薬事・食品衛生審議会

平成２５年度第１回血液事業部会運営委員会

議 事 次 第

日時：平成２５年６月１２日（水）
１６：００～１８：００
場所：厚生労働省１９階 専用第２３会議室

議題：

1. 委員長の選出及び委員長代理の指名
2. 議事要旨の確認
3. 感染症定期報告について
4. 血液製剤に関する報告事項について
5. その他

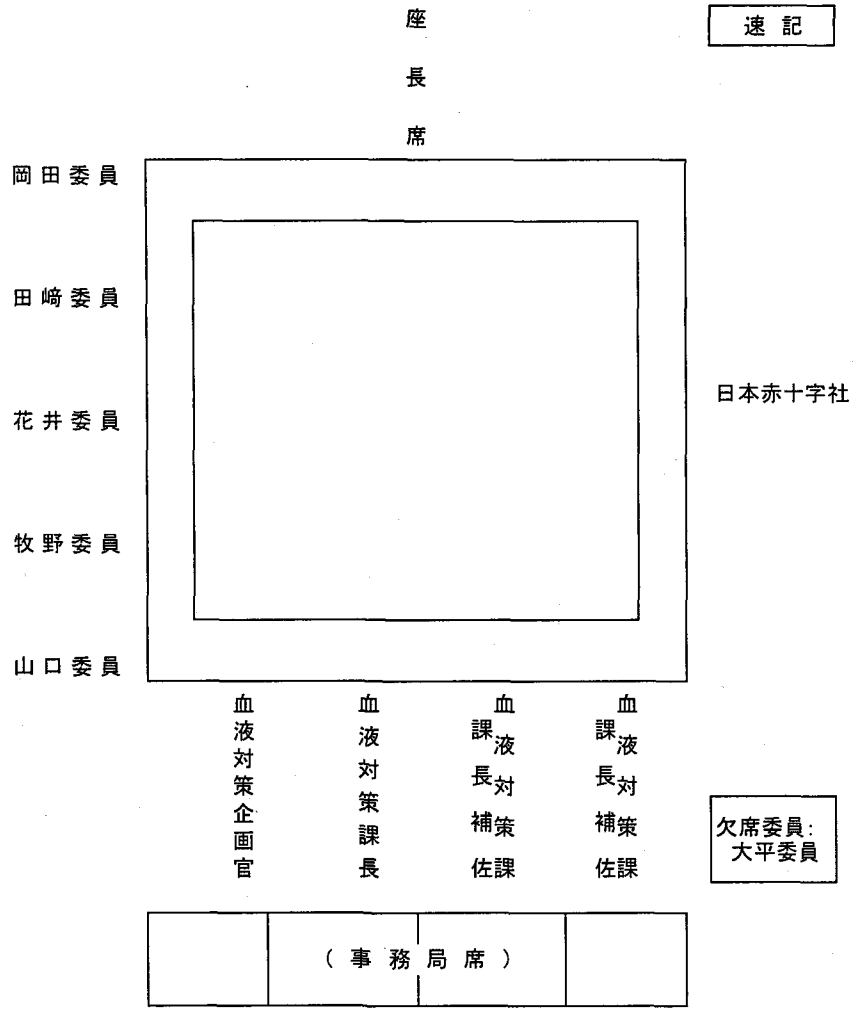
配付資料：

座席表
委員名簿
設置要綱
資　料　１　平成２４年度第４回血液事業部会運営委員会議事要旨（案）
資料２－１　感染症定期報告（研究報告概要一覧表及び個別症例報告概要）
資料２－２　感染症定期報告（研究報告詳細版）
資料３－１　供血者からの遡及調査の進捗状況について
資料３－２　血液製剤に関する医療機関からの感染症報告事例等について
資料３－３　献血件数及びＨＩＶ抗体・核酸増幅検査陽性件数について
資　料　４　フィブリノゲン製剤納入先医療機関の追加調査について

平成２５年度第１回 薬事・食品衛生審議会薬事分科会 血液事業部会運営委員会 座席表

平成25年6月12日(水)
厚生労働省19階
専用第23会議室
16:00～18:00

速記



薬事・食品衛生審議会薬事分科会
血液事業部会運営委員会 委員名簿

1. 大平 勝美 （おおひら　かつみ）
   はばたき福祉事業団理事長

2. 岡田 義昭 （おかだ　よしあき）
   国立感染症研究所血液・安全性研究部第一室長

3. 田﨑 哲典 （たさき　てつのり）
   東京慈恵会医科大学附属病院輸血部診療部長 教授

4. 花井 十伍 （はない　じゅうご）
   ネットワーク医療と人権理事

5. 牧野 茂義 （まきの　しげよし）
   国家公務員共済組合連合会虎の門病院輸血部長

6. 山口 照英 （やまぐち　てるひで）
   国立医薬品食品衛生研究所生物薬品部研究員

（50音順、敬称略）

薬事分科会血液事業部会運営委員会規程

（目的）

第１条　この規程は薬事分科会規程（以下「規程」という。）第２条第１項に基づき設置される血液事業部会（以下「部会」という。）に置かれる運営委員会（以下「委員会」という。）の運営に関し必要な事項を定め、審議の円滑な実施を図ることを目的とする。

（所掌）

第２条　委員会は、規程第３条第５項に規定する部会が調査審議すべき血液製剤（血液製剤代替医薬品を含む。以下同じ。）に係る事項（以下「部会で調査審議すべき事項」という。）を検討するとともに、以下に掲げる事項を確認し、部会に報告するものとする。

一　安全な血液製剤の安定供給の確保等に関する法律（以下「血液法」という。）第２６条第１項に規定する血液製剤の製造又は輸入の実績に係る報告

二　血液法第２９条に規定する薬事法第６８条の８第１項に規定する生物由来製品（血液製剤に限る。）の評価に係る報告

三　規程第４条第１項の規定に基づき部会に置かれる調査会における調査審議の状況

四　その他部会で調査審議する事項のうち特別の事項についての状況

（委員会への所属）

第３条　委員会に所属すべき委員は、部会に所属する委員、臨時委員及び専門委員（以下「委員等」という。）の中から、部会長が指名する。

２　部会長は、前項の規定により委員会に属すべき委員等を指名する場合は、血液製剤を使用する患者の代表、医療関係者、血液事業の専門家を含め、数名を指名する。

３　部会長は、第一項の規定により委員会に属すべき委員等を指名した場合は、部会においてその旨を報告しなければならない。

（委員長の選任）

第４条　委員会に委員長を置き、委員会に属する委員等の互選により選任する。

２　委員長は、委員会の事務を掌理する。

３　委員長に事故があるときは、委員会に属する委員等のうちから委員長があ

らかじめ指名する者が、その職務を代理する。

（委員会の開催）

第５条　委員会は、四半期（１月から３月まで、４月から６月まで、７月から９月まで及び１０月から１２月までの各期間をいう。）ごとに開催する。

２　前項に規定する場合のほか、委員等が必要と認めるときは委員会を開催することができる。

（議決）

第６条　部会への報告の要否等、議決を行う必要がある委員会の議事は、委員会に属する委員等で会議に出席したものの過半数で決し、可否同数のときは、委員長の決するところによる。

（議事の公開）

第７条　委員会は原則として公開する。ただし、公開することにより、委員の自由な発言が制限され公正かつ中立な審議に著しい支障をおよぼすおそれがある場合、又は、個人の秘密、企業の知的財産等が開示され特定の者に不当な利益又は不利益をもたらすおそれがある場合については、委員長は、これを非公開とすることができる。

（雑則）

第８条　この規程に定めるもののほか、委員会の運営に関し必要な事項は、部会長が部会に諮り決定するものとする。

附則

この規程は、平成１５年７月３０日から施行する。

資料１

平成24年度第4回　血液事業部会運営委員会議事要旨（案）

日時：平成25年3月29日（金）10:00～13:00
場所：厚生労働省17階　専用第18～20会議室
出席者：（委員）
半田委員長、大平委員、岡田委員、花井委員、牧野委員、山口委員
（日本赤十字社血液事業本部）
田所経営会議委員、日野副本部長、豊田副本部長、河島製造管理課長、石川研究一課長
（バクスター株式会社）
川本バイオサイエンス事業部長、村井品質保証部員
（事務局）
加藤課長、丈達企画官、笠松補佐

議　題：1. 議事要旨の確認
2. 感染症定期報告について
3. 血液製剤に関する報告事項について
4.「献血血液の研究開発等での使用に関する指針」に基づく公募に係る事前評価について（非公開）
5. その他

【審議概要】

議題1について

議事要旨に関する意見等については、事務局まで連絡することとされた。

議題2について

感染症定期報告について、事務局から説明後、質疑応答がなされた。

議題3について

事務局より、供血者からの遡及調査の進捗状況、血液製剤に関する報告事項、献血件数及びHIV抗体・核酸増幅検査陽性件数について説明がなされた。

議題4について

「献血血液の研究開発等での使用に関する指針」に基づき、公募申請に対する事前評価が実施された。運営委員会での事前評価が必要とされた113件のうち、「承認」89件、「修正の上で承認」8件、「却下」16件であった。なお、事前評価が不要とされた140件については、いずれも「承認」とされた。

（参考）掲載アドレス（厚生労働省ホームページ）
http://www.mhlw.go.jp//new-info/kobetu/iyaku/kenketsugo/5-121127_1/kekka_h25.html

議題5について

（フィブリノゲン製剤に係る調査）

事務局より、フィブリノゲン製剤の調査に係る公表事項の報告がなされた。

（バクスター社からの報告事項）

バクスター社の人血清アルブミン製剤については、品質上の問題が生じたため平成23年11月から製造が中止されていたが、バクスター社の方針により、供給再開はせずに日本市場から撤退するとの報告がなされた。

血液対策課より、製造販売業者の責務である安定供給が果たされなかったこと及び需給計画が尊重されなかったことは非常に遺憾であり、日本での海外製剤の供給については、血液製剤の安定供給を図る上で、時として大きなリスクになりうるとの意見が示された。また、委員から、バクスター社を含め血液製剤の製造販売業者においては、血液法の趣旨に則り、血液製剤の安定的かつ適切な供給に努めて欲しいとの意見が出された。

以上

資料２－１

Ａ　研究報告（概要一覧表）

平成２５年６月１２日
（平成２５年２月～平成２５年４月受理分）

研究報告のまとめ方について

１　平成２５年２月１日～平成２５年４月３０日までに提出された感染症定期報告に含まれる研究報告（論文等）について、重複している分を除いた報告概要一覧表を作成した。

２　一覧表の後に、個別の研究報告の詳細を添付した。

感染症定期報告の報告状況(2013/2/1～2013/4/30)

【血液製剤、輸血の安全性に関する報告】

血対課Id	感染症(PT)	出典	概要	新規文献番号
130019	E型肝炎	Hepatoogy. 56(2012) SUPPL1-1100A	フランスにおけるE型肝炎ウイルス(HEV)感染の報告。47歳肝移植患者は急性肝炎を発症し、E型肝炎と診断された。ウイルスの部分配列決定により、中国のウサギから分離された株と高い相同性が示された。患者の血清による子ブタ及びウサギの感染実験では、子ブタは感染しなかったが、ウサギではHEV RNAの検出はなかったものの、抗HEV抗体が一過性に検出された。患者は海外渡航しておらず、レストランでシェフとして働き、ウサギを含む複数の死亡動物と接触していた。フランスの食用新鮮ウサギ及び冷凍ウサギの最大の供給国が中国であることは注目に値する。また、免疫抑制によりウイルス感染が起こった可能性がある。	1
130019	E型肝炎	AABB Annual Meeting & CTTXPO 2012	E型肝炎ウイルス(HEV)の培養系及びHEV感染価定量系の確立の報告。HEV遺伝子型3型は世界で最も多くみられ、HEV遺伝子型4型は日本を含めたアジアに多く、ときに重症肝炎を引き起こす。3型及び4型のHEVを含む、HEV特異的IgM及びIgGに陽性又は陰性の血液検体を用い、ヒト肝癌細胞株(PLC/PRF/5)及びヒト肺腺癌細胞株(A549)に接種した。HEVに感染させた細胞を維持培地にて培養し、HEV RNAコピー数をRT-PCR法で測定し、培養3週間後に回収した培地における子孫ウイルスの検出により感染性を確認した。本感染価定量系をMirasol感染性低減化技術に応用したところ、2Log以上の感染性低減化が確認された。このシステムはウイルス不活化技術の性能評価にも有用と考えられる。	2
130047	パルボウイルス	Br J Haematol. 159(2012)385-393	血液製剤によるパルボウイルスに関する最近の知見をまとめた報告。現在実施可能なウイルス不活化の方法は、パルボウイルスB19やプリオンを除去することができず、血液製剤を介したこれらの伝播について理論的な懸念が残されている。本報告は最近の知見をまとめた総説であり、近年見出されたパルボウイルスB19の新たな遺伝子型やヒトパルボウイルス4(PARV4)に関する知見のほか、パルボウイルスにより引き起こされる臨床症状、並びに血液製剤の製造所で現在行われている検査手法などが紹介されている。	3
130049	パルボウイルス感染	http://wwwnc.cdc.gov/eid/article/18/10/11-1373_article.htm	ガーナにおけるヒトパルボウイルス4(PARV4)感染に関する報告。サハラ以南のアフリカ諸国では、経口伝播がPARV4感染に関与している可能性がある。ガーナにおいて、気道症状を呈する15歳未満の小児1,904人から鼻腔検体または糞便検体を採取し検査を行った結果、鼻腔検体961例中8例(0.83%)及び糞便検体943例中5例(0.53%)からPARV4のDNAが検出された。ウイルス濃度は、鼻腔検体では1.3×10^3～1.8×10^7コピー/mL、糞便検体では2.3×10^3～4.6×10^8コピー/mLであり、全てPARV4ジェノタイプ3と分類された。PARV4感染経路として気道あるいは糞口経路が示唆される。	4
130053	ウイルス感染	AABB Annual Meeting & CTTXPO 2012	米国におけるHTLV-I/IIの抗体陽性率に関する報告。近年、米国ではHTLV-I/IIの検査結果が陽性となる供血者数が減少しているが、感染者は今も確認されている。2009～11年、米国赤十字社で確認されたHTLV-I/II陽性供血者のデータを検索し、米国国勢調査の性別と地域分布に基づき、地域ごとの抗体陽性率を計算した。3年間で、HTLV陽性の443人を含む7,098,612人の同種血供血者からの1,900万を超える供血を調査した。HTLV供血の総抗体陽性率は10万人当たり2.3人であった。そのうち女性供血者は72%(443人中319人)、男性は28%(443人中124人)であり、抗体陽性率はそれぞれ女性が3.6/10万人、男性が1.2/10万人であり、男女差が認められた。さらに、供血者10万人当たりのHTLV 陽性供血者数には地域差が認められ、北東部と西部、中西部と北東部、中西部と西部、中西部と南部の間で有意差があった。なお、中西部は抗体陽性率が最も低かった。複数回供血者のうち、36人がHTLV陽性であり、14人は3年以内の供血が陰性であった。3年以内の新規感染が14例確認されたことから、HTLV単回抗体検査のみで供血者スクリーニングを行うことは有効とは言えない。	5
130053	ウエストナイルウイルス感染	Transfusion. 52(2012)2664-2670	米国におけるウエストナイルウイルス(WNV)の核酸増幅検査(NAT)に関する報告。ニューヨーク血液センターにおいて、2010年7月1日～10月31日の期間中、供血サンプルについてNATスクリーニングを行い、NAT陽性の血液についてWNV-IgM及びIgG抗体の有無を調べた。WNVウイルス血症であるとみられる血液20本(0.0129%、1/7752)が確認され、そのうち、ミニプールのNAT(MP-NAT)では検出されなかった可能性がある9本が同定された。また、供血者個別に実施するNAT(ID-NAT)を遡及的に実施したところ、2本の陽性血液が確認された。ニューヨーク州におけるNAT陽性血液の多くは、州の中でもWNV症例の多い郡(ナッソー郡及びサフォーク郡)の居住者から採血されたものであった。	6
130053	デング熱	ProMED-mail 20121007.1328469	カンボジアにおけるデング熱の発生状況に関するの報告。2012年9月第1週までに、少なくとも34,483人のデング熱症例が報告され、昨年の同時期の12,972人と比較して2.66倍に増加した。また、今年9月までに146人の子どもがデング熱で死亡し、昨年の同期間の死亡者数59人と比較して2.47倍に増加した。デング熱により1週間に3～5人の子どもが死亡し続けていると言う。保護者が患児を個人医院に連れて行き、治療が無効で疾患がより重篤になってから公立病院を訪れるため、その時には既に手遅れとなっていること多く、そのため死亡者数の増加につながったと保健当局の専門家は考えている。	7
130053	エプスタイン・バーウイルス感染	Transfusion. 52(2012)2653-2663	カナダにおける幹細胞移植レシピエントの輸血関連エプスタイン・バーウイルス(EBV)感染に関する研究報告。リンパ増殖性疾患と関連するEBVについては、現在スクリーニング検査が行われていない。本研究では、造血幹細胞(HSC)移植を受ける小児における移植後のEBV感染率及び血液製剤の投与と感染との関連性について分析を行った。移植前のEBV抗体陽性率は、レシピエントで77.9%、ドナーで61.8%であった。レシピエントの全員が移植前後の期間に血液製剤の投与を受けていた。抗体陰性患者における30日及び60日のEBVの移植後累積感染率は、それぞれ4.6%(95%CI、1.2－17.3%)、13.4%(95%CI、5.8－29.4%)であった。また、分析を臍帯血移植を受けた抗体陰性患者のみに限定した場合、60日の累積感染率は8.3%(95%CI、2.2－29.4%)であった。EBV感染と輸血量の関連性を肯定する傾向も認められており、これらの結果からHSC移植レシピエントにおける輸血と移植後EBV感染の関連性が示唆された。	8

