. Because of the short storage time for WB-derived PLTs (i.e., 5 days), the blood center in our investigation performs sampling within 2 hours after separation of the components. This technique does not allow for an additional 24-hour holding period to improve the sensitivity of the test. Both the shorter holding period and the smaller sampling size (i.e., 1.6 to 2.4 mL in each tubing segment) are likely to decrease the sensitivity of the method when compared to comparable apheresis testing procedures. The sensitivity of the method described, however, is likely to be superior to pH and glucose measurements commonly used for WB-derived PLTs QC. At the blood center reporting this case, the overall incidence of true-positive bacterial contamination (i.e., confirmed by replicate growth on the units from which the tubing segment was obtained) using the method described is 1 in 21,000 WB-derived PLT units[18] (which can be estimated as 1 in 3500 WB-derived PLT pools if we assume that 1 segment in each pool of 6 was contaminated), whereas the incidence of true-positive bacterial detection on apheresis PLT units at this same institution is 1 in 2700.

Although alternative devices for prepooling and sampling for culture have been approved by the FDA,[31] these alternatives, as currently configured, require the use of proprietary blood collection bags, leukoreduction filter, and bacterial growth detection systems that are not compatible with the bacterial detection systems used at all blood establishments, including the blood establishment where the PLTs in this report were prepared.

The BacT/ALERT culture method was approved by the FDA in 2002 for QC of bacterial contamination of single-donor PLT (SDP) units only. Because use of the BacT/ALERT method for individual WB-derived PLT units is not practical due to the small volume of each unit, a study was conducted in 2005 to validate the use of this method for the detection of bacterial contamination in WB-derived PLTs in a pooled format.[32] This study demonstrated that the BacT/ALERT method is capable of detecting very low concentrations of bacteria in a single WB-derived PLT unit when the contaminated unit is pooled with 5 other sterile units for culturing. In this validation study, both aerobic and anaerobic bottles were used. Although the use of one aerobic bottle and one anaerobic bottle is strongly recommended by the manufacturers of BacT/ALERT, the majority of the blood centers only use one aerobic bottle[33] as reported in our investigation. A recent study done by Brecher and Hay[34] using *Staphylococcus lugdunensis* suggested that the use of both aerobic and anaerobic bottles may significantly increase sensitivity of screening, particularly when the inoculum is low. It is unclear, however, whether this increase in sensitivity is due to the use of anaerobic media or simply reflects an increase in total volume inoculated.

Non-culture-based screening methods have been suggested for detection of bacterial contamination in WB-derived PLT units;[5] however, these methods are typically less sensitive than culture. FDA recently approved a rapid test to be used to supplement current screening strategies for detection of bacterial contamination in PLTs.[35] This supplemental test is to be used near the time of transfusion and can detect bacterial contamination that was not detected by culture. The performance of this new test in WB-derived PLTs is unknown, however, since studies were conducted using leukoreduced apheresis PLTs.

Our report and others[2,6,7,36] indicate that current screening methods to prevent transfusion of bacterially contaminated PLTs can be improved. Further studies to evaluate the sensitivity of culture and non-culture-based screening methods for detection of bacterial contamination in WB-derived PLTs are needed. Efforts to improve recognition of bacterial contamination of PLTs also need to continue. If transfusion-related bacteremia is suspected, the residual blood product unit should be saved by the hospital and the blood center immediately informed. Timely information will allow blood centers to rapidly trace and quarantine potentially contaminated cocomponents made from the same donation. Finally, the BacT/ALERT package insert's recommendations should be followed, particularly concerning the use of one aerobic and one anaerobic culture bottle with sufficient volume. β-Hemolytic streptococci are facultative anaerobes and may be better recovered under anaerobic conditions.[37]

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