130053	HHV-8感染	Transfusion. 52(2012)2294-2299	ガーナにおけるヒトヘルペスウイルス8(HHV-8)の伝播に関する報告。HHV-8の抗体陽性率は、欧州や北米では5%未満、サハラ以南アフリカでは50～70%と地域によって開きがある。今回、供血者と全血輸血を受けた免疫正常受血者のペア252組から得た検体に対して血清学的検査及び分子生物学的検査を行った。その結果、受血者28人(11%)及び供血者16人(6%)がHHV-8抗体陽性であり、抗体陽性の血液を輸血された抗体陰性受血者12人中1人に感染の疑いが高いことが確認された。当該供血者の血液にはHHV-8のDNAが含まれており、当該供血者を含む5人のHHV-8のDNA配列は、ブートストラップ値97%で既知のジェノタイプとは異なるクラスターを形成していた。今回のHHV-8伝播は、受血者の多くが免疫正常者であったため、臨床的影響はなかった。しかし、サハラ以南アフリカでは、免疫抑制剤の使用の増加に伴い、臨床的なリスクが懸念される。	9
130053	HHV-8感染	J Infect Dis. 206(2012)1497-1503	ウガンダにおけるヒトヘルペスウイルス8(HHV-8)の伝播に関する報告。6カ月の追跡期間を設けた前向きコホート研究により、HHV-8抗体陽性血液の輸血が受血者へ及ぼした影響について調べた。1092人の受血者中471人(43.1%)にHHV-8抗体陽性血液が輸血された。年齢中央値は1.8歳(0.1～78歳)で、111人(10.2%)が追跡期間中に死亡した。交絡因子(年齢、HIV感染、マラリア以外の疾患、輸血回数)を調整したところ、保管期間が短い(4日以内)HHV-8抗体陽性血液の受血者は、HHV-8抗体陰性血液の受血者と比較して死亡率が高かった(補正ハザード比 1.92、95%信頼区間 1.21～3.05、p = 0.01)。一方、保管期間が長い(5日以上)HHV-8陽性血液の受血者では、輸血と死亡率の増加との間に有意な関連はなかった。急性HHV-8感染と若年死亡率の間に観察された関連性を検証するために更なる研究が必要である。	10
130053	ヒトアナプラズマ症	AABB Annual Meeting & CTTXPO 2012	米国におけるヒト顆粒球アナプラズマ症(HGA)感染者の報告。64歳男性患者は、3日間続く倦怠感、労作時呼吸困難、下血により入院し、保存前白血球除去赤血球製剤5ユニットが輸血され、その後容態は安定し退院し、その2日後、頭痛、発熱、悪寒により再入院した。末梢血スメアによりHGAと一致する桑実胚を持つ多形核白血球が確認された。受血者及び全5ユニットの白血球除去赤血球製剤の供血者セグメント検体について*Anaplasma phagocytophilum*の検査を行ったところ、1名の供血者に感染が確認された。当該供血者は媒介ダニの多発地帯であるロードアイランド州在住の81歳健常男性で、屋外活動は行うがダニ刺咬歴はなかった。白血球除去はHGA伝播の予防とはならず、赤血球製剤がHGA多発地帯から非多発地帯へ供給されて輸血される例は多いため、受血者が予期せず発熱した場合は地域に関係なく輸血伝播によるアナプラズマ症の可能性を考慮すべきである。	11
130053	リーシュマニア症	Vox Sang.. 103(2012)356-358	リーシュマニアの病原体不活化技術に関する報告。無症候性リーシュマニア感染症は、流行地域における輸血感染の主な原因となっている。スペイン・バレアレス諸島では、供血者の無症候性*Leishmania.infantum*の感染率は高い(調査対象供血者の5.9%)。現在、血液銀行の基準を満たす供血者のためのリーシュマニアスクリーニング検査は存在しないため、血液製剤中のリーシュマニアの除去のために数種類の方法が用いられている。今回、アモトサレンとUVA照射(INTERCEPT)を用いて無症候性*L..infantum*感染供血者から採取した血液製剤を用いて病原体除去技術の能力を調査した。病原体不活化処理実施前の血小板製剤6例中5例で、RT-PCR結果が陽性であった。INTERCEPTでの不活化後、これらの血小板製剤はRT-PCRで陰性となり、*in vitro*培養において6カ月後も全て陰性であった。これは供血者の血液成分から原虫を除去する目的でINTERCEPTが用いられた初の報告である。	12
130053	リーシュマニア症	ProMED-mail 20130217.1546451	スペインにおけるリーシュマニア症の発生状況に関する報告。マドリッド南部の市町村(Bosquesurの緑地帯周辺のFuenlabrada、Leganes、Getafe、Humanes等)で発生しているリーシュマニア症のアウトブレイクは、2012年の150症例を含めて過去3年間に500症例が報告されており、まだ継続しているとみられる。Bosquesurで増加しているウサギが保有宿主となっている可能性がある。2012年、Fuenlabradaは緊急事態地域と宣言され、事態を制御するためにウサギの駆除が許可された。スペイン当局は、2012年末までに報告された内臓リーシュマニア症は3例で、2011年の47例と比べて改善したと述べたが、2013年の現時点で既に3例の新規症例が報告されており、アウトブレイクはまだ終了していないとみられる。	13
130053	異型クロイツフェルト・ヤコブ病	Transfusion. 52(2012)2285-2293	赤血球のプリオンろ過の費用対効果に関する報告。変異型クロイツフェルトヤコブ病(vCJD)の危険を低減するため、プリオンを取り除くフィルター(The P-Capt filter)が開発され、アイルランドにおいてプリオンろ過を実行することについての費用効果が評価された。その結果、プリオンろ過を行わなければ、10年間に2人が赤血球輸注によるvCJDを発症し、失われる寿命は18.5年となると推定された。また、プリオンろ過の実装にかかる費用は68.2百万ユーロで、寿命1年あたりのコストは3.7百万ユーロであると推定された。	14
130053	異型クロイツフェルト・ヤコブ病	ProMED-mail 20121208.1443015	ブラジルで発生したBSEに関する報告。ブラジルSertanopolisにおいて、四肢を硬直させ横臥状態のウシが発見され、その後死亡が確認された。当該ウシは肉牛繁殖用に飼育され、死亡時の年齢は13歳であった。ブラジル国内の研究所で実施された病理組織学的検査では、牛海綿状脳症は陰性であるとの結果であった。しかし、ブラジル国内の別の研究施設で実施された免疫組織化学的検査では、牛海綿状脳症が陽性であるとの結果が得られた。さらに、同試料はイギリスにある国際獣疫事務局の付託研究施設にも送付され、免疫組織化学的検査により牛海綿状脳症陽性であると確認された。今回の牛海綿状脳症は、ブラジル国内における最初の症例であった。	15

【その他の報告】

130006	ウイルス感染	http://www.mhlw.go.jp/stf/houdou/2r9852000002u1pm.html	山口県における重症熱性血小板減少症候群(SFTS)に関する報告。厚生労働省は、新規のフレボウイルス属ウイルスによるSFTSが国内で初めて確認されたことを発表した。患者に最近の海外渡航歴はなく、昨秋に死亡している。SFTSは、2009年頃より中国で発生が報告され、2011年に初めて原因ウイルスが特定された、ダニ媒介性疾患である。厚生労働省はSFTSに関して都道府県等に情報提供を行うとともに、医療機関に対して、同様の患者を診察した際は情報提供するよう、自治体を通じて協力を要請した。厚生労働省は、本疾患に関する情報収集や調査研究を実施している。	16

130015	ウイルス感染	http://www.nih.go.jp/niid/ja/sfts/sfts-iasrs/3142-pr3963.html	国内における重症熱性血小板減少症候群ウイルス(SFTSV)感染者の報告。患者は発熱、嘔吐、下痢(黒色便)を呈し入院したが、全身状態が悪化し死亡した。患者に海外渡航歴はなかった。入院中に採取された血液からウイルスが分離され、SFTSVと同定された。また血液中にSFTSV遺伝子が含まれることが確認された。中国からの報告ではマダニからウイルスが分離されており、SFTSVの宿主はダニであると考えられている。ヒトへの感染はSFTSVを有するダニに咬まれることによるが、患者血液や体液との直接接触による感染も報告されている。有効性が確認された治療法やワクチンはない。SFTSVに感染しないようにするには、ダニに咬まれないようにすることが重要である。	17
130050	ウイルス感染	J Infect Dis. 207(2012)736-739	中国における重症熱性血小板減少症候群(SFTS)ブニヤウイルスのヒトからヒトへの伝播に関する報告。2010年5月～6月に発生したSFTSのアウトブレイクの感染経路を同定するために調査が行われた。発端患者は58歳男性で、2010年5月20日に発熱、疲労、筋肉痛、咳及び悪心を呈し、5月30日に死亡した。6月6日～8日、発端患者と接触があった31人のうち4人(3人は家族)が二次感染し、SFTSと一致する症状を発症した。調査によると、二次感染には患者血液との接触が有意に関連していたが、呼吸器からの分泌物、尿、便との接触とは有意な関連性はなかった。二次感染患者4人全員の回復期血清において、IgG抗体が陽性であり、無症状の接触者27人では、ウイルスRNA及びIgG抗体のいずれも陰性であった。これらのことから、発端患者の血液との接触が二次感染者の感染源と推定できるが、他の感染源がある可能性もある。患者の家族及び医療従事者は、患者の血液や体液に直接触れないよう、防護策をとることが推奨される。	18
130053	コロナウイルス感染	WHO/GAR Background and summary of novel coronavirus infection-as of 21 December 2012	サウジアラビア、ヨルダン及びカタールにおけるコロナウイルス感染の報告。WHOは、2012年10月から12月までの間に、9症例の新種のコロナウイルスによる重症急性呼吸器感染症の報告を受けていることを発表した。カタール(2例)、サウジアラビア(5例)及びヨルダン(2例)において感染確定症例が報告され、すべてが重症の症例であり、5例が死亡している。確定症例又は可能性症例と個人的な接触があった家族の少なくとも2人及び医療介護を提供した数名が肺炎症状を示しており、ヒト-ヒト感染の可能性を示唆しているが、共通の感染源への暴露があった可能性を完全に排除することはできない。この新種ウイルスはSARSコロナウイルスの遠縁に該当するが、SARSコロナウイルスとは異なり、ヒト集団内で容易に感染したり、持続的に感染が拡大することはないと考えられるとしている。	19
130053	コロナウイルス感染	ProMED-mail 20130213.1541531	英国における新型コロナウイルス(NCoV)感染者の報告。英国健康保護局(HPA)は2013年2月13日、NCoV感染が既に確定している患者の家族1人に、NCoV感染が確認されたと発表した。新たに感染が確認されたこの患者は、呼吸器感染症に罹患しやすい基礎疾患があり、最近の海外渡航歴はなかったが、短期間の呼吸器症状を呈して2月9日に入院し、現在は集中治療を受けている。この時点で、NCoVによる重症肺炎の確定患者は計11名となった。HPAは英国国際ガイドラインに従い、2患者と密接に接触した家族及び治療に携わった医療従事者の監視を継続中であると発表した。家族内でのNCoVヒト－ヒト感染を考慮し、欧州疾病管理センターは2012年12月7日に発表されたリスクアセスメントの更新を現在進めている。	20
130053	インフルエンザ	共同通信(2013年1月16日配信)	米国におけるインフルエンザ予防接種の呼びかけに関する報告。疾病予防管理センター(CDC)によると、47州においてインフルエンザの広範な流行が見られ、前週の41州から拡大している。これを受け、CDCは国民にワクチン接種を呼びかけた。また、生後6カ月以上の国民は例外なくワクチンを接種すべきだとし、幼児、妊婦のほか、ぜんそくや糖尿病などの既往歴を有する人及び65歳以上の高齢者は、特にワクチン接種が重要だと警告している。	21
130053	ウイルス感染	PLOS Pathogens. 8(2012)1-14	コンゴ民主共和国における急性出血熱ヒト症例に関連した新型ラブドウイルス(BASV)に関する報告。2009年にコンゴ民主共和国で発生した3症例の急性出血熱に関連し、大規模シークエンシングを用いて新型BASVを発見した。症例は、突然の発症、高熱、吐血及び血性下痢を特徴とし、3症例中2例は3日以内に死亡した。BASVは唯一の生存者の血液中に100万コピー/mL以上の濃度で存在していた。本症例のBASVのゲノムは他のどのBASVとも異なっていた。唯一の生存者と看護した看護師から高レベルのBASV抗体が発見され、両者ともウイルスに感染したことが明らかとなった。ウイルスの発生源は不明であるものの、本調査の知見では、BASVがアフリカの急性出血熱に関連し、ヒト-ヒト接触で伝播する可能性があることが示唆される。	22
130053	ウイルス感染	ProMED-mail 20120929.1315179	豪州におけるロスリバーウイルス感染の発生状況に関する報告。西オーストラリア州において重大な蚊媒介性疾患が流行しており、ロスリバーウイルス感染者については3年前の5倍に増加している。生涯継続する後遺症をのこすこともあるこのウイルスに、2011年から2012年にかけて州全域で1,570人が感染した。西オーストラリア州のロスリバーウイルス感染者は2009年－2010年の332人から2010年－2011年には770人へ2倍以上に増加している。過去2年間の感染者数の増加は、ラニーニャ現象によって蚊が多く発生したことが原因であると考えられている。	23
130053	ウイルス感染	ProMED-mail 20130215.1544648	豪州におけるオーストラリアコウモリリッサウイルス感染者の報告。クイーンズランド州北部在住の8歳男児が、オーストラリアコウモリリッサウイルスを保有しているコウモリまたはオオコウモリにより咬傷あるいは擦過傷を受け、ウイルスに感染し、現在危篤状態である。これは狂犬病様のオーストラリアコウモリリッサウイルスに感染した3人目の確定症例である。過去(1996年と1998年)に感染した2人は死亡した。クイーンズランド州の保健担当官は、ウイルスからの最良の防護策はコウモリやオオコウモリとの接触を避けることであると注意を呼びかけている。	24
130053	エボラ出血熱	WHO GAR 2012 8 Oct.	コンゴ民主共和国におけるエボラ出血熱の発生状況に関する報告。WHOは、2012年10月7日時点で、コンゴ民主共和国内でエボラ出血熱が49例(うち、確定例31例、疑い例18例)報告されていると発表した。このうち24例が既に死亡している(うち、確定例10例、疑い例14例)。これらの症例は、Orientale州Haut-Uele地区のIsiro及びViadanaで報告されている。エボラウイルスのヒト-ヒト感染は、主に血液又は体液の直接接触に関連するものであり、適切な感染防御措置が講じされていない場合のヘルスケア担当者への感染が報告されている。	25
130053	ニパウイルス感染	ProMED-mail 20130205.1530748	バングラディッシュにおけるニパウイルス脳炎の発生状況に関する報告。バングラデシュで再流行した致死性のニパウイルスによって、2013年2月3日現在、感染者12人中10人が死亡した。バングラデシュの疫学疾病対策研究所(IEDCR)によると、死亡者はダッカ県、パブナ県、ナトール県から各2名、ラジバリ県、ジェナイダ県、ナオガオン県、ラジシャヒ県から各1名が報告されている。ダッカ県の死亡者2名は、ミメシング県バールカでナツメヤシの生ジュースを飲んだと報告している。IEDCRの担当官は、感染したコウモリの尿や唾液で汚染された生のナツメヤシジュースや果物を飲食しないように警告した。患者を介護する者も予防策をとる必要がある。バングラデシュでのアウトブレイクではこれまでに感染者180人中139人が死亡している。	26

130053	ボレリア感染	N Engl J Med. 17(2013)291-293	米国におけるボレリア感染に関する報告。回帰熱を引き起こす*Borrelia miyamotoi*はライム病を媒介するダニから検出されている。米国において*B. miyamotoi*感染が存在する可能性について検討した。ライム病浸淫地域在住者の3群（第1群：ロードアイランド州及びマサチューセッツ州でダニ媒介性感染症の血清検査を受けた584人、第2群：ニューイングランド州南部でライム病が疑われた277人、第3群：ニューヨーク州南部でウイルス感染様症状を呈しライム病検査を行った14人）から1990年～2010年の間に採取した血清保管検体について、ELISA法及びウエスタンブロット法を用いて*B.miyamotoi*のGlpQタンパク質抗体の検査を行った。その結果、抗体陽性率は第1群で1.0%、第2群で3.2%、第3群で21.0%であった。第2群の1人及び第3群の2人の回復期の抗体価は、急性期の抗体価と比べて4倍以上高かった。この所見から、これらの患者は最近*B.miyamotoi*に感染したと考えられる。この3人の患者はいずれも免疫不全ではない。米国のライム病浸淫地域で*B.miyamotoi*感染が広がっている可能性が示唆される。	27
130053	トキソプラズマ症	Transpl Infect Dis. 14(2012)496-501	スペインにおけるトキソプラズマ症感染に関する報告。トキソプラズマ症は臍帯血移植（CBT）レシピエントのような免疫不全患者に影響を及ぼす重篤な日和見感染症である。この病院で治療された4人及び文献から収集した5人（計9人）のトキソプラズマ症CBT患者について再評価を実施した。播種性疾患を呈した患者は全てトキソプラズマ感染症により死亡した。彼らの移植前の血清学検査結果は、陽性1人、陰性3人、不明1人であった。CBTレシピエントにおける播種性トキソプラズマ症の死亡率は容認出来ないほどに高いが、これらの患者の多くは血清学的検査で陰性となり、臨床症状が明確ではないため診断が難しい。CBTレシピエントにおいて、より良い診断検査と予防戦略が必要と考えられる。	28
130053	ロッキー山紅斑熱	ProMED-mail 20121114.1409214	メキシコにおけるロッキー山紅斑熱の発生状況に関する報告。メキシコ保健省はダニ媒介性のリケッチア感染症であるロッキー山紅斑熱の4症例を確定し、別の4症例を疑いありと発表した。Coahuila州Saltilloの4集落で既に防疫線が設けられた。確定した4症例のうち1例がSaltillo、1例がParras de la Fuente、2例がTorreonからで、疑い例は全てSaltilloでの発生であった。Valle de las Aves集落において少なくとも2人の女児が死亡するというこの緊急事態に直面し、当該集落及びLomas de Zapaliname、Pedregal、Nueva Imagenでは2012年11月10日又は11日から予防措置が実施された。	29

B　個別症例報告概要

○ 総括一覧表
○ 報告リスト

平成２５年６月１２日
（平成２５年２月～平成２５年４月受理分）

個別症例報告のまとめ方について

個別症例報告が添付されているもののうち、個別症例報告の重複を除いたものを一覧表の後に添付した（国内症例については、資料３において集積報告を行っているため、添付していない）。

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期	転帰	出典	区分	備考
		器官別大分類	基本語								
第13回	13-1	感染症および寄生虫症	慢性Ｃ型肝炎	イギリス	男性	不明	不明	死亡	症例報告	当該製品	報告日： 識別番号：C-12000037 MedDRA/J Version 15.1
	13-2	感染症および寄生虫症	慢性Ｃ型肝炎	イギリス	男性	不明	不明	死亡	症例報告	当該製品	報告日： 識別番号：C-12000036 MedDRA/J Version 15.1

血対課ID	受理日	番号	報告者名	一般名	生物由来成分名	原材料名	原産国	含有区分	文献	症例	適正措置報告
130005	20-Feb-13	120940	バクスター	ルリオクトコグ アルファ(遺伝子組換え)	ルリオクトコグ アルファ(遺伝子組換え)	遺伝子組換えチャイニーズハムスター卵巣細胞株	該当なし	有効成分	なし	あり	なし

	番号		感染症の種類		発現国	性別	年齢（歳）	発現時期（年/月/日）	転帰	出典	区分	備考		
			器官別大分類	基本語								識別番号	報告日	MedDRA (Ver.)
第20回	20-	1	10021881/ 感染症および寄生虫症 /Infections and infestations	10019744/ C型肝炎 /Hepatitis C	アルゼンチン	男性	36歳	不明	不明	症例報告	外国製品	12000033	2012/11/15	15.1

血対課ID	受理日	番号	報告者名	一般名	生物由来成分名	原材料名	原産国	含有区分	文献	症例	適正措置報告
130013	22-Mar-13	121087	バクスター	乾燥濃縮人血液凝固第Ⅷ因子	乾燥人血液凝固第Ⅷ因子	人血漿	米国	有効成分	なし	あり	なし
130014	22-Mar-13	121088	バクスター	乾燥濃縮人血液凝固第Ⅷ因子	人血清アルブミン	人血漿	米国	添加物	なし	あり	なし

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期	転帰	出典	区分	備考
		器官別大分類	基本語								
第 20 回	20-1	感染症および寄生虫症	Ｂ型肝炎	ドイツ	男	17	2013/01/17	不明	自発報告	外国製品	12000040、1 回(完了) 平成 25 年 2 月 7 日 MedDRA ver.15.1
	20-1	感染症および寄生虫症	医薬品を介する感染因子の伝播	ドイツ	男	17	2013/01/17	不明	自発報告	外国製品	12000040、1 回(完了) 平成 25 年 2 月 7 日 MedDRA ver.15.1

血対課ID	受理日	番号	報告者名	一般名	生物由来成分名	原材料名	原産国	含有区分	文献	症例	適正措置報告
130030	10-Apr-13	130037	化学及血清療法研究所	乾燥濃縮人アンチトロンビンⅢ	アンチトロンビンⅢ	ヒト血液	日本	有効成分	あり	あり	なし

	番号	感染症の種類		発現国	性別	年齢	発現時期	転帰	出典	区分	備考		
		器官別大分類	基本語								識別番号	報告日	MedDRA Version
第20回	20-1	感染症および寄生虫症	Ｃ型肝炎	中国	女性	30歳	2010年7月9日	未回復	症例報告	外国製品	12000044	2013年3月5日	15.1

血対課ID	受理日	番号	報告者名	一般名	生物由来成分名	原材料名	原産国	含有区分	文献	症例	適正措置報告
130038	19-Apr-13	130066	バクスター	人血清アルブミン	人血清アルブミン	人血漿	米国	有効成分	なし	あり	なし

別紙(4)

感染症発生症例一覧

	番号	感染症の種類		発生国	性別	年齢	発現時期	転帰	出典	区分	備考
		語官別大分類	基本語								
第20回	1	感染検査	C型肝炎抗体陽性	米国	女	不明	2008/8/8	不明	症例報告	外国製品	識別番号3-12000045 報告日:2013年3月8日

血対課ID	受理日	審号	報告者名	一般名	生物由来成分名	原材料名	原産国	含有区分	文献	症例	適正措置報告
130039	22-Apr-13	130073	CSLベーリング	乾燥pH4処理人免疫グロブリン	人免疫グロブリンG	ヒト血液	ドイツ	有効成分	あり	あり	なし
130040	22-Apr-13	130074	CSLベーリング	乾燥pH4処理人免疫グロブリン	ベプシン	ブタ胃粘膜	米国	製造工程	なし	あり	なし

別紙様式第4

感染症定期報告に関する今後の対応について

平成１６年度第５回
運営委員会確認事項
（平成１６年９月１７日）

1 基本的な方針

運営委員会に報告する資料においては、

(1) 文献報告は、同一報告に由来するものの重複を廃した一覧表を作成すること。

(2) 8月の運営委員会において、国内の輸血及び血漿分画製剤の使用した個別症例の感染症発生報告は、定期的にまとめた「感染症報告事例のまとめ」を運営委員会に提出する取り扱いとされた。これにより、感染症定期報告に添付される過去の感染症発生症例報告よりも、直近の「感染症報告事例のまとめ」を主として利用することとすること。

2 具体的な方法

(1) 感染症定期報告の内容は、原則、すべて運営委員会委員に送付することとするが、次の資料概要を作成し、委員の資料の確認を効率的かつ効果的に行うことができるようにする。

① 研究報告は、同一文献による重複を廃した別紙のような形式の一覧表を作成し、当該一覧表に代表的なものの報告様式（別紙様式第2）及び該当文献を添付した**「資料概要A」**を事務局が作成し、送付する。

② 感染症発生症例報告のうち、発現国が「外国」の血漿分画製剤の使用による症例は、同一製品毎に報告期間を代表する感染症発生症例一覧（別紙様式第4）をまとめた**「資料概要B」**を事務局が作成し、送付する。

③ 感染症発生症例報告のうち、発現国が「国内」の輸血による症例及び血漿分画製剤の使用による感染症症例については、「感染症報告事例のまとめ」を提出することから、当該症例にかかる「資料概要」は作成しないこととする。ただし、運営委員会委員から特段の議論が必要との指摘がなされたものについては、別途事務局が資料を作成する。

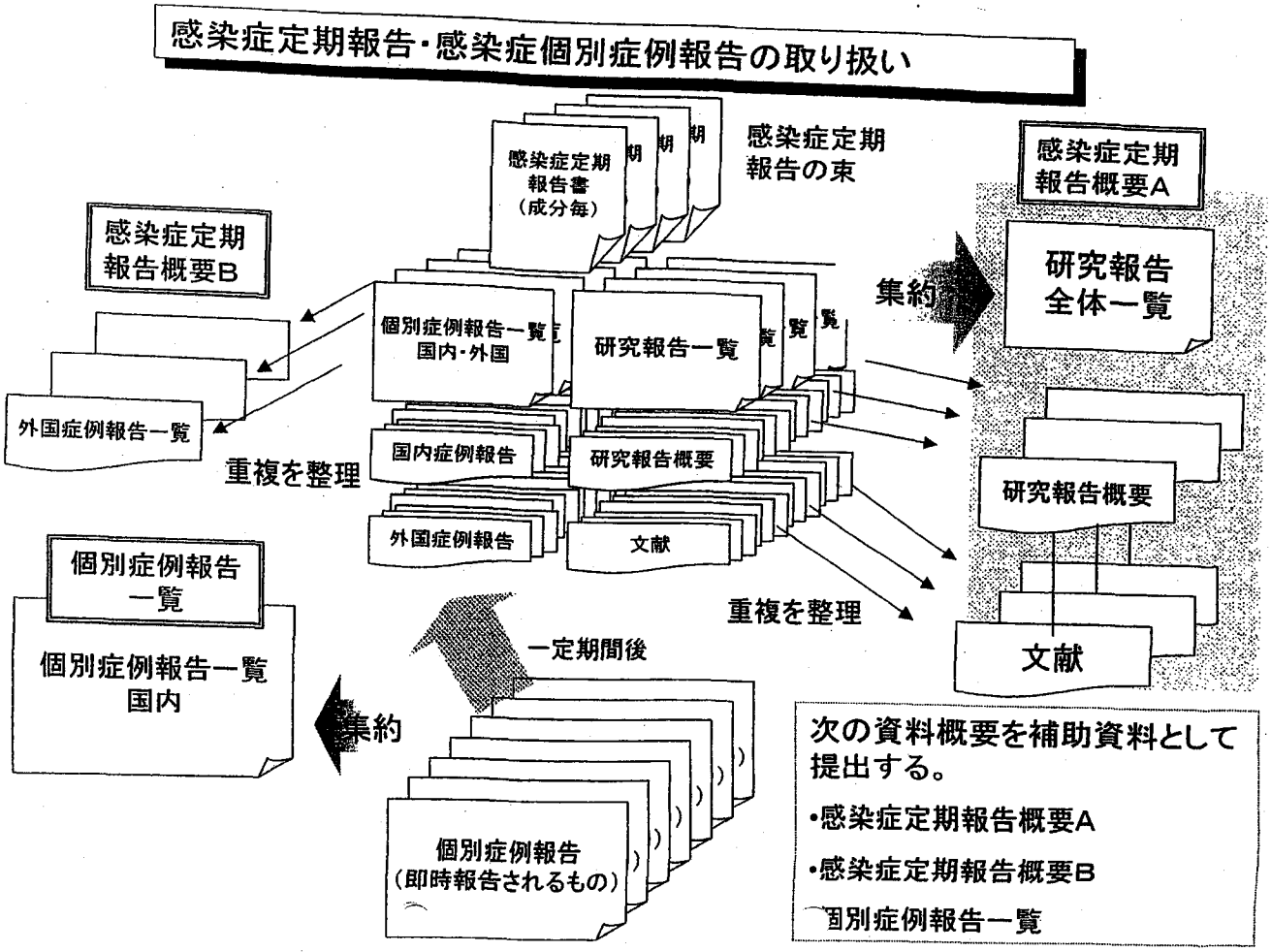
(2) 発現国が「外国」の感染症発生症例報告については、国内で使用しているロットと関係がないもの、使用時期が相当程度古いもの、因果関係についての詳細情報の入手が困難であるものが多く、必ずしも緊急性が高くないと考えられるものも少なくない。また、国内症例に比べて個別症例を分析・評価することが難しいものが多いため、緊急性があると考えられるものを除き、その安全対策への利用については、引き続き、検討を行う。

(3) 資料概要A及びBについては、平成16年9月の運営委員会から試験的に作成し、以後「感染症的報告について（目次）」資料は廃止することとする。

資料２－２

A　研究報告（詳細版）

平成２５年６月１２日
（平成２５年２月～平成２５年４月受理分）



別紙様式第２

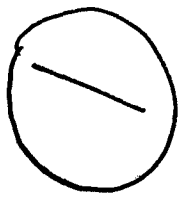
医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日 2013/01/16	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	フィブリノゲン配合剤		研究報告の公表状況	Hepatology2012:56(SUPPL.1)1100A	公表国 フランス	
販売名（企業名）	①タココンプ ②タココンプ組織接着用シート （CSLベーリング株式会社）					

研究報告の概要

47歳肝移植患者は急性肝炎を発症し急性肝炎Eウイルス(HEV)感染と診断された。部分配列決定では中国のウサギから分離された株と高い相同性が示された。
患者の血清を子ブタおよびウサギに静脈内投与し、感染実験を実施した。陰性対照として、それぞれの第三種の動物を用いた。ブタはDay32まで、ウサギはDay39まで、血清学およびリアルタイムHEV RNA検査で感染をモニターした。
全配列は、ウサギ株と平均81%の相同性、人と動物のジェノタイプ3株と平均77%の相同性を示した。子ブタは感染しないままであった。接種ウサギは感染後Day7～14の間、抗HEV抗体の一過性の内分泌を示したが、HEV RNAは検出できないままであった。配列相同性およびウサギの抗体検出の変動は、ウサギ由来であるか議論がある。これらの動物はヒトのジェノタイプ3および4の株に容易に感染するので、ブタが非感染であることは、非定型的な特徴である。
患者は海外渡航しておらず、レストランでシェフとして働き、ウサギを含む複数の死亡動物と接触した。フランスのウサギのHEV感染のデータはない。しかし、中国はフランスの食用新鮮ウサギおよび冷凍ウサギの第一のサプライヤーであることは注目に値する。類似株がヒトで報告されていないので、ウサギからヒトへの感染が稀なのは確かである。免疫抑制によりウイルス感染が起こった可能性がある。

使用上の注意記載状況・その他参考事項等



報告企業の意見	今後の対応
本剤の原薬の製造工程中で用いられるトロンボプラスチンの原料としてウサギが用いられている。 原薬製造工程および製剤製造工程にウイルス除去/不活化工程（ナノフィルトレーション、γ線滅菌等）を含むことから、HEV に対する本剤の安全性への影響は取られていると考える。	今後とも新しい感染症に関する情報収集に努める所存である。

temic and neuroinflammation) was observed in saline-injected BDL, compared to sham pigs. In BDL pigs, brain water was increased with altered cerebral haemodynamics. In BDL pigs PDGFRb expression was significantly increased and highly localised to surrounding small microvessels at the level of capillary beds, with no phosphorylated PDGFRb detected in any sham pig. Conclusion: The results suggest that in our porcine model of cirrhosis, persistent pericyte activation within the capillary bed of the brain, may influence cerebral haemodynamics and play a pivotal role in the cirrhotic brain phenotype. Whether this is a compensatory mechanism to limit severe brain oedema, or causal, along with any interaction with inflammation and hyperammonaemia is the focus of ongoing study.

Disclosures:
Rajiv Jalan - Consulting: Ocera Therapeutics, Conatus
The following people have nothing to disclose: Gavin Wright, D. Bloett, P. Siegler, Vanessa Stadlbauer

1951
Myeloid cells require IL-6/gp130 signaling for protective anti-inflammatory functions during bacterial peritonitis

Sara D. Sackett, Leif E. Sander, Antje Mohs, Sonja Strauch, Daniela C. Kroy, Konrad L. Streetz, Christian Trautwein; RWTH University Hospital Aachen, Aachen, Germany

Background: Sepsis and sepsis related deaths represent a major complication of patients suffering from liver cirrhosis. In particularly, spontaneous bacterial peritonitis (SBP) is a severe bacterial complication of liver cirrhosis and the mechanisms involved in SBP are not completely understood. We recently reported that gp130, the signaling receptor for IL-6 family cytokines, is important for crosstalk between hepatocytes and myeloid derived suppressor cells to control inflammation during sepsis. *Objective:* We hypothesized that gp130 signaling plays a role in the differentiation of innate immune cells during bacterial peritonitis (BP) and is therefore involved in controlling the host inflammatory response during infections of the peritoneum. *Methods:* to elucidate the role of gp130/IL6 signaling in hematopoietic cells we generated bone marrow specific gp130 knockout mice and their respective controls by bone marrow transplantation. Following re-engraftment of the hematopoietic system, cecal ligation and puncture, a murine model of BP, was performed to evaluate survival, organ damage, cytokine production and regulation as well as immune cell response. *Results:* Deficiency of gp130 in hematopoietic cells caused increased liver apoptosis and kidney damage and rendered mice more susceptible to sepsis-induced mortality due to unrestrained inflammation. Gp130 deficient myeloid cells failed to induce the expression of arginase1 and IL10, important immunosuppressive components, and instead express high levels TNF-α and IL-12 during peritonitis. Results from bone marrow derived macrophage (BMDM) experiments and gene expression analysis of FACS sorted exudate cells further demonstrate the defect in activation of anti-inflammatory programming. Furthermore, we show that the IL4 receptor, a downstream target of IL10, is reduced in BMDM. Additional in vivo and in vitro experiments show that this gene expression defect can be rescued by the exogenous addition of IL10 and significantly improves survival. *Conclusions:* Here we demonstrate a unique function of gp130 in promoting an anti-inflammatory phenotype and as a critical element for immune homeostasis in myeloid derived cells during BP. These results demonstrate that gp130 signals are required for efficient M2 skewing in vitro and in vivo. These data highlight the importance of gp130 regulation in the innate immune response during bacterial peritonitis and in macrophage activation and may provide a novel therapeutic approach for treatment of SBP.

Disclosures:
Christian Trautwein - Grant/Research Support: BMS, Novartis, BMS, Novartis; Speaking and Teaching: Roche, BMS, Roche, BMS
The following people have nothing to disclose: Sara D. Sackett, Leif E. Sander, Anje Mohs, Sonja Strauch, Daniela C. Kroy, Konrad L. Streetz

1952
First description of human infection due to a rabbit hepatitis E virus strain

Deborah Delaune[1], Nicole Pavio[2], Eric Marchadier[1], Olivier Chazouillères[3], Anne-Marie Roque-Afonso[4,5], [1]AP-HP, Hopital Paul Brousse, National Reference Centre for HAV and HEV, Villejuif, France; [2]ANSES, Maison-Alfort, France; [3]AP-HP, Hopital Saint Antoine, Hepatology, Paris, France; [4]Inserm, Unité 785, Villejuif, France

A 47-years-old liver-transplant patient developed an acute hepatitis in June 2011. Acute hepatitis E virus (HEV) infection was diagnosed on the basis of positive anti-HEV IgM and detectable HEV RNA. Partial sequencing revealed a high homology with strains isolated from Chinese rabbits. To date, no human infections due to rabbit strains have been described. Full-length sequence was obtained and experimental infection of 2 piglets and 2 rabbits was performed by intravenous administration of patient's sera. A third animal of each species was used as a negative control. Infection was monitored by serology and real-time HEV RNA testing up to doy 32 for pigs and doy 39 for rabbits. The full length sequence presented a mean of 81 % homology with rabbit strains and a mean of 77 % homology with human and animal genotype 3 strains. Piglets remained uninfected as assessed by undetectable HEV RNA and negative serology. Inoculated rabbits presented a transitory secretion of anti-HEV antibodies between doy 7 and 14 post infection, but HEV RNA remained undetectable in all samples. Sequence homology and transitory detection of antibodies in rabbits are arguments for its rabbit's origin. Absence of pig infection further confirms atypical characteristics since these animals are readily infected by human genotypes 3 and 4 strains. The patient did not travel abroad. He works as a chef in a restaurant and is in contact with several dead animals, including rabbits. No data are available on HEV infection of French rabbits. However, it is noteworthy that China is the first supplier of fresh and frozen rabbits used in catering in France. Rabbit to human transmission is certainly a rare event since no similar strains have been reported in humans. Virus transmission may have been favoured by immunosuppression.

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The following people have nothing to disclose: Deborah Delaune, Nicole Pavio, Eric Marchadier, Olivier Chazouillères, Anne-Marie Roque-Afonso

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研究報告の概要

○ヒト血液由来E型肝炎ウイルス(HEV)ジェノタイプ3、4の培養系の確立ならびに本系を応用したHEV感染価定量系の構築について

背景:HEVは特に発展途上国や地域において経口感染すると考えられているが、近年先進国を含め世界中で拡がりを見せ、また輸血による感染が明らかとなり、懸念事項となっている。本研究はHEVの培養系を確立し、感染性因子低減化技術の有効性を評価するin vitroの感染価定量系を構築することを目的とした。HEVジェノタイプは4つに分類され、ジェノタイプ3(G3)が世界で最も多く見られる。G4は日本を含めアジアに多く、時に重症肝炎を引き起こす。また、血液から検出されたウイルスは、糞便から得たウイルスとは異なる表面構造を持つことが近年報告されている。それゆえ、培養系の確立と、それを応用した感染価定量系の構築において、日本のHEV RNA陽性血液検体から一般的に分離されるG3とG4を用いるのは妥当である。

方法:G3またはG4のHEVを含むHEV特異的IgM及びIgG陽性または陰性の血液検体14例を用い、ヒト肝癌細胞株(PLC/PRF/5)及びヒト肺腺癌細胞株(A549)に接種した。HEVに感染させた細胞は維持培地にて培養した。HEV RNAコピー数はRT-PCR法で測定した。HEVの感染性については培養3週間後に回収した培地における子孫ウイルスの検出により確認した。

結果:特異的IgMを含む血液検体由来のJRC-HE3株(G3)及びIgMとIgGを含むUA1株(G4)を用いた培地から子孫ウイルスが検出された。いずれの検体でも細胞変性は確認されなかった。極めて感染性の高いJRC-HE3株の感染効率(1組織培養感染量)としては、$10^{5.5}$RNAコピー数が必要であった。電子顕微鏡による観察で、感染細胞の培養上清中にHEVと見られる球形粒子が確認された。数回のウイルス継代と長期の培養を行っても、JRC-HE3にアミノ酸変異はほとんど認められなかった。本感染価定量系をMirasol感染性低減化技術に応用したところ、2log以上の感染性低減化が確認された。

結論:HEV RNA陽性血液検体から得たHEV G3とG4を用いてHEV培養系及びHEV感染性の力価測定を評価するin vitroシステムを確立した。このシステムはウイルス不活化技術の性能評価にも有用であろう。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血
新鮮凍結血漿-LR「日赤」120
新鮮凍結血漿-LR「日赤」240
新鮮凍結血漿-LR「日赤」480

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
HEV RNA陽性血液検体から得たHEVジェノタイプ3と4を用いてHEV培養系を確立し、それを応用した感染価定量系を構築したとの報告である。	今後も引き続き情報の収集に努める。

別紙3

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that in the *env* region, regardless of the results of WB (Table). Conclusion: It is speculated that the amino acid substitutions including the start-codon, stop-codon, and deletion mutations in the *env* region are associated with the indeterminate or negative WB results of HTLV-1-infected individuals.

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Frequencies of Amino Acid Substitutions

Results of WB	Total Number of Blood Donors	Observed Number of Amino Acid Substitutions	
		env	*gag*
Indeterminate	45	72	20
Negative	4	8	1
Positive	37	25	11

SP455

Establishment of Culture System for Hepatitis E Virus (HEV) Genotypes 3 and 4 Originating from Human Blood, and Application of this System to the Assessment of HEV Infectious Titer

T Owada[1], (t-owada@jrc.or.jp), M Kaneko[1], C Matsumoto[1], K Mio[2], S Uchida[1], M Satake[1], K Tadokoro[1]; [1]Central Blood Institute, Japanese Red Cross Society, Tokyo, Japan; [2]Biomedicinal Information Research Center, National Institute of Advanced Industrial Science and Technology, Tokyo, Japan

Background/Case Studies: The hepatitis E virus (HEV) has been considered to be orally transmitted particularly in developing countries and regions. However, recently, HEV has been found to be spreading worldwide including in industrialized nations and has also been demonstrated to be transmitted by blood transfusion. Thus, the possibility of HEV infection via transfusion is a major concern globally. The objectives of this study are to establish a culture system for HEV and an in vitro system for the evaluation of the efficacy of pathogen reduction technology. HEV is classified into four genotypes, among which genotype 3 (G3) is one of the most common worldwide, and G4 is mainly spreading in Asia, including Japan, and occasionally causes severe hepatitis. Moreover, it has recently been reported that the virus obtained from blood has a different composition of the envelope-like structure from that obtained from feces. Hence, it is reasonable to use G3 and G4 from HEV-RNA-positive blood specimens commonly isolated in Japan when we try to develop a culture system and to expand this system to a methodology for evaluating HEV infectious titer referred to as 'tissue culture infectious dose (TCID). **Study Design/Methods:** Fourteen specimens containing HEV of the G3 or G4 were used, which were either positive or negative for HEV-specific IgM and IgG. The cell lines of human hepatoma cells (PLC/PRF/5) and human lung adenocarcinoma cells (A549) were inoculated with viral specimens for 2 h at 37°C. The cells infected with HEV were incubated in a maintenance medium containing 30 mM Mg^{2+} and 2% FCS. The medium was collected and replaced with a fresh one every week. HEV RNA copies were determined by real-time RT-PCR analysis. The establishment of HEV infectivity was confirmed by the detection of viral progenies in recovered media after 3 weeks of incubation. **Results/Findings:** Viral progenies were detected in recovered media when the JRC-HE3 strain (G3) from blood specimens containing specific IgM or the UA1 strain (G4) containing IgM and IgG was used. No cytopathic effect was observed in any specimens. We found 1 TCID of the highly infectious strain JRC-HE3 corresponds to approximately $10^{4.4}$ RNA copies. Clear spherical particles that were likely to be HEV were found in the culture supernatant of infected cell lines by electron microscopy. Amino acid substitution in JRC-HE3 scarcely occurred after several viral passages and long-term cultivation. Using this titration system, we found a log reduction greater than 2 for HEV infectivity when the Mirasol Pathogen Reduction System (Terumo BCT) was used. **Conclusion:** We established an HEV culture system using G3 and G4 from HEV-RNA-positive blood specimens and an in vitro system for the titration of HEV infectivity. These systems could be useful for assessing the performance of a viral inactivation technology.

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SP457

Flow Cytometry Shows Biochemical Variations and Structural Changes in RBC Upon Binding of West Nile and Dengue Viruses

C Chancey[1] (caren.chancey@fda.hhs.gov), A Teixeira-Carvalho[2], G Añez[1], L M Espina[1], M Ríos[1]; [1]DETTD, FDA-CBER, Bethesda, MD, United States; [2]CPqRR, FIOCRUZ, Belo Horizonte, Brazil

Background/Case Studies: *West Nile (WNV) and Dengue virus (DENV)* are enveloped positive-strand RNA viruses from the genus *Flavivirus*, family Flaviviridae. WNV and DENV are primarily transmitted to hosts by mosquito bites, but transmission can also occur from human-to-human by blood transfusion. In specimens from WNV- or DENV-positive blood donors, infectious virus bound to red blood cells (RBC) has been detected at levels equivalent to those found in plasma (for DENV) or up to one log higher than in plasma (WNV). We hypothesize that flavivirus-RBC adherence has a role in viral dissemination and pathogenesis, and the understanding of this adherence is important for development of improved diagnostics and therapeutics. In this study, we used flow cytometry to investigate the nature of WNV and DENV binding to RBC. **Study Design/Methods:** DENV-2 (New Guinea C strain) and WNV/DENV4-E30 chimera (attenuated virus containing structural proteins of WNV and non-structural proteins of DENV4-E30 vaccine construct) were grown in Vero cells, purified from culture supernatants, and labeled with a fluorescent probe. RBC were separated from whole blood collected in either ACD or EDTA, washed, and resuspended in 0.9% saline. Labeled virus was allowed to bind to RBC for 1 hour, RBC were washed, and data was collected using a digital flow cytometer. **Results/Findings:** Both DENV and *WNV/DENV4-E30 bound to RBC under varying buffer and anti-coagulant conditions.* DENV showed greater binding when EDTA was used as the anti-coagulant, and WNV/DENV4-E30 showed greater binding when ACD was used as the anti-coagulant and when Ca^{2+}/Mg^{++} were absent from the binding buffer. Approximately four times as much DENV was required to bind RBCs to yield the same mean fluorescence intensity (MFI) as observed when WNV/DENV4-E30 binds. Both viruses caused damage to RBC at virus: RBC ratios greater than 10:1, and WNV caused lysis of RBCs at 25:1. **Conclusion:** Flow cytometry is useful for investigating the frequency and intensity of WNV and DENV binding to RBC, and the consequent morphological alterations. The optimal conditions for WNV and DENV binding to washed RBC are slightly different, suggesting that there may be biochemical differences between the viruses and potential RBC ligands. The difference between DENV and WNV in amount of input virus required to reach the same MFI suggests that WNV binds RBC more efficiently than does DENV, which is consistent with previous studies on DENV and WNV distribution in blood donor samples. Although the amounts of virus used are higher than those that would be encountered in clinical samples, the damage to RBC caused by WNV and DENV is notable and future studies into the mechanism of membrane disruption are needed.

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SP458

Influenza A Virus (H5N1) Can Be Transmitted Through Blood Transfusion in Ferrets

X Wang[1,2] (xue.wang@fda.hhs.gov), J Tan[1,2], P Zhang[1,2], J Zhao[1,2], I Hewlett[1,2]; [1]CBEER/DETTD, FDA, Bethesda, MD, United States; [2]FDA, Bethesda, MD, United States

Background/Case Studies: Some highly pathogenic strains of Influenza A virus can infect multiple organs other than the respiratory system. It has been reported that virus can be detected in blood during infection with both *avian H5N1 viruses and pandemic H1N1 (swine)* viruses, and is associated with severe case. It is unknown that virus infectivity in blood. **Study Design/Methods:** Using the susceptible ferret animal model, we studied the infectiv-

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研究報告の概要

凝固因子濃縮製剤の複数の不活性化の導入は、実質的に過去 20 年間の重要な病原性に関連付けられているウイルスによる感染症を排除している。
現時点で導入可能なウイルス不活化の方法は、パルボウイルス B19 やプリオンを除去することができないことが知られており、感染の伝播に関する理論的な懸念が残っている。
血液製剤における新しいパルボウイルスであるヒトパルボ 4 (PARV4) や新しい遺伝子型のパルボウイルス B19 の発見後、懸念されている。
パルボウイルスは、ヒト免疫不全ウイルスやＣ型肝炎ウイルスのように慢性的な病原性はないが、免疫の抑制された患者では臨床症状を引き起こす可能性がある。
製造業者は既知のウイルスが含まれていないことを保証するために、ミニプールでのポリメラーゼ連鎖反応テストなどの対策を講じている。
これまでのところ、ヒトのボカウイルス、パルボウイルスは、分画された血液製剤で検出されておらず、それらの存在が実証されない限り、製造中のルーチン検査は必須ではない。
患者と血液製剤の安全性の継続的な監視は重要な事項である。

報告企業の意見	今後の対応
血漿分画製剤におけるパルボウイルスなどに関する懸念の情報である。 献血血漿については採血時に日本赤十字社でヒトパルボウイルス B19 の抗原検査(RHA 法)を輸入血漿については当社で NAT 検査を実施している。 また、当社血漿分画製剤は最終製品において NAT 検査を行い、パルボウイルス B19DNA 陰性であることを確認している。	今後ともパルボウイルスなどに関する安全性情報等に留意していく。

使用上の注意記載状況・その他参考事項等

慎重投与(次の患者には慎重に投与すること)
・溶血性・失血性貧血の患者［ヒトパルボウイルスＢ19 の感染を起こす可能性を否定できない。感染した場合には、発熱と急激な貧血を伴う重篤な全身症状を起こすことがある。］
・免疫不全患者・免疫抑制状態の患者［ヒトパルボウイルスＢ19 の感染を起こす可能性を否定できない。感染した場合には、持続性の貧血を起こすことがある。］
重要な基本的注意
(1)本剤の原材料となる‥［スクリーニング項目、不活化・除去工程］‥投与に際しては、次の点に十分注意すること。
1)血漿分画製剤の現在の製造工程では、ヒトパルボウイルス B19 等のウイルスを完全に不活化・除去することが困難であるため、本剤の投与によりその感染の可能性を否定できないので、投与後の経過を十分に観察すること。
妊婦、産婦、授乳婦等への投与
妊婦又は妊娠している可能性のある婦人には治療上の有益性が危険性を上回ると判断される場合にのみ投与すること。［妊娠中の投与に関する安全性は確立していない。本剤の投与によりヒトパルボウイルス B19 の感染の可能性を否定できない。感染した場合には胎児への障害（流産、胎児水腫、胎児死亡）が起こる可能性がある。］

bjh review

Parvovirus transmission by blood products – a cause for concern?

Päivi Norja,[1] Riitta Lassila[2] and Mike Makris[3,4]

[1]Department of Virology, Haartman Institute, Helsinki University, [2]Coagulation disorders Unit, Division of Haematology, Department of Internal Medicine, Helsinki University Central Hospital, Helsinki, Finland, [3]Department of Cardiovascular Science, University of Sheffield, and [4]Sheffield Haemophilia and Thrombosis Centre, Royal Hallamshire Hospital, Sheffield, UK

Summary

The introduction of dual viral inactivation of clotting factor concentrates has practically eliminated infections by viruses associated with significant pathogenicity over the last 20 years. Despite this, theoretical concerns about transmission of infection have remained, as it is known that currently available viral inactivation methods are unable to eliminate parvovirus B19 or prions from these products. Recently, concern has been raised following the identification of the new parvoviruses, human parvovirus 4 (PARV4) and new genotypes of parvovirus B19, in blood products. Parvoviruses do not cause chronic pathogenicity similar to human immunodeficiency virus or hepatitis C virus, but nevertheless may cause clinical manifestations, especially in immunosuppressed patients. Manufacturers should institute measures, such as minipool polymerase chain reaction testing, to ensure that their products contain no known viruses. So far, human bocavirus, another new genus of parvovirus, has not been detected in fractionated blood products, and unless their presence can be demonstrated, routine testing during manufacture is not essential. Continued surveillance of the patients and of the safety of blood products remains an important ongoing issue.

Keywords: haemophilia, coagulation, parvovirus, parvovirus 4, clotting factor concentrate.

Patients with inherited bleedings disorders, such as severe haemophilia A, B, von Willebrand disease (VWD), as well as other rare bleeding disorders suffer recurrent spontaneous and traumatic bleeds and are treated with intravenous infusions of the missing clotting factor, so-called replacement therapy.

The treatment of bleeding disorders has developed enormously from the use of fresh frozen plasma in the 1940s, cryoprecipitate in the 1960s, and clotting factor concentrates since the early 1970s. Over the last 20 years recombinant technology, aimed at avoidance of all animal- or human-derived proteins during the manufacture or final formulation of the coagulation factor concentrates, has been developed. Until recently, the major drive in concentrate development has been the reduction in infective risk. Today, the development of allo-antibodies to factor VIII (FVIII) (inhibitors) has taken over as the main problem in haemophilia management.

Plasma-derived clotting factor concentrates are prepared by fractionation of up to 30 000 pooled plasma units. Until viral inactivation was introduced in 1985, viral infections present in the donors could easily be transmitted to the recipient. The result was that virtually all recipients of concentrates prior to 1985 were infected with hepatitis C virus (HCV) and many were also infected with human immunodeficiency virus (HIV) and hepatitis B virus (HBV) (Makris *et al*, 1996). The viral elimination processes are based on destruction of the viruses with dry or wet heat treatment (sometimes under pressure), chemical treatment with combination of solvent and detergent (S/D), and nanofiltration (Mannucci & Tuddenham, 2001).

Viral elimination processes proved to be highly successful in virtually abolishing the risk of infection with HBV, HCV and HIV. However, in 1992, a number of outbreaks of hepatitis A transmission by concentrates were reported (Richardson & Evatt, 2000). This occurred due to the fact that hepatitis A, which does not have a lipid envelope, was resistant to the viral elimination by the S/D method used during manufacture. Subsequently, new regulations require that all plasma-derived clotting factor concentrates undergo two different viral elimination procedures before release.

Despite the success of the currently used viral elimination techniques, two infectious agent problems have remained, parvovirus B19 (B19) and prion transmission. As B19 is relatively resistant to all the currently available elimination methods, manufacturers introduced screening of mini-pools by the polymerase chain reaction (PCR). Positive minipools are not used in fractionation of blood products, but despite this, recent evidence suggests that the risk of B19 transmission is

Correspondence: Dr Mike Makris, Department of Cardiovascular Science, University of Sheffield, Royal Hallamshire Hospital, Sheffield S10 2JF, UK. E-mail: m.makris@sheffield.ac.uk

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real (Soucie *et al*, 2011). Variant Creutzfeld Jacob Disease (vCJD), a prion disease, is the human form of the Bovine Spongiform Encephalopathy (BSE) that appeared in the UK cattle population in the 1980s. Transmission by blood products and clotting factor concentrates has been demonstrated (Peden *et al*, 2010). Prions are highly resistant to all currently used elimination techniques but because of geographical restriction of vCJD, some countries have chosen to avoid using plasma collected in those countries, e.g., the UK (Millar *et al*, 2010).

Further viral newcomers in this field include human parvovirus 4 (PARV4), which has been linked with intravenous drug administration, both for therapeutic use, such as for bleeding disorders, and recreational abuse. In recognition of the difficulty of eliminating infective agents from clotting factor concentrates, some countries (e.g., UK, Canada and Ireland) decided to use exclusively recombinant clotting factors, when these became available. This can be envisioned as protectionism in the countries where this kind of national transitions have not been undertaken. The current plasma-derived products have proven safe, at least so far.

Our objective here is to illustrate that, in association with intravenous repetitive coagulation factor replacement therapies – despite the current precautions – the risk of viral transmittance cannot be completely excluded. The surveillance of both old and new patients and concentrates remains our continued task.

We live in a world where new disease entities and viral epidemics continue to emerge in various locations and, with the current air travel frequency, these diseases can spread quite rapidly. The recently identified west Nile virus and swine influenza are examples of suddenly emerging pathogenic viruses that have a relatively strong penetrance and cause local and even worldwide epidemics. Additionally, prions are an example of pathogens, which are very hard to detect and the success of their elimination remains unclear for many years, with potentially serious consequences.

Parvovirus taxonomy and basic features

The name *parvovirus* originates from the Latin word *parvum*, which means small; parvoviruses are among the smallest known viruses with a virion diameter of 18–26 nm. Parvoviruses infect a wide range of vertebrates and insects causing systemic infections. The family *Parvoviridae* is divided into two sub-families: Parvovirinae and Densovirinae (http://ictvonline.org/virusTaxonomy.asp?version=2009). Viruses from the sub-family Parvovirinae infect vertebrate cells and are divided further into five genera (Table I): *Parvovirus*, *Dependovirus*, *Erythrovirus*, *Bocavirus*, and *Amdovirus*. Of these, *Erythrovirus*, *Dependovirus* and *Bocavirus* genera contain viruses infecting humans. In addition, a sixth genus, *Partetravirus*, containing human PARV4 and human PARV4-like viruses, has been proposed. The sub-family of Densovirinae contains viruses of invertebrates.

Table I. The taxonomy of parvoviruses.

Subfamily	Genus	Example virus, abbreviation
Parvovirinae	*Parvovirus*	*Minute virus of Mice, MVM*
		Canine parvovirus, CPV
	Dependovirus	*Adeno-assosiated virus AAV*
		Goose parvovirus
	Erythrovirus	*Human parvovirus, B19*
		Simian parvovirus, SPV
	Bocavirus	*Bovine parvovirus, BPV*
		Human bocaviruses 1-4, HBoV1-4
	Amdovirus	*Aleutian mink disease virus, AMVD*
	Partetravirus	*Human partetravirus, PARV4*
	(proposed)	*Porcine hokovirus, PHoV/PPV3*
Densovirinae	*Densovirus*	*Junonia coenia densovirus*
	Brevidensovirus	*Aedes aegypti densovirus*
	Iteravirus	*Bombyx mori densovirus*
	Pefudensovirus	*Periplanta fuliginosa densovirus*

Fig 1. Genome structure and protein encoding reading frames of B19.

The structure of parvoviruses is simple; the icosahedral virion consists of only proteins and linear single-stranded-DNA genome with hairpin structures at both ends. The hairpins are palindromic and the 3′-end can fold and function as a primer during viral replication (Fig 1). The length of the DNA genome is approximately 5–6 kb. In the parvovirus infection cycle, the virus attaches to its receptor, e.g. globoside (P-antigen) in case of human B19, on the surface of host cells (Brown *et al*, 1993) and is transported into the cell by endocytosis. Inside the host cell, the virion is transported to the nucleus where parvovirus replication takes place. Parvoviruses do not encode their own DNA-polymerase, indicating that all parvoviruses are dependent on (i) host cell polymerase and (ii) S-phase of dividing cells. In the case of dependoviruses, co-infection with another virus is needed for efficient DNA replication (Atchison *et al*, 1965).

General aspects and epidemiology of human and porcine parvoviruses

Parvovirus B19

Human parvovirus B19 (B19) is the type species of the *Erythrovirus* and representative member of parvoviruses. B19 was discovered when a serum sample from an asymptomatic blood donor gave a false-positive result in an immunoelectrophoresis assay for HBV (Cossart *et al*, 1975). The virus was detected in panel B and was coded 19, from which its name originates. The most common transmission route of B19 is respiratory, but it can also transmit via

plasma-derived medical products and from mother to fetus. B19 infections are prevalent worldwide, and seroprevalence studies based on B19 IgG have shown that in Europe 60–80% of adults have been infected with this virus during their lifetime (Mossong *et al*, 2008). In Asia the B19 seroprevalence in blood donors was found to be 25–40% (Kishore *et al*, 2010, Ke *et al*, 2011).

B19 DNA prevalence in blood donors has been reported by a number of studies. The rates of positivity were 0·88% in the USA (Kleinman *et al*, 2007), 0·2% in the Netherlands (Koppelman *et al*, 2011) and 0·55–1·3% in the UK and Africa (Candotti *et al*, 2004).

B19 is erythrotropic and replicates in erythroid progenitor cells in human bone marrow. After short viraemia the virus is eliminated from the blood circulation by neutralizing antibodies produced by the host. However, B19 genomic DNA remains detectable in solid tissues of seropositive individuals. Initially, B19 DNA was detected in the synovium of patients with rheumatoid arthritis but, in 1997, the viral DNA was also detected in 48% of synovia collected from healthy controls (Saal *et al*, 1992; Soderlund *et al*, 1997). Later, B19 was shown to persist with full-length coding capacity in several tissue types of both symptomatic and asymptomatic persons, most probably for a lifetime (Soderlund-Venermo *et al*, 2002; Norja *et al*, 2006; Manning *et al*, 2007).

B19 strains have been divided into three divergent genotypes according to their genomic sequence. Genotype 1 is the prototypic virus and is nowadays globally the most predominant circulating virus (Hubschen *et al*, 2009). The genotype 2 virus was first identified in human skin and in the serum of an Italian HIV-positive patient with chronic anaemia (Hokynar *et al*, 2002; Nguyen *et al*, 2002). Genotype 2 has since been found in human solid tissues but only sporadically in blood and seems to have disappeared from wide circulation after the 1970s (Blumel *et al*, 2005; Norja *et al*, 2006; Manning *et al*, 2007; Grabarczyk *et al*, 2011, Koppelman *et al*, 2011). The genotype 3 virus was found in France in the serum and bone marrow of a child with transient aplastic anaemia (Nguyen *et al*, 1999). Following its discovery, genotype 3 has been reported to be endemic in Ghana and Brazil (Candotti *et al*, 2004; Sanabani *et al*, 2006; Freitas *et al*, 2008; Keller *et al*, 2009). Since identification of the genotypes 2 and 3, many commercial and in-house PCR-methods have been shown to detect these B19 genotypes with lower sensitivity or fail to detect either or both of these genotypes (Hokynar *et al*, 2004; Baylis, 2008).

Bocavirus

Human bocavirus 1 (HBoV1) was first identified in 2005 by random molecular screening and large-scale sequencing. HBoV1 was discovered in Sweden, in nasopharyngeal aspirates of children with respiratory tract infections (Allander *et al*, 2005). It belongs to the genus *Bocavirus* and its closest relatives are the bovine parvovirus (BPV) and the minute virus of canines (MVC). The receptors and target cells of HBoV1 are unknown and, to date, HBoV1 has been cultured only in pseudo-stratified human airway epithelium cell culture system (Dijkman *et al*, 2009). Using similar methods of random amplification, three additional human bocaviruses were identified in faecal samples in 2009 and 2010 (Arthur *et al*, 2009; Kapoor *et al*, 2009, 2010). These new HBoVs were named HBoV2, HBoV3 and HBoV4. Of these HBoV2 seems to be the most prevalent and circulates globally (Arthur *et al*, 2009; Kapoor *et al*, 2009, 2010; Chow *et al*, 2010; Kantola *et al*, 2010).

The seroprevalence of HBoV1 has been reported to be more than 90% in adults. However, the HBoV1-4 viral-like particles used in the enzyme-linked immunosorbent assay (ELISA) have been shown to cross-react, which might affect the serological assays. Norja *et al* (2012) detected an HBoV1 seroprevalence of 94·9%, but after removing cross-reacting antibodies the rate was 68·4%. Similar results were obtained by Kantola *et al* (2011), who observed that adult HBoV1 seroprevalence decreased, from 96 to 59%, after removing the cross-reacting antibodies. The Kantola study reported HBoV2-4 seroprevalences among adults of 34% for HBoV2, 15% for HBoV3, and 2% for HBoV4 (Kantola *et al*, 2011). As far as we are aware, human bocavirus DNA has not been detected in blood donations.

Parvovirus 4

PARV4 was identified in 2005 in a HBV-positive intravenous drug abuser with various viral infection-related symptoms by similar methods to the HBoVs (Jones *et al*, 2005). During the following year, a related virus variant (PARV5) was identified in plasma pools used in the manufacture of plasma-derived medicinal products (Fryer *et al*, 2006). Subsequently, the name PARV5 was changed to PARV4 genotype 2 (Fryer *et al*, 2007a). In 2008, a third genotype of PARV4 was identified in HIV-infected African patients (Simmonds *et al*, 2008). DNAs for PARV4 genotype-1 and -2 have been found in bone marrow, lymphoid tissue, and liver of subjects with a history of intravenous drug use, or HIV, or HCV infection (Manning *et al*, 2007; Simmonds *et al*, 2007; Longhi *et al*, 2007; Schneider *et al*, 2008a). In addition, several studies described PARV4 DNA in donor blood samples and coagulation factor concentrates (Fryer *et al*, 2006, 2007a,b; Lurcharchaiwong *et al*, 2008; Schneider *et al*, 2008b). Initially the parenteral transmission route was proposed for PARV4, but the genotype 3 of PARV4 has also been found in subjects without a risk of parenteral exposure (Simmonds *et al*, 2008; Panning *et al*, 2010).

Porcine parvovirus

Porcine parvovirus (PPV1) was first isolated in Germany and the USA in 1965 and today it is found worldwide

(Csagola *et al*, 2012). PPV1 belongs to the genus *Parvovirus*. PPV1 is transmitted oronasally among seronegative dams (female parents) and the virus is then further transmitted through the placenta to fetus, causing reproductive failure. During the last decade, several new parvoviruses have been identified in pigs, including porcine hokovirus (PHoV/PPV3), which is related to PARV4 (Csagola *et al*, 2012).

Human diseases caused by parvoviruses

Until the discovery of HBoVs and PARV4, human B19 was considered to be the only pathogenic parvovirus to humans. The adeno-associate viruses of *Dependovirus* genus are non-pathogenic and are studied as vectors for gene-therapy. Although B19 is associated with various clinical manifestations, subclinical infection is a common finding among both children and adults. In healthy, immunocompetent subjects, B19 infection is usually mild and transient, requiring no treatment.

The most common B19 manifestation among children is a rash causing the disease *Erythema infectiosum* (EI), fifth disease or 'slapped cheek', and arthritis among adults (Anderson *et al*, 1984). In EI, the rash typically appears first on the cheeks, spreading to the neck, trunk, and limbs. In addition, the patient may have headache, fever, nausea, and diarrhoea. Among adults, arthritis can be the only manifestation of B19 infection, affecting 45–80% of infected subjects (Anderson *et al*, 1985; Reid *et al*, 1985; White *et al*, 1985). Joint symptoms are symmetrical and affect fingers, wrists, ankles, and knees. Arthritis is usually transient but in some cases it may be prolonged and fulfill the criteria of rheumatoid arthritis (Naides *et al*, 1990).

Women without B19-specific antibodies are at risk of primary B19 infection and trans-placental transmission. During maternal infection, the risk of vertical transmission is approximately 30% (Brown, 2010). Intrauterine B19 infection has been associated with fetal anaemia, hydrops, miscarriage, and fetal death (Enders *et al*, 2006, 2008).

In subjects with shortened red-cell survival, such as sickle cell disease, B19-infection may lead to aplastic crisis (Pattison *et al*, 1981). Among immunosuppressed subjects with decreased ability to produce antibodies, including patients with leukaemia or lymphoma (Kurtzman *et al*, 1988, 1989) or in the HIV/HCV-infected, the B19 infection may become persistent causing chronic anaemia.

Following the HBoV1 discovery, a large number of studies of the prevalence of HBoV1 have been undertaken in respiratory secretions of young children. According to recent studies, primary infections of HBoV1 are significantly associated with respiratory illnesses, including wheezing, pneumonia, and otitis media (Soderlund-Venermo *et al*, 2009; Don *et al*, 2010; Meriluoto *et al*, 2012). HBoV1 has also been detected in faeces from children with symptoms of gastroenteritis. However, the significance of HBoV1 as an enteric virus is questionable, because in many subjects another enteric virus was detected simultaneously with HBoV1, and there is a lack of evidence of replication of HBoV1 in the enteric tract (Albuquerque *et al*, 2007; Yu *et al*, 2008, Szomor *et al*, 2009). HBoV2 instead may cause gastroenteritis in young children (Kapoor *et al*, 2009; Chow *et al*, 2010; Kantola *et al*, 2010).

So far, no disease associations have been confirmed for PARV4 (Lahtinen *et al*, 2011). The virus has been linked to encephalitis (Benjamin *et al*, 2011),. and detected in the blood of three mothers bearing newborns with hydrops (Chen *et al*, 2011). Among patients with haemophilia, clinical presentations concurrent with PARV4 seroconversion were rash and unexplained hepatitis (Sharp *et al*, 2012). The individual in whom PARV4 was first identified presented with fatigue, vomiting, arthralgia, neck stiffness, night sweats, and diarrhoea, but this patient was lost to follow up, and it is not known if the described symptoms were associated with the PARV4 infection (Jones *et al*, 2005).

There are no antiviral drugs or vaccines against human parvovirus infection. However, among immunocompetent patients, treatment is unnecessary and infections are self cleared. Immunodeficient patients with chronic B19 infection, and patients with transient aplastic anaemia and B19, can be managed with intravenous immunoglobulin or erythrocyte transfusions (Frickhofen *et al*, 1990; Koduri *et al*, 1999).

Parvoviruses in blood products

Parvovirus B19

The B19 titre in blood is at its highest, up to 10^{13} genome equivalents/ml blood, during the first days of acute infection. Infected subjects are usually asymptomatic when the viral titres are at their highest. This creates a risk of contaminating blood products by blood donors with asymptomatic B19 infection. Siegl and Cassinotti (1998) reported B19 DNA in 50–80% and in 30–50% of non-virally inactivated VIII concentrates and S/D-inactivated coagulation factor IX (FIX) concentrates, respectively. A more recent German study detected B19 DNA in 26% of coagulation factor concentrates of different types, collected between 2007 and 2008 (Modrow *et al*, 2011). The highest viral loads were observed in the intermediate purity FVIII /VWF concentrates. Because of its small and non-enveloped structure, B19 is relatively resistant to most viral inactivation procedures used in the manufacturing of medical blood-derived products (Willkommen *et al*, 1999; Koenigbauer *et al*, 2000; Schmidt *et al*, 2001) and B19 is only partially removable with small pore size nanofiltration (Burnouf-Radosevich *et al*, 1994).

In Europe, in an attempt to reduce the risk of B19 transmission by blood products, the blood derived products manufactured after 2004 are not allowed to contain B19 DNA of more than 10^4 iu/ml, and nucleic acid testing for B19 is

obligatory for S/D-treated human plasma products (European Pharmacopoeia Commission, Council of Europe European Directorate for the Quality of Medicines, 2011). Similar instructions are given by United States Food and Drug Administration (http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm). B19 DNA concentrations below the 10^4 iu/ml limit are not considered to lead to seroconversion (Brown *et al*, 2001). However, Soucie *et al* (2011) reported 1·7 times higher B19 IgG seroconversion rates among children who received plasma-derived factor concentrates screened for B19 levels than among children receiving recombinant products. The infectivity of B19 in the blood products is affected by both the level B19-specific IgG in the products and the immune status of the recipient.

In order to quantify the B19 positive units, the quantitative DNA amplification method is used, and primers designed to detect all three genotypes are required. Many in-house PCR methods and one commercial PCR method, designed before the identification of B19 genotypes, are prone to miss B19 genotypes 2 and 3 (Hokynar *et al*, 2004; Baylis *et al*, 2004; Koppelman *et al*, 2004, 2007). Consequently, some plasma pools have remained contaminated with an excessive level of B19. All three genotypes of B19 have been reported in clotting factor concentrates, however, with a reduced frequency for genotypes 2 and 3 (Schneider *et al*, 2004; Modrow *et al*, 2011). To obtain an accurate diagnosis and safety of blood products, methods with capability to detect all B19 genotypes should be used. A reference panel for B19 DNA genotypes by the World Health Organization Expert Committee on Biological Standardization (ECBS) was established at the end of 2009, and it is available for validation of B19 PCR-based detection assays for all three genotypes (Baylis *et al*, 2012). Furthermore, the most recent study of B19 levels in plasma donations described a commercial method for simultaneous B19 and HAV screening (Koppelman *et al*, 2012).

Human bocaviruses

Three studies have analysed whether HBoV1 occurs in blood donor plasma and plasma-derived coagulation factor concentrates (Fryer *et al*, 2007b; Eis-Hubinger *et al*, 2010; Modrow *et al*, 2011). None of the studies reported positive cases of HBoV1. The absence of detectable HBoV1 DNA in blood or plasma donors, however, may be due to the fact that HBoV1 infections are most common among young children (Soderlund-Venermo *et al*, 2009; Meriluoto *et al*, 2012) and less frequent among the blood donor population. Negative results could also be explained by low HBoV1 titres in donors, as possible low-level viraemia could remain undetectable in manufactured plasma pools containing hundreds or thousands of donations.

PARV4 DNA in blood-derived medical products

The first study of PARV4 in blood products was published soon after the virus was discovered and reported PARV4 DNA with prevalence of 5·1% in manufacturing plasma pools (Fryer *et al*, 2006). In this study both PARV4 genotype 1 and 2 DNAs were amplified, and the virus titres ranged between < 500 copies to 10^6 copies/ml. A year later, the same authors reported PARV4 genotype 1 and 2 DNAs in 4% of recently sourced plasma pools collected in Europe and the USA, in 21% of the older plasma pools collected between 1990 and 1993, in 2% of the blood collected from healthy blood donors and in 6% of febrile patients (Fryer *et al*, 2007b). Schneider *et al* (2008a) reported PARV4 in 1–33% of randomly selected plasma-derived concentrate pools. The higher frequency of the PARV4 was detected in the older concentrates manufactured 10 years earlier, but smaller amounts of PARV4 were also detectable in the currently used concentrates. Recently, a study from China reported PARV4 DNA in the blood of 16–22% healthy subjects, in 33% of HBV-infected subjects and in 41% of HCV-infected subjects (Yu *et al*, 2012). In addition, PARV4 DNA has been detected in 26% of blood donor plasma pools collected between 2007 and 2010 in China (Ma *et al*, 2012). Table II summarizes PARV4 DNA detected in plasma- and blood-derived medical products. Even if the disease associations of PARV4 are not currently known, the prevalences of PARV4, especially those detected most recently in France and China, raise a question of whether blood donor minipools should be tested by screening for PARV4 DNA similar to B19.

In contrast to the studies described in Table II, three studies performed in France and Germany analysed high numbers of blood donor plasma donations, minipools or coagulation factor concentrates and failed to detect any

Table II. PARV4 DNA findings in blood donor samples and coagulation factor concentrates.

References	Blood product	PARV4 DNA prevalence (%)
Fryer *et al* (2006)	Plasma pools	5·1
Fryer *et al* (2007a,b)	Plasma pools,	8·7
	individual plasma	4
Fryer *et al* (2007c)	FVIII concentrates	16
Lurcharchaiwong *et al* (2008)	Blood donor sera	3·95
Schneider *et al* (2008b)	Coagulation FFII, FVIII, FIX, activated prothrombin complex concentrates	21
Vallerini *et al* (2008)	Blood donor sera	1
Touinssi *et al* (2010)	Blood donor plasma	24·6
Ma *et al* (2012)	Plasma pools	26

PARV4 DNA positive samples (Servant-Delmas *et al*, 2009; Eis-Hubinger *et al*, 2010; Modrow *et al*, 2011). Whether these negative results are due to seasonal or geographical reasons need further studies. The unanswered question also is, whether the higher frequency of PARV4 in older blood products (Fryer *et al*, 2007a,b; Schneider *et al*, 2008b) represents a timely population-based hazard or whether the manufacturing processes, i.e., nanofiltration, have improved the elimination of the viruses more comprehensively. On the other hand, demonstration of virus genome in plasma-derived products does not translate to infectivity.

PARV4 and haemophilia

In a study of 35 persons with haemophilia from the UK and USA receiving replacement therapy, 15/35 (43%) were positive for the PARV4 IgG whereas only 1/35 (3%) of untreated family members were positive (Sharp *et al*, 2009). The concentrates involved in treatment were non-virally inactivated clotting factors issued from the late 1970s to the early 1980s. The methods of detection were serological, ELISA-type assays, detecting both anti-PARV4 IgG and IgM, developed by the group of Simmonds.

In a 5-year follow-up of a cohort of 194 haemophilia patients who were born between 1972 and 1982, 1–7% of patients/year seroconverted for PARV4 (Sharp *et al*, 2012). They were followed between 1989 and 1994 by 6 monthly blood sampling. At cohort enrolment, almost all patients were HCV-positive and 43% of patients were PARV4 IgG-positive. Among PARV4 seropositive subjects, 46% were HIV-positive and 38% HIV-negative, thus PARV4 exposure did not significantly associate with HIV infection. The active disease forms related to PARV4 detection were rash and exacerbation of hepatitis. PARV4 IgM became positive during acute infections. The concentrates involved were plasma-derived and had undergone S/D treatment and dry or wet heating processes. Overall, the seroprevalence and the risk of seroconversion are significantly higher in patients having replacement therapy than the background population or sibling controls.

Transmission of porcine parvovirus by the old Hyate C porcine concentrate

A serious complication of therapy of persons with haemophilia is the development of antibodies (inhibitors) against the clotting factor, which renders the concentrates ineffective in controlling bleeding. Porcine FVIII concentrate, Hyate C, has been used as a treatment of patients with congenital haemophilia and inhibitory antibodies. Hyate C was developed in 1980 and was manufactured by Ipsen Ltd (Slough, UK) from pig plasma. During the manufacturing process Hyate C underwent a number of purification steps, and cell culture was used to confirm the absence of viruses but, in contrast to human coagulation factor concentrates, it did not undergo viral inactivation. In 1996, PPV1 was found in several Hyate C products and its supply was suspended. The knowledge that many recipients of Hyate C were already infected by HIV and were immunosuppressed led to concerns that PPV1 could in some cases infect humans. Soucie *et al* (2000) detected PPV1 DNA in 95% of porcine FVIII concentrates and confirmed that PPV1 is a common, low level contaminant in Hyate C. However, none of the 98 recipients of Hyate C tested positive for PPV1 antibodies. Most pigs naturally have antibodies to PPV1, but there is no evidence of transmission to humans from physical contact between pigs and humans. In addition to PPV1, porcine hokovirus, closely related to PARV4, has recently been found in porcine plasma and FVIII preparations (Szelei *et al*, 2010). The theoretical risk that porcine parvoviruses could infect humans remains a concern, but, if employed, PCR screening and discarding the porcine parvovirus DNA positive samples could eliminate the risk of transmission. Porcine plasma-derived FVIII concentrates are no longer available and to our knowledge are not being developed but a recombinant porcine FVIII concentrate has recently started clinical trials.

Implications

New viruses and other disease-inducing agents will always continue to evolve. In the first 10 months of 2010 there were 2350 reports of outbreaks of infectious diseases in humans, plants and animals (www.promedmail.org). It must be appreciated that the identification of new infections internationally is the norm rather than the exception. The studies of PARV4 and haemophilia have shown that the virus can be transmitted via blood donations and plasma products, at least when the viral inactivation steps include the methods of S/D and heating. We do not know the infectivity frequency following nanofiltration, but it may eliminate the majority of the viral load (Schneider *et al*, 2008b). To date, the parvoviruses have not been proven to cause significant chronic pathogenesis in patients with a healthy immunological system. However, in patients already infected with HIV or otherwise immunocompromised, B19 has pathogenic consequences. Overall, the current data imply that viruses are able to escape the current plasma fractionation and purification steps.

For infections that are potentially transmissible by clotting factor concentrates, blood donors should be screened serologically and mini-pools of plasma should be genotyped with the virus load measured with PCR (EMA, 2010). The safety of the plasma-derived concentrates demands continuous watchful strategies and surveillance. The regulatory studies required for registration occur early in the introduction of the products onto the market and are not optimal at detecting infection transmission by agents other than hepatitis A/B/C, HIV and Parvovirus where acute seroconversion detection is possible. Continuous vigilance by the haemophilia community is required to identify infective problems early. Adverse event reporting studies, such as the European Haemophilia Safety Surveillance (EUHASS) system, or national spontaneous reporting schemes have the potential to identify problems, but alertness to new or unusual problems is required for unexpected events (Makris *et al*, 2011). In this way any unexpected clinically significant transmission of infection by plasma products can be traced and eliminated as rapidly as possible.

References

Albuquerque, M.C., Rocha, L.N., Benati, F.J., Soares, C.C., Maranhao, A.G., Ramirez, M.L., Erdman, D. & Santos, N. (2007) Human bocavirus infection in children with gastroenteritis, Brazil. *Emerging Infectious Diseases*, 13, 1756–1758.

Allander, T., Tammi, M.T., Eriksson, M., Bjerkner, A., Tiveljung-Lindell, A. & Andersson, B. (2005) Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 12891–12896.

Anderson, M.J., Lewis, E., Kidd, I.M., Hall, S.M. & Cohen, B.J. (1984) An outbreak of erythema infectiosum associated with human parvovirus infection. *The Journal of Hygiene*, 93, 85–93.

Anderson, M.J., Higgins, P.G., Davis, L.R., Willman, J.S., Jones, S.E., Kidd, I.M., Pattison, J.R. & Tyrrell, D.A. (1985) Experimental parvoviral infection in humans. *The Journal of Infectious Diseases*, 152, 257–265.

Arthur, J.L., Higgins, G.D., Davidson, G.P., Givney, R.C. & Ratcliff, R.M. (2009) A novel bocavirus associated with acute gastroenteritis in Australian children. *PLoS Pathogens*, 5, e1000391.

Atchison, R.W., Casto, B.C. & Hammon, W.M. (1965) Adenovirus-associated defective virus particles. *Science*, 149, 754–756.

Baylis, S.A. (2008) Standardization of nucleic acid amplification technique (NAT)-based assays for different genotypes of parvovirus B19: a meeting summary. *Vox Sanguinis*, 94, 74–80.

Baylis, S.A., Shah, N. & Minor, P.D. (2004) Evaluation of different assays for the detection of parvovirus B19 DNA in human plasma. *Journal of Virological Methods*, 121, 7–16.

Baylis, S.A., Ma, L., Padley, D.J., Heath, A.B., Yu, M.W.; Collaborative Study Group. (2012) Collaborative study to establish a World Health Organization International genotype panel for parvovirus B19 DNA nucleic acid amplification technology (NAT)-based assays. *Vox Sanguinis*, 102, 204–211.

Benjamin, L.A., Lewthwaite, P., Vasanthapuram, R., Zhao, G., Sharp, C., Simmonds, P., Wang, D. & Solomon, T. (2011) Human parvovirus 4 as potential cause of encephalitis in children, India. *Emerging Infectious Diseases*, 17, 1484–1487.

Blumel, J., Eis-Hubinger, A.M., Stuhler, A., Bonsch, C., Gessner, M. & Lower, J. (2005) Characterization of parvovirus B19 genotype 2 in KU812Ep6 cells. *Journal of Virology*, 79, 14197–14206.

Brown, K.E. (2010) The expanding range of parvoviruses which infect humans. *Reviews in Medical Virology*, 20, 231–244.

Brown, K.E., Anderson, S.M. & Young, N.S. (1993) Erythrocyte P antigen: cellular receptor for B19 parvovirus. *Science*(New York, N.Y.), 262, 114–117.

Brown, K.E., Young, N.S., Alving, B.M. & Barbosa, L.H. (2001) Parvovirus B19: implications for transfusion medicine. Summary of a workshop. *Transfusion*, 41, 130–135.

Burnouf-Radosevich, M., Appourchaux, P., Huart, J.J. & Burnouf, T. (1994) Nanofiltration, a new specific virus elimination method applied to high-purity factor IX and factor XI concentrates. *Vox Sanguinis*, 67, 132–138.

Candotti, D., Etiz, N., Parsyan, A. & Allain, J.P. (2004) Identification and characterization of persistent human erythrovirus infection in blood donor samples. *Journal of Virology*, 78, 12169–12178.

Chen, M.Y., Yang, S.J. & Hung, C.C. (2011) Placental transmission of human parvovirus 4 in newborns with hydrops, Taiwan. *Emerging Infectious Diseases*, 17, 1954–1956.

Chow, B.D., Ou, Z. & Esper, F.P. (2010) Newly recognized bocaviruses (HBoV, HBoV2) in children and adults with gastrointestinal illness in the united states. *Journal of Clinical Virology: The Official Publication of the Pan American Society for Clinical Virology*, 47, 143–147.

Cossart, Y.E., Field, A.M., Cant, B. & Widdows, D. (1975) Parvovirus-like particles in human sera. *Lancet*, 1, 72–73.

Csagola, A., Lorincz, M., Cadar, D., Tombacz, K., Biksi, I. & Tuboly, T. (2012) Detection, prevalence and analysis of emerging porcine parvovirus infections. *Archives of Virology*, 157, 1003–1010.

Dijkman, R., Koekkoek, S.M., Molenkamp, R., Schildgen, O. & van der Hoek, L. (2009) Human bocavirus can be cultured in differentiated human airway epithelial cells. *Journal of Virology*, 83, 7739–7748.

Don, M., Soderlund-Venermo, M., Valent, F., Lahtinen, A., Hedman, L., Canciani, M., Hedman, K. & Korppi, M. (2010) Serologically verified human bocavirus pneumonia in children. *Pediatric Pulmonology*, 45, 120–126.

Eis-Hubinger, A.M., Drexler, J.F., Reber, U., Panning, M., Drosten, C., Weber-Schehl, M., Hedges, D., Baylis, S.A. & Roth, W.K. (2010) Absence of detection of novel human parvoviruses in german plasma donations. *Transfusion*, 50, 266–267.

EMA (2010). Guideline on plasma-derived medicinal products. Document reference: EMA/CHMP/BWP/706271/2010. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/07/WC500109627.pdf

Enders, M., Schalasta, G., Baisch, C., Weidner, A., Pukkila, L., Kaikkonen, L., Lankinen, H., Hedman, L., Soderlund-Venermo, M. & Hedman, K. (2006) Human parvovirus B19 infection during pregnancy – value of modern molecular and serological diagnostics. *Journal of Clinical Virology: the Official Publication of the Pan American Society for Clinical Virology*, 35, 400–406.

Enders, M., Weidner, A., Rosenthal, T., Baisch, C., Hedman, L., Soderlund-Venermo, M. & Hedman, K. (2008) Improved diagnosis of gestational parvovirus B19 infection at the time of nonimmune fetal hydrops. *The Journal of Infectious Diseases*, 197, 58–62.

European Pharmacopoeia Commission, Council of Europe European Directorate for the Quality of Medicines (2011) Human plasma (pooled and treated for virus inactication), monograph 1646 (01/2011). In: European Pharmacopeia, 7th edn. Council of Europe, Strasbourg.

Freitas, R.B., Melo, F.L., Oliveira, D.S., Romano, C.M., Freitas, M.R., Macedo, O., Linhares, A.C., de A Zanotto, P.M. & Durigon, E.L. (2008) Molecular characterization of human erythrovirus B19 strains obtained from patients with several clinical presentations in the Amazon region of Brazil. *Journal of Clinical Virology: the Official Publication of the Pan American Society for Clinical Virology*, 43, 60–65.

Frickhofen, N., Abkowitz, J.L., Safford, M., Berry, J.M., Antunez-de-Mayolo, J., Astrow, A., Cohen, R., Halperin, I., King, L. & Mintzer, D. (1990) Persistent B19 parvovirus infection in patients infected with human immunodeficiency virus type 1 (HIV-1): a treatable cause of anemia in AIDS. *Annals of Internal Medicine*, 113, 926–933.

Fryer, J.F., Kapoor, A., Minor, P.D., Delwart, E. & Baylis, S.A. (2006) Novel parvovirus and related variant in human plasma. *Emerging Infectious Diseases*, 12, 151–154.

Fryer, J.F., Delwart, E., Bernardin, F., Tuke, P.W., Lukashov, V.V. & Baylis, S.A. (2007a) Analysis of two human parvovirus PARV4 genotypes identified in human plasma for fractionation. *The Journal of General Virology*, 88, 2162–2167.

Fryer, J.F., Delwart, E., Hecht, F.M., Bernardin, F., Jones, M.S., Shah, N. & Baylis, S.A. (2007b) Frequent detection of the parvoviruses, PARV4 and PARV5, in plasma from blood donors and symptomatic individuals. *Transfusion*, 47, 1054–1061.

Fryer, J.F., Hubbard, A.R. & Baylis, S.A. (2007c) Human parvovirus PARV4 in clotting factor VIII concentrates. *Vox Sanguinis*, 93, 341–347.

Grabarczyk, P., Kalinska, A., Kara, M., Wieczorek, R., Ejduk, A., Sulkowska, E., Golebiowska-Staroszczyk, S., Matysiak, M., Baylis, S.A. & Brojer, E. (2011) Identification and characterization of acute infection with parvovirus B19 genotype 2 in immunocompromised patients in Poland. *Journal of Medical Virology*, 83, 142–149.

Hokynar, K., Soderlund-Venermo, M., Pesonen, M., Ranki, A., Kiviluoto, O., Partio, E.K. & Hedman, K. (2002) A new parvovirus genotype persistent in human skin. *Virology*, 302, 224–228.

Hokynar, K., Norja, P., Laitinen, H., Palomaki, P., Garbarg-Chenon, A., Ranki, A., Hedman, K. & Soderlund-Venermo, M. (2004) Detection and differentiation of human parvovirus variants by commercial quantitative real-time PCR tests. *Journal of Clinical Microbiology*, 42, 2013–2019.

Hubschen, J.M., Mihneva, Z., Mentis, A.F., Schneider, F., Aboudy, Y., Grossman, Z., Rudich, H., Kasympekova, K., Sarv, I., Nedeljkovic, J., Tahita, M.C., Tarnagda, Z., Ouedraogo, J.B., Gerasimova, A.G., Moskaleva, T.N., Tikhonova, N.T., Chitadze, N., Forbi, J.C., Faneye, A.O., Otegbayo, J.A., Charpentier, E. & Muller, C.P. (2009) Phylogenetic analysis of human parvovirus b19 sequences from eleven different countries confirms the predominance of genotype 1 and suggests the spread of genotype 3b. *Journal of Clinical Microbiology*, 47, 3735–3738.

Jones, M.S., Kapoor, A., Lukashov, V.V., Simmonds, P., Hecht, F. & Delwart, E. (2005) New DNA viruses identified in patients with acute viral infection syndrome. *Journal of Virology*, 79, 8230–8236.

Kantola, K., Sadeghi, M., Antikainen, J., Kirveskari, J., Delwart, E., Hedman, K. & Soderlund-Venermo, M. (2010) Real-time quantitative PCR detection of four human bocaviruses. *Journal of Clinical Microbiology*, 48, 4044–4050.

Kantola, K., Hedman, L., Arthur, J., Alibeto, A., Delwart, E., Jartti, T., Ruuskanen, O., Hedman, K. & Söderlund-Venermo, M. (2011) Seroepidemiology of human bocaviruses 1–4. *Journal of Infectious Diseases*, 9, 1403–1412.

Kapoor, A., Slikas, E., Simmonds, P., Chieochansin, T., Naeem, A., Shaukat, S., Alam, M.M., Sharif, S., Angez, M., Zaidi, S. & Delwart, E. (2009) A newly identified bocavirus species in human stool. *The Journal of Infectious Diseases*, 199, 196–200.

Kapoor, A., Simmonds, P., Slikas, E., Li, L., Bodhidatta, L., Sethabutr, O., Triki, H., Bahri, O., Oderinde, B.S., Baba, M.M., Bukbuk, D.N., Besser, J., Bartkus, J. & Delwart, E. (2010) Human bocaviruses are highly diverse, dispersed, recombination prone, and prevalent in enteric infections. *The Journal of Infectious Diseases*, 201, 1633–1643.

Ke, L., He, M., Li, C., Liu, Y., Gao, L., Yao, F., Li, J., Bi, X., Lv, Y., Wang, J., Hirsch, M.L. & Li, W. (2011) The prevalence of human parvovirus B19 DNA and antibodies in blood donors from four Chinese blood centers. *Transfusion*, 51, 1909–1918.

Keller, L.W., Barbosa, M.L., Melo, F.L., Pereira, L. M., David-Neto, E., Lanhez, L.E. & Durigon, E. L. (2009) Phylogenetic analysis of a near-full-length sequence of an erythrovirus genotype 3 strain isolated in Brazil. *Archives of Virology*, 154, 1685–1687.

Kishore, J., Srivastava, M. & Choudhary, N. (2010) Standardization of B19 IgG ELISA to study the seroepidemiology of parvovirus B19 in North Indian voluntary blood donors. *Asian Journal of Transfusion Science*, 2, 86–90.

Kleinman, S.H., Glynn, S.A., Lee, T.H., Tobler, L., Montalvo, L., Todd, D., Kiss, J.E., Shyamala, V. & Busch, M.P.National Heart, Lung, Blood Institute Retrovirus Epidemiology Donor Study (REDS-II) (2007) Prevalence and quantitation of parvovirus B19 DNA levels in blood donors with a sensitive polymerase chain reaction screening assay. *Transfusion*, 10, 1756–1764.

Koduri, P.R., Kumapley, R., Valladares, J. & Teter, C. (1999) Chronic pure red cell aplasia caused by parvovirus B19 in AIDS: use of intravenous immunoglobulin – a report of eight patients. *American Journal of Hematology*, 61, 16–20.

Koenigbauer, U.F., Eastlund, T. & Day, J.W. (2000) Clinical illness due to parvovirus B19 infection after infusion of solvent/detergent-treated pooled plasma. *Transfusion*, 40, 1203–1206.

Koppelman, M.H., Cuypers, H.T., Emrich, T. & Zaaijer, H.L. (2004) Quantitative real-time detection of parvovirus B19 DNA in plasma. *Transfusion*, 44, 97–103.

Koppelman, M.H., Rood, I.G., Fryer, J.F., Baylis, S. A. & Cuypers, H.T. (2007) Parvovirus B19 genotypes 1 and 2 detection with real-time polymerase chain reaction assays. *Vox Sanguinis*, 93, 208–215.

Koppelman, M.H., van Swieten, P. & Cuijpers, H. T. (2011) Real-time polymerase chain reaction detection of parvovirus B19 DNA in blood donations using a commercial and an in-house assay. *Transfusion*, 51, 1346–1354.

Koppelman, M.H., Cuijpers, H.T., Wessberg, S., Valkeajarvi, A., Pichl, L., Schottstedt, V. & Saldanha, J. (2012) Multicenter evaluation of a commercial multiplex polymerase chain reaction test for screening plasma donations for parvovirus B19 DNA and hepatitis A virus RNA. *Transfusion*, 7, 1498–1508.

Kurtzman, G.J., Cohen, B., Meyers, F., Amunullah, A. & Young, N.S. (1988) Persistent B19 parvovirus infection as a cause of severe chronic anaemia in children with acute lymphocytic leukaemia. *Lancet*, 2, 1159–1162.

Kurtzman, G., Frickhofen, N., Kimball, J., Jenkins, D.W., Nienhuis, A.W. & Young, N.S. (1989) Pure red-cell aplasia of 10 years' duration due to persistent parvovirus B19 infection and its cure with immunoglobulin therapy. *New England Journal of Medicine*, 321, 519–523.

Lahtinen, A., Kivela, P., Hedman, L., Kumar, A., Kantele, A., Lappalainen, M., Liitsola, K., Ristola, M., Delwart, E., Sharp, C., Simmonds, P., Soderlund-Venermo, M. & Hedman, K. (2011) Serodiagnosis of primary infections with human parvovirus 4, Finland. *Emerging Infectious Diseases*, 17, 79–82.

Longhi, E., Bestetti, G., Acquaviva, V., Foschi, A., Piolini, R., Meroni, L., Magni, C., Antinori, S., Parravicini, C. & Corbellino, M.(2007) Human parvovirus 4 in the bone marrow of italian patients with AIDS. *AIDS* (London, England), 21, 1481–1483.

Lurcharchaiwong, W., Chieochansin, T., Payungporn, S., Theamboonlers, A. & Poovorawan, Y. (2008) Parvovirus 4 (PARV4) in serum of intravenous drug users and blood donors. *Infection*, 36, 488–491.

Ma, Y.Y., Guo, Y., Zhao, X., Wang, Z., Lv, M.M., Yan, Q.P. & Zhang, J.G. (2012) Human parvovirus PARV4 in plasma pools of Chinese origin. *Vox Sanguinis*, doi: 10.1111/j.1423-0410.2012.01605.x. [Epub ahead of print].

Makris, M., Preston, F.E., Rosendaal, F.R., Underwood, J.C., Rice, K.M. & Triger, D.R. (1996) The natural history of chronic hepatitis C in haemophiliacs. *British Journal of Haematology*, 94, 746–752.

Makris, M., Calizzani, G., Fischer, K., Gilman, E. A., Hay, C.R.M., Lambert, T., Lassila, R., Ludlam, C.A. & Mannucci, P.M. (2011) EUHASS: The European Haemophilia Safety Surveillance system. *Thrombosis Research*, 127, 22–25.

Manning, A., Willey, S.J., Bell, J.E. & Simmonds, P. (2007) Comparison of tissue distribution, persistence, and molecular epidemiology of parvovirus B19 and novel human parvoviruses PARV4 and human bocavirus. *The Journal of Infectious Diseases*, 195, 1345–1352.

Mannucci, P.M. & Tuddenham, E.G. (2001) The hemophilias – from royal genes to gene therapy. *The New England Journal of Medicine*, 344, 1773–1779.

Meriluoto, M., Hedman, L., Tanner, L., Simell, V., Makinen, M., Simell, S., Mykkanen, J., Korpelainen, J., Ruuskanen, O., Ilonen, J., Knip, M., Simell, O., Hedman, K. & Soderlund-Venermo, M. (2012) Association of human bocavirus 1 infection with respiratory disease in childhood follow-up study, Finland. *Emerging Infectious Diseases*, 18, 264–271.

Millar, C.M., Connor, N., Dolan, G., Lee, C.A., Makris, M., Wilde, J., Winter, M., Ironside, J.W., Gill, N. & Hill, F.G. (2010) Risk reduction strategies for variant creutzfeldt-jakob disease transmission by UK plasma products and their impact on patients with inherited bleeding disorders. *Haemophilia: The Official Journal of the World Federation of Hemophilia*, 16, 305–315.

Modrow, S., Wenzel, J.J., Schimanski, S., Schwarzbeck, J., Rothe, U., Oldenburg, J., Jilg, W. & Eis-Hubinger, A.M. (2011) Prevalence of nucleic acid sequences specific for human parvoviruses, hepatitis A and hepatitis E viruses in coagulation factor concentrates. *Vox Sanguinis*, 100, 351–358.

Mossong, J., Hens, N., Friederichs, V., Davidkin, I., Broman, M., Litwinska, B., Siennicka, J., Tracinska, A., VAN Damme, P., Beutels, P., Vyse, A., Shkedy, Z., Aerts, M., Massari, M. & Gabutti, G. (2008) Parvovirus B19 infection in five European countries: seroepidemiology, force of infection and maternal risk of infection. *Epidemiology and Infection*, 136, 1059–1068.

Naides, S.J., Scharosch, L.L., Foto, F. & Howard, E.J. (1990) Rheumatologic manifestations of human parvovirus B19 infection in adults. initial two-year clinical experience. *Arthritis and Rheumatism*, 33, 1297–1309.

Nguyen, Q.T., Sifer, C., Schneider, V., Allaume, X., Servant, A., Bernaudin, F., Auguste, V. & Garbarg-Chenon, A. (1999) Novel human erythrovirus associated with transient aplastic anemia. *Journal of Clinical Microbiology*, 37, 2483–2487.

Nguyen, Q.T., Wong, S., Heegaard, E.D. & Brown, K.E. (2002) Identification and characterization of a second novel human erythrovirus variant, A6. *Virology*, 301, 374–380.

Norja, P., Hokynar, K., Aaltonen, L.M., Chen, R., Ranki, A., Partio, E.K., Kiviluoto, O., Davidkin, I., Leivo, T., Eis-Hubinger, A.M., Schneider, B., Fischer, H.P., Tolba, R., Vapalahti, O., Vaheri, A., Soderlund-Venermo, M. & Hedman, K. (2006) Bioportfolio: lifelong persistence of variant and prototypic erythrovirus DNA genomes in human tissue. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 7450–7453.

Norja, P., Hedman, L., Kantola, K., Kemppainen, K., Suvilehto, J., Pitkäranta, A., Aaltonen, L.M., Seppänen, M., Hedman, K. & Söderlund-Venermo, M. (2012) Occurrence of human bocaviruses and parvovirus 4 in solid tissues. *Journal of Medical Virology*, 8, 1267–1273.

Panning, M., Kobbe, R., Vollbach, S., Drexler, J.F., Adjei, S., Adjei, O., Drosten, C., May, J. & Eis-Hubinger, A.M. (2010) Novel human parvovirus 4 genotype 3 in infants, Ghana. *Emerging Infectious Diseases*, 16, 1143–1146.

Pattison, J.R., Jones, S.E., Hodgson, J., Davis, L.R., White, J.M., Stroud, C.E. & Murtaza, I. (1981) Parvovirus infections and hypoplastic crisis in sickle-cell anaemia. *Lancet*, 1, 664–665.

Peden, A., McCardle, L., Head, M.W., Love, S., Ward, H.J., Cousens, S.N., Keeling, D.M., Millar, C.M., Hill, F.G. & Ironside, J.W. (2010) Variant CJD infection in the spleen of a neurologically asymptomatic UK adult patient with haemophilia. *Haemophilia: The Official Journal of the World Federation of Hemophilia*, 16, 296–304.

Reid, D.M., Reid, T.M., Brown, T., Rennie, J.A. & Eastmond, C.J. (1985) Human parvovirus-associated arthritis: a clinical and laboratory description. *Lancet*, 1, 422–425.

Richardson, L.C. & Evatt, B.L. (2000) Risk of hepatitis A virus infection in persons with hemophilia receiving plasma-derived products. *Transfusion Medicine Reviews*, 14, 64–73.

Saal, J.G., Steidle, M., Einsele, H., Muller, C.A., Fritz, P. & Zacher, J. (1992) Persistence of B19 parvovirus in synovial membranes of patients with rheumatoid arthritis. *Rheumatology International*, 12, 147–151.

Sanabani, S., Neto, W.K., Pereira, J. & Sabino, E.C. (2006) Sequence variability of human erythroviruses present in bone marrow of brazilian patients with various parvovirus B19-related hematological symptoms. *Journal of Clinical Microbiology*, 44, 604–606.

Schmidt, I., Blumel, J., Seitz, H., Willkommen, H. & Lower, J. (2001) Parvovirus B19 DNA in plasma pools and plasma derivatives. *Vox Sanguinis*, 81, 228–235.

Schneider, B., Becker, M., Brackmann, H.H. & Eis-Hubinger, A.M. (2004) Contamination of coagulation factor concentrates with human parvovirus B19 genotype 1 and 2. *Thrombosis and Haemostasis*, 92, 838–845.

Schneider, B., Fryer, J.F., Reber, U., Fischer, H.P., Tolba, R.H., Baylis, S.A. & Eis-Hubinger, A.M. (2008a) Persistence of novel human parvovirus PARV4 in liver tissue of adults. *Journal of Medical Virology*, 80, 345–351.

Schneider, B., Fryer, J.F., Oldenburg, J., Brackmann, H.H., Baylis, S.A. & Eis-Hubinger, A.M. (2008b) Frequency of contamination of coagulation factor concentrates with novel human parvovirus PARV4. *Haemophilia: The Official Journal of the World Federation of Hemophilia*, 14, 978–986.

Servant-Delmas, A., Laperche, S., Mercier, M., Elghouzzi, M.H., Lionnet, F., Stankovic, K., Girot, R. & Lefrere, J.J. (2009) Human parvovirus 4 in recipients of cellular products and in blood donors: epidemiologic similarity with B19 parvovirus. *Transfusion*, 49, 1771–1773.

Sharp, C.P., Lail, A., Donfield, S., Simmons, R., Leen, C., Klenerman, P., Delwart, E., Gomperts, E.D. & Simmonds, P. (2009) High frequencies of exposure to the novel human parvovirus PARV4 in hemophiliacs and injection drug users, as detected by a serological assay for PARV4 antibodies. *The Journal of Infectious Diseases*, 200, 1119–1125.

Sharp, C.P., Lail, A., Donfield, S., Gomperts, E.D. & Simmonds, P. (2012) Virologic and clinical features of primary infection with human parvovirus 4 in subjects with hemophilia: frequent transmission by virally inactivated clotting factor concentrates. *Transfusion*, 52, 1482–1489.

Siegl, G. & Cassinotti, P. (1998) Presence and significance of parvovirus B19 in blood and blood products. *Biologicals: Journal of the International Association of Biological Standardization*, 26, 89–94.

Simmonds, P., Manning, A., Kennell, R., Carnie, F.W. & Bell, J.E. (2007) Parenteral transmission of the novel human parvovirus PARV4. *Emerging Infectious Diseases*, 13, 1386–1388.

Simmonds, P., Douglas, J., Bestetti, G., Longhi, E., Antinori, S., Parravicini, C. & Corbellino, M. (2008) A third genotype of the human parvovirus PARV4 in sub-saharan africa. *The Journal of General Virology*, 89, 2299–2302.

Soderlund, M., von Essen, R., Haapasaari, J., Kiistala, U., Kiviluoto, O. & Hedman, K. (1997) Persistence of parvovirus B19 DNA in synovial membranes of young patients with and without chronic arthropathy. *Lancet*, 349, 1063–1065.

Soderlund-Venermo, M., Hokynar, K., Nieminen, J., Rautakorpi, H. & Hedman, K. (2002) Persistence of human parvovirus B19 in human tissues. *Pathologie-Biologie*, 50, 307–316.

Soderlund-Venermo, M., Lahtinen, A., Jartti, T., Hedman, L., Kemppainen, K., Lehtinen, P., Allander, T., Ruuskanen, O. & Hedman, K. (2009) Clinical assessment and improved diagnosis of bocavirus-induced wheezing in children, Finland. *Emerging Infectious Diseases*, 15, 1423–1430.

Soucie, J.M., Erdman, D.D., Evatt, B.L., Anderson, L.J., Torok, T.J., El-Jamil, M., Barnhart, E., Tepper, M., Burrill, H.N., Pickett, A.M. & Mengeling, W.L. (2000) Investigation of porcine parvovirus among persons with hemophilia receiving hyate: C porcine factor VIII concentrate. *Transfusion*, 40, 708–711.

Soucie, J.M., De Staercke, C., Chitlu, R.M.B., Gruppo, R.A., Hooper, W.C., Kessler, C.M., Roshni Kulkarni, R., Manco-Johnson, M.J., Monahan, P.E., Oakley, Meredith., Powell, J., Pyle, M., Recht, M., Riske, B., Sabio, H. & Trimble, S. (2011) Continued Transmission of Parvovirus B19 in Plasma-Derived Factor Concentrates After the Implementation of B19 Nucleic Acid Plasma Minipool Screening. *Blood (ASH Annual Meeting Abstracts)*, 118, 3378.

Szelei, J., Liu, K., Li, Y., Fernandes, S. & Tijssen, P. (2010) Parvovirus 4-like virus in blood products. *Emerging Infectious Diseases*, 16, 561–564.

Szomor, K.N., Kapusinszky, B., Rigo, Z., Kis, Z., Rozas, M., Farkas, A., Szilagyi, A., Berencsi, G. & Takacs, M. (2009) Detection of human bocavirus from fecal samples of Hungarian children with acute gastroenteritis. *Intervirology*, 52, 17–21.

Touinssi, M., Brisbarre, N., Picard, C., Prassati, C., Dussol, B., Uch, R., Gallian, P., Cantaloube, J.F., Micco, P. & Biagini, P. (2010) Parvovirus 4 in blood donors, France. *Emerging Infectious Diseases*, 16, 165–166.

Vallefrini, D., Barozzi, P., Quadrelli, C., Bosco, R., Potenza, L., Riva, G., Gregorini, G., Sandrini, S., Tironi, A., Montagnani, G., De Palma, M., Torelli, G., Delwart, E. & Luppi, M. (2008) Parvoviruses in blood donors and transplant patients, Italy. *Emerging Infectious Diseases*, 14, 185–186.

White, D.G., Woolf, A.D., Mortimer, P.P., Cohen, B.J., Blake, D.R. & Bacon, P.A. (1985) Human parvovirus arthropathy. *Lancet*, 1, 419–421.

Willkommen, H., Schmidt, I. & Lower, J. (1999) Safety issues for plasma derivatives and benefit from NAT testing. *Biologicals: Journal of the International Association of Biological Standardization*, 27, 325–331.

Yu, J.M., Li, D.D., Xu, Z.Q., Cheng, W.X., Zhang, Q., Li, H.Y., Cui, S.X., Miao-Jin, H., Yang, S.H., Fang, Z.Y. & Duan, Z.J. (2008) Human bocavirus infection in children hospitalized with acute gastroenteritis in China. *Journal of Clinical Virology: The Official Publication of the Pan American Society for Clinical Virology*, 42, 280–285.

Yu, X., Zhang, J., Hong, L., Wang, J., Yuan, Z., Zhang, X. & Ghildyal, R. (2012) High prevalence of human parvovirus 4 infection in HBV and HCV infected individuals in shanghai. *PLoS ONE*, 7, e29474.

別紙様式第2-1

No. 20

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
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研究報告の概要

○ガーナの小児の鼻腔及び糞便検体から分離されたヒトパルボウイルス4

サハラ以南のアフリカ諸国では、経口伝播がヒトパルボウイルス4(PARV4)の感染に関与している可能性がある。ガーナにおいて、気道症状を呈する15歳未満の小児1,904人から鼻腔検体または糞便検体を採取し検査を行った結果、鼻腔検体961例中8例(0.83%)及び糞便検体943例中5例(0.53%)からPARV4 DNAが検出された。ウイルス濃度は、鼻腔検体では1.3×10^{3}～1.8×10^{7}コピー/mL(中央値1.0×10^{4}コピー/mL)、糞便検体では2.3×10^{3}～4.6×10^{6}コピー/mL(中央値6.8×10^{4}コピー/mL)であり、全てPARV4ジェノタイプ3と分類された。ウイルス濃度が約6－7$\log_{10}$コピー/mLの場合もあり、PARV4感染経路として気道あるいは糞口経路が示唆される。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血
新鮮凍結血漿-LR「日赤」120
新鮮凍結血漿-LR「日赤」240
新鮮凍結血漿-LR「日赤」480

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
ガーナにおいて気道症状を呈する小児の鼻腔及び糞便検体からPARV4が検出され、PARV4感染経路として経口経路が示唆されるとの報告である。	ヒトパルボウイルス4の病原性について未だ詳細は不明であるが、今後も本ウイルスについての情報収集に努める。

MedDRA/J Ver.15.1J

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DISPATCHES

Human Parvovirus 4 in Nasal and Fecal Specimens from Children, Ghana

Jan Felix Drexler, Ulrike Reber, Doreen Muth, Petra Herzog, Augustina Annan, Fabian Ebach, Nimarko Sarpong, Samuel Acquah, Julia Adlkofer, Yaw Adu-Sarkodie, Marcus Panning, Egbert Tannich, Jürgen May, Christian Drosten, and Anna Maria Eis-Hübinger

Nonparenteral transmission might contribute to human parvovirus 4 (PARV4) infections in sub-Saharan Africa. PARV4 DNA was detected in 8 (0.83%) of 961 nasal samples and 5 (0.53%) of 943 fecal samples from 1,904 children in Ghana. Virus concentrations ≈6–7 $\log_{10}$ copies/mL suggest respiratory or fecal–oral modes of PARV4 transmission.

Human parvovirus 4 (PARV4; human paretravirus) is a single-stranded DNA virus discovered in 2005 (*1*). PARV4 has been detected in persons at risk for parenteral infections, suggesting blood-borne transmission (*2,3*) although other transmission routes have not been ruled out. Studies in northern Europe demonstrated a high prevalence of antibodies against PARV4 in injection drug users, persons co-infected with HIV and hepatitis C virus, and persons with hemophilia who were exposed to nonvirally inactivated clotting factors; however, antibodies were not detected in the general population (*4,5*).

In contrast, PARV4 seroprevalence was 25%–37% in adults in the Democratic Republic of Congo, Cameroon, and Burkina Faso who were not infected with HIV and hepatitis C virus, (*6*). PARV4 DNA was detected in blood of *8.6% of children 15 or 24 months* of age in Ghana (*7*). There was no history of exposure to multiple-use needles or blood transfusion in any of these children. These data suggested alternative modes of PARV4 transmission in countries in Africa. Nonparenteral modes of transmission of PARV4 have also been suggested in South Africa (*6*), Taiwan (*8*), India, (*9*), China (*10*), and Thailand (*11*).

PARV4 has been classified into 3 genotypes. Genotypes 1 and 2 are found in North America, Europe, and Asia (*1–3,9–11*), and genotype 3 is found in in sub-Saharan Africa (*7,12*). To investigate whether PARV4 is found in the respiratory or intestinal tract, we analyzed previously collected specimens from 1,904 children in Ghana.

The Study

Ethical approval for this study was provided by the Committee on Human Research Publication and Ethics, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. Informed consent was obtained from parents or guardians of all children.

A total of 1,904 anonymous nasal and fecal specimens were obtained during a study on molecular diagnostics for respiratory and enteric tract infections in symptomatic children <15 years of age at the Presbyterian Hospital in Agogo, Ghana. Nasal swab specimens were obtained from children with upper or lower respiratory tract symptoms. Fecal samples were obtained from 504 children with gastrointestinal symptoms (53.4% of sampled children; 294 [58.3%] of symptomatic children with vomiting, 190 [37.7%] with diarrhea, and 144 [28.6%] with acute malnutrition; 9 [1.8%] with incomplete clinical data) and 439 (46.6%) children without gastrointestinal symptoms.

A total of 961 nasal swabs were obtained during February–November 2008 from 520 boys and 441 girls (median age 19 months, range 0–162 months, interquartile range 8–38 months). Nasal swabs were placed in 1.5 mL of RNAlater (QIAGEN, Hilden, Germany). A total of 943 fecal samples were obtained during May–October 2009 from 500 boys and 443 girls (median age 36 months, range 0–165 months, interquartile range 17–78 months). Fecal samples were prepared as 10% suspensions in phosphate-buffered saline. No paired nasal and fecal specimens were available from individual patients.

Viral DNA was purified from 140 μL of nasal swab suspension or 200 μL of fecal suspension by using QIAamp Viral RNA and DNA Stool Mini Kits (QIAGEN), respectively. Two real-time PCRs were performed. One primer/probe set was designed to detect PARV4 genotypes 1 or 2 viruses (*13*), and a second primer set was designed to detect PARV4 genotype 3 viruses (*7*). The sensitivity of both protocols was 1–2 genome copies/reaction. Absolute quantification of PARV4 genome copy numbers relied on photometrically quantified genotype 3 plasmid standards, as described (*7*).

To exclude bias from DNA purification methods, PARV4-negative nasal and fecal specimens were spiked with quantified plasmid standards. Subsequent

Author affiliations: University of Bonn Medical Centre, Bonn, Germany (J.F. Drexler, U. Reber, D. Muth, A. Annan, F. Ebach, C. Drosten, A.M. Eis-Hübinger); Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany (P. Herzog, J. Adlkofer, E. Tannich, J. May); Kumasi Centre for Collaborative Research in Tropical Medicine, Kumasi, Ghana (A. Annan, N. Sarpong, S. Acquah); Kwame Nkrumah University of Science and Technology, Kumasi (Y. Adu-Sarkodie); and Freiburg University Medical Center, Freiburg, Germany (M. Panning)

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Table. Nucleotide sequence divergence of parvovirus 4 strains from nasal swab and fecal samples from children, Ghana, from genotype 1, 2, and 3 prototype strains*

Specimen type and no.	Nucleotide position according to GenBank accession no. EU874248	Nucleotide sequence divergence from parvovirus 4 reference strains, %		
		Genotype 1 (GenBank AY622943)	Genotype 2 BR10627–5 (GenBank DQ873390)	Genotype 3 NG-OR (GenBank EU874248)
Nasal swab				
N1	1700–4660	6.56	7.39	0.92
N2	299–4660	7.51	8.07	0.88
N3	50–4660	7.37	8.38†	0.83
N4	1962–2056‡	9.16	6.73	2.14
N4	2117–3413	4.97	5.31	0.93
N5	1962–2056	9.16	6.73	2.14
N5	2117–4183	5.50	6.34	0.98
N6	299–4660	7.51	8.10	0.90
N7	1962–2056	9.16	6.73	2.14
N7	2431–2914	6.24	7.01	1.25
N7	3068–3246	4.61	5.19	1.12
N8	624–3246	7.36	7.84	0.84
Feces				
F1	1700–4183	6.20	6.82	0.89
F2	1700–4460	6.56	7.39	0.92
F3	1700–3716	6.08	6.52	0.85
F4	1700–4183	6.02	6.78	0.89
F5	1700–4183	6.93	6.73	1.04

*Pairwise nucleotide divergence was calculated by using the DNA distance matrix in BioEdit (www.mbio.ncsu.edu/BioEdit/bioedit.html).
†Because the homologs of the first 92 nt of strain N3 are not given in the prototype strain BR10627–5, calculation of divergence started at N3 nt position 93.
‡Nucleotide sequence of the PCR product (primer sequences trimmed) was amplified by using screening PCR designed for detection of PARV4 genotype 3 as described (7).

quantification was equivalent between techniques and specimens, and differences between specimen types in several experiments were <0.5 $\log_{10}$ copies/mL. Standard procedures were used to prevent PCR contamination. Determination of PARV4 genotypes was conducted by nucleotide sequencing of several genomic target regions (Table).

Eight (0.83%) of 961 nasal swabs and 5 (0.53%) of 943 fecal samples tested were positive for PARV4 DNA. Virus concentrations ranged from 1.3×10^3 to 1.8×10^7 copies/mL (median 1.0×10^4 copies/mL) in nasal swab suspensions and from 2.3×10^3 to 4.6×10^6 copies/mL (median 6.8×10^4 copies/mL) in fecal suspensions (Figure 1). The difference in virus concentrations between the 2 groups was not significant (p = 0.056, by Mann-Whitney U test).

Nucleotide sequencing of amplicons generated by screening PCRs and sequencing of additional genomic regions classified all viruses as PARV4 genotype 3 (Table) (GenBank accession numbers JN183920–JN183932). This result was confirmed by phylogenetic analysis of a 483-nt fragment of the capsid-encoding open reading frame 2 (Figure 2).

Ages of the 8 children with PARV4-positive nasal swab specimens ranged from 9 to 58 months (median 32 months). Ages of the 5 children with PARV4-positive fecal samples were 1, 36, 43, 57, and 124 months. Nasal swab specimens with the highest viral loads were from a 9-month-old boy and a 29-month-old girl. Fecal samples with the highest viral loads were from 2 boys 43 and 57 months of age.

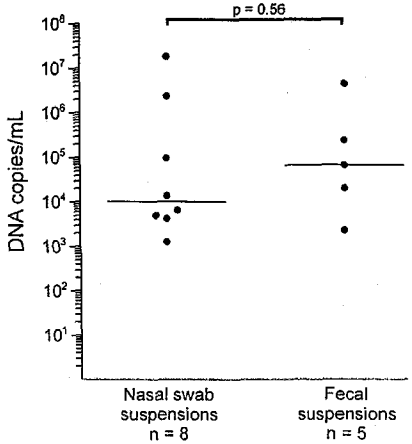

Figure 1. Parvovirus 4 DNA loads in virus-positive nasal and fecal specimens from children, Ghana. Virus concentrations are given on a log scale on the y-axis. Each dot represents 1 specimen. Horizontal lines represent median values for each sample type. For calculation of statistical significance of the difference in viral quantities between sample types, the Mann-Whitney U test was used. Virus quantities in nasal swabs and feces are given for sample suspensions (nasal swabs in 1.5 mL of stabilizing reagent and feces in a 10% suspension in phosphate-buffered saline).

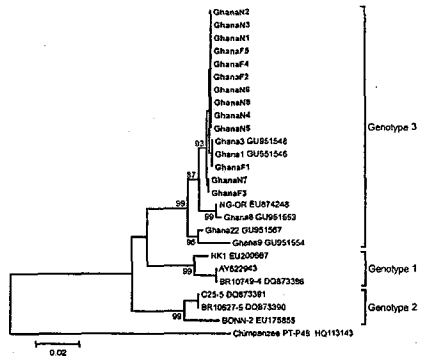

Figure 2. Phylogenetic analysis of a 483-nt fragment of the parvovirus 4 (PARV4) capsid-encoding open reading frame (ORF) 2 for PARV4 strains identified in children, Ghana. Neighbor-joining phylogeny was conducted in MEGA5.05 (www.megasoftware.net) by using a gap-free ORF2 fragment corresponding to positions 2,432–2,914 in the PARV4 genotype 3 prototype strain NG-OR (GenBank accession no. EU874248) with a nucleotide percentage distance substitution model and 1,000 bootstrap replicates. Scale bar indicates percentage uncorrected nucleotide distance. Previously published PARV4 sequences are given with strain names (if available) and GenBank accession numbers. Viruses newly identified are in **boldface**. The source of PARV4 strains identified in the study is indicated by capital letters (N, nasal specimen; F, fecal specimen). PARV4 genotypes are given to the right of taxa. A chimpanzee partetravirus was used as the outgroup.

Conclusions

We found PARV4 in 0.8% of nasal swab specimens and 0.5% of fecal specimens from 2 groups of children in Ghana symptomatic for respiratory illness and with or without diarrheal illness, respectively. Our results provide evidence to suggest that the higher prevalence of PARV4 reported among adults in countries in western Africa (*6*) might be caused by transmission by the respiratory or fecal–oral route.

However, demonstration of PARV4 in the respiratory tract and feces does not identify a transmission route. PARV4 in the respiratory tract could be caused by high viremia, which was recently reported in a child in India with a genotype 2 infection (*9*) and in 2 patients with hemophilia in the United Kingdom, 1 with a genotype 1 infection and 1 with a genotype 2 infection (*14*).

It is unclear to what extent the putative nonparenteral transmission routes of PARV4 genotype 3 in western Africa apply to other areas. Markedly lower PARV4 antibody prevalences observed in Europe (*4,5*) argue against PARV4 spread by nonparenteral routes, e.g., from infected injection drug users to the general population. Likewise, the higher prevalence of PARV4 antibodies in HIV-infected blood donors in South Africa compared with uninfected donors (*6*) appears incompatible with PARV4 transmission primarily by the respiratory route. Therefore, our results do not contradict those of a study conducted in Scotland, which showed no PARV4 in respiratory specimens (*15*).

Because of the small number of children with PARV4 DNA in nasal or fecal specimens, correlation of infection with age groups was not possible. A limitation of our study was the lack of blood specimens from children with current respiratory or fecal PARV4 shedding, and serologic studies are needed to evaluate susceptibility of different age groups to PARV4 infection. Furthermore, detection of PARV4 in patients with respiratory disease does not indicate that PARV4 was the cause of the disease. In 5 of 8 PARV4-positive nasal swabs, typical respiratory viruses (parainfluenza virus, influenza A virus, rhinovirus) were also detected and the pattern of symptoms in PARV4-positive children did not differ from symptoms in PARV4-negative children. Similarly, 3 of 5 children with PARV4-positive feces did not have gastrointestinal symptoms at the time of fecal sampling. One child had vomiting and another child had vomiting and diarrhea. Moreover, in 3 of these 5 children, in addition to PARV4, *Giardia lamblia*, a potential cause of diarrhea, was also detected.

Although data for exposure and risk factors and paired samples were not available, suggested transmission routes might explain the high infection rates in western Africa. Further studies are needed to assess the effect of PARV4 excretion on virus epidemiology and the chronology of PARV4 infection.

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Dr Drexler is a physician and clinical virologist at the University of Bonn. His research interest is characterization of novel human and zoonotic viruses.

References

1. Jones MS, Kapoor A, Lukashov VV, Simmonds P, Hecht F, Delwart E. New DNA viruses identified in patients with acute viral infection syndrome. J Virol. 2005;79:8230–6. http://dx.doi.org/10.1128/JVI.79.13.8230-8236.2005

2. Fryer JF, Lucas SB, Padley D, Baylis SA. Parvoviruses PARV4/5 in hepatitis C virus–infected patient. Emerg Infect Dis. 2007;13:175–6. http://dx.doi.org/10.3201/eid1301.060856
3. *Simmonds P, Manning A, Kenneil R, Carnie FW, Bell JE. Parenteral* transmission of the novel human parvovirus PARV4. Emerg Infect Dis. 2007;13:1386–8. http://dx.doi.org/10.3201/eid1309.070428
4. Sharp CP, Lail A, Donfield S, Simmons R, Leen C, Klenerman P, *et al.* High frequencies of exposure to the novel human parvovirus PARV4 in hemophiliacs and injection drug users, as detected by a serological assay for PARV4 antibodies. J Infect Dis. 2009;200:1119–25. http://dx.doi.org/10.1086/605646
5. Lahtinen A, Kivela P, Hedman L, Kumar A, Kantele A, Lappalainen M, et al. Serodiagnosis of primary infections with human parvovirus 4, Finland. Emerg Infect Dis. 2011;17:79–82. http://dx.doi.org/10.3201/eid1701.100750
6. Sharp CP, Vermeulen M, Nebie Y, Djoko CF, LeBreton M, Tamoufe U, et al. Epidemiology of human parvovirus 4 infection in sub-Saharan Africa. Emerg Infect Dis. 2010;16:1605–7. http://dx.doi.org/10.3201/eid1701.100750
7. Panning M, Kobbe R, Vollbach S, Drexler JF, Adjei S, Adjei O, et al. Novel human parvovirus 4 genotype 3 in infants, Ghana. Emerg Infect Dis. 2010;16:1143–6. http://dx.doi.org/10.3201/eid1607.100025
8. Yang SJ, Hung CC, Chang SY, Lee KL, Chen MY. Immunoglobulin G and M antibodies to human parvovirus 4 (PARV4) are frequently detected in patients with HIV-1 infection. J Clin Virol. 2011;51:64–7. http://dx.doi.org/10.1016/j.jcv.2011.01.017
9. Benjamin LA, Lewthwaite P, Vasanthapuram R, Zhao G, Sharp C, Simmonds P, et al. Human parvovirus 4 as potential cause of encephalitis in children, India. Emerg Infect Dis. 2011;17:1484–7. http://dx.doi.org/10.3201/eid1708.110165
10. Yu X, Zhang J, Hong L, Wang L, Yuan Z, Zhang X, et al. High prevalence of human parvovirus 4 infection in HBV and HCV infected individuals in Shanghai. PLoS ONE. 2012;7:e29474. http://dx.doi.org/10.1371/journal.pone.0029474
11. Lurcharchaiwong W, Chieochansin T, Payungporn S, Theamboonlers A, Poovorawan Y. Parvovirus 4 (PARV4) in serum of intravenous *drug users and blood donors. Infection.* 2008;36:488–91. http://dx.doi.org/10.1007/s15010-008-7336-4
12. Simmonds P, Douglas J, Bestetti G, Longhi E, Antinori S, Parravicini C, et al. A third genotype of the human parvovirus PARV4 in sub-Saharan Africa. J Gen Virol. 2008;89:2299–302. http://dx.doi.org/10.1099/vir.0.2008/001180-0
13. Fryer JF, Delwart E, Hecht FM, Bernardin F, Jones MS, Shah N, et al. Frequent detection of the parvoviruses, PARV4 and PARV5, in plasma from blood donors and symptomatic individuals. Transfusion. 2007;47:1054–61. http://dx.doi.org/10.1111/j.1537-2995.2007.01235.x
14. Sharp CP, Lail A, Donfield S, Gomperts ED, Simmonds P. Virologic and clinical features of primary infection with human parvovirus 4 in subjects with hemophilia: frequent transmission by virally inactivated clotting factor concentrates. Transfusion. 2012;52:1482–9. http://dx.doi.org/10.1111/j.1537-2995.2011.03420.x
15. Manning A, Russell V, Eastick K, Leadbetter GH, Hallam N, Templeton K, *et al. Epidemiological profile and clinical associations* of human bocavirus and other human parvoviruses. J Infect Dis. 2006;194:1283–90. http://dx.doi.org/10.1086/508219

Address for correspondence: Anna Maria Eis-Hübinger, Institute of Virology, University of Bonn Medical Centre, Sigmund-Freud-Str. 25, D-53127 Bonn, Germany; email: anna-maria.eis-huebinger@ukb.uni-bonn.de

別紙様式第2-1 No. 21

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 2012. 10. 20	新医薬品等の区分 該当なし		総合機構処理欄
一般的名称	新鮮凍結人血漿						
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)		研究報告の公表状況	E P Notari, J Brodsky, W R Steele, R Y Dodd, S L Stramer. AABB Annual Meeting&CTTXPO 2012; October 6–9, 2012, BOSTON.	公表国 米国		使用上の注意記載状況・その他参考事項等 新鮮凍結血漿-LR「日赤」 新鮮凍結血漿-LR「日赤」成分採血 新鮮凍結血漿-LR「日赤」120 新鮮凍結血漿-LR「日赤」240 新鮮凍結血漿-LR「日赤」480

研究報告の概要

○大規模かつ様々な地域の供血者サンプルにおける2009-2011年のHTLV-I/IIの抗体陽性率

背景/症例報告: 近年、米国ではHTLV-I/IIの検査結果が陽性となる供血者数が減少しているが、感染は今も確認されており、性別と地域によって異なっている。今回、複数回供血者においてHTLV単回検査が実施可能か評価を行った。

研究デザイン/方法: 2009～11年、米国赤十字社で確認されたHTLV-I/II陽性供血者のデータを検索した。HTLV-I/II抗体検査に繰り返し反応を示した供血者は、EIAで確認し、IFA、ウェスタンブロット(WB)及びRIPAの併用で最終確認した。抗体陽性率は米国国勢調査の性別と地域分布に基づいて計算した。北東部(NE)、南部(S)、中西部(MW)、及び西部(W)の地域についてカイ二乗検定とボンフェローニ補正による多重比較を行った。罹患密度(ID)は、過去の供血歴が1095日(3年)を超えない供血者人年(PY)に対する抗体陽転者の人数とした。

結果/所見: 3年間で、HTLV陽性の443人を含む7,098,612人の同種血供血者からの1,900万を超える供血を調査した。HTLV供血の総抗体陽性率は10万人当たり2.3人であった。うち女性供血者は72%(443人中319人)、男性は28%で、抗体陽性率はそれぞれ3.6/10万人、1.2/10万人であった(p<0.0001、OR= 2.9、95%CI 2.4及び3.6)。全体として、地域及び供血者10万人当たりのHTLV陽性供血者数は、NE対W(p=0.013)、MW対NE(p=0.002)、MW対W(p=0.0001)で有意差があった;SとNE、SとWの間では差は認められず、NWは抗体陽性率が最も低かった。複数回供血者のうち、36人がHTLV陽性であり、22人は3年を超えた供血、14人は3年以内の供血が陰性であった。同14人の総IDは0.18/10万人年(95%CI、0.10、0.30)であり、女性供血者(13人)のIDが0.34/10万人年(95%CI、0.18、0.58)、男性供血者(1人)が0.03/10万人年であった(95%CI、0.001、0.141)。14人中7人がHTLV-I/II、5人がHTLV-I、2人がHTLV-IIであった。14人中11人がIFA陽性(エンドポイント1:64－2:1024)であり、残りの3人がWB/RIPAで確認された。

結論: HTLVの抗体陽性率と性別に有意な差が観察された。3年以内の新規感染14例が確認されたことから、HTLV単回抗体検査のみで供血者スクリーニングを行うことは有効とは言えない。

報告企業の意見

大規模かつ様々な地域の供血者においてHTLV-I/IIの抗体陽性率を評価したところ、抗体陽性率と性別に有意な差が確認され、また複数回供血者におけるHTLV単回検査は、供血者スクリーニングとしては有効ではないことが明らかとなったとの報告である。

今後の対応

日本赤十字社では、献血時のスクリーニング法として、化学発光酵素免疫測定法(CLEIA)によるHTLV-I抗体のスクリーニング検査を行い、確認検査としてウエスタンブロット法による検査を行っている。今後も引き続き情報の収集に努める。

5

MedDRA/J Ver.15.1J

Plenary

P1-030A

Transfused RBCs Can Be Immunogenic in Splenectomized Mice: of Inflammation, Adjuvants, and Anamnestic Responses

J. E. Hendrickson (jeanna.hendrickson@emory.edu), S. R. Stowell, N. H. Smith, K. Girard-Pierce, K. Hudson, J. C. Zimring. [1]Pediatrics and Pathology, Emory University, Atlanta, GA, United States; [2]Pathology, Emory University, Atlanta, GA, United States; [3]Pediatrics, Emory University, Atlanta, GA, United States; [4]Scripps Research Institute, San Diego, CA, United States; [5]Puget Sound Blood Center, Seattle, WA, United States

Background/Case Studies: Patients with thalassemia major may have higher rates of RBC alloimmunization than the general transfused population, and splenectomy has been reported to be associated with alloimmunization in this patient population. Previous murine studies with RBCs expressing the mHEL model antigen have shown that splenectomy largely abrogates alloimmunization. However, many factors (donor as well as recipient) may influence RBC alloimmunization, and we hypothesize that some RBC antigens under some conditions have the capacity to be immunogenic upon initial exposure in splenectomized animals. **Study Design/Methods:** Blood from donor mice expressing the HOD (lysozyme, ovalbumin, and human Duffy b), the hGPA (human glycophorin A), or the KEL2 (human Cellano) antigen on their RBCs was transfused into C57BL/6 or FVB recipients who had been surgically splenectomized; control animals were sham splenectomized. A subset of recipients were pretreated with poly (I:C) or CFA prior to transfusion; and a subset of recipients received multiple KEL2 transfusions. Two weeks after transfusion, alloimmunization (IgG, IgM) was assessed by flow cytometric crossmatch, utilizing transfused RBCs and antigen negative RBCs as targets. **Results/Findings:** No splenectomized mice transfused with HOD or KEL2 RBCs in the absence of induced inflammation made a detectable IgM or IgG alloantibody response (n = 3 experiments, 60 animals total). However, 100% of control, sham splenectomized animals made detectable alloantibodies, and control animals multiply transfused with KEL2 RBCs demonstrated a boostable response. In contrast to findings in the mHEL system, HOD, hGPA, and KEL2 expressing RBCs led to detectable alloantibodies following transfusion into recipients pretreated with CFA (HOD) or poly (I:C) (hGPA, KEL2) (n = 7 independent experiments, 70 animals total). **Conclusion:** Although a spleen plays a critical role in primary RBC alloimmune responses in mice transfused in their baseline state, a spleen is not essential for responses to 3 different RBC antigens when transfusion occurs in the presence of adjuvants or recipient inflammation. Under no studied condition, however, did splenectomized animals make higher levels of RBC alloantibodies than their control counterparts. It has been suggested that splenectomized thalassemia patients may have elevated IL-6 levels, and the presumed alteration in immune function in such patients may be involved in the reported findings; other factors to consider in this patient population include RBC transfusion burden, timing of initial RBC antigen exposure (pre vs post-splenectomy), and transfused RBC life span. A better understanding of the potential impact of splenectomy (be it surgical or autosplenectomy) on RBC alloimmunization may benefit thalassemia major and sickle cell anemia patients alike.

Disclosure of Commercial Conflict of Interest

K. Girard-Pierce: Nothing to disclose; J. E. Hendrickson: Nothing to disclose; K. Hudson: Nothing to disclose; N. H. Smith: Nothing to disclose; S. R. Stowell: No Answer; J. C. Zimring: Nothing to disclose

Disclosure of Grants Conflict of Interest

K. Girard-Pierce: Nothing to disclose; J. E. Hendrickson: Immucor, Grants or Research Support; K. Hudson: Nothing to disclose; N. H. Smith: Nothing to disclose; S. R. Stowell: No Answer; J. C. Zimring: Immucor, Grants or Research Support

P2-030A

HTLV-I/II Prevalence and Incidence from 2009-2011 In a Large, Geographically Diverse Sample of US Blood Donors

E P Notari (ed.notari@redcross.org), J Brodsky, W R Steele, R Y Dodd, S L Stramer. [1]Transmissible Diseases, American Red Cross, Rockville, MD, United States; [2]Scientific Support Office, American Red Cross, Gaithersburg, MD, United States; [3]Quality Analytics Inc., Riverwood, IL, United States

Background/Case Studies: The number of US blood donors testing positive (pos) for HTLV-I/-II infection has decreased in recent years, but prevalent and incident infections are still detected and differ by gender and geographic distribution. The feasibility of one-time testing for HTLV was assessed by evaluating HTLV incidence in repeat donors. **Study Design/Methods:** Data for HTLV-I/II pos donors identified by the American Red Cross (ARC) from 2009-2011 were retrieved. Antibody in donors testing anti-HTLV-I/II repeat reactive (Abbott PRISM) were confirmed by a 2nd EIA followed by a combination of IFA, western blot (WB) and RIPA (OA Viral & Rickettsial Diseases Lab). Prevalence rates were calculated by gender and geographic distribution based on US Census Regions. Rates for the Northeast (NE), South (S), Midwest (MW) and West (W) were compared using multiple pairwise chi-square comparisons with Bonferroni adjustment. Incidence density (ID) was calculated as the number of seroconverters over total donor person years (PY) with prior donation histories not to exceed 1095 days (3 years). **Results/Findings:** For the 3-year period, >19 million donations were tested from 7,098,612 allogeneic donors including 443 HTLV pos. Overall HTLV donation prevalence was 2.3 per 100,000 (PHT). Female donors accounted for 72% (319/443) and males 28% with prevalence rates of 3.6 PHT and 1.2 PHT, respectively (p < 0.0001, OR = 2.9, 95% CI 2.4, 3.6). Overall, the number of HTLV pos donors by region and prevalence rates of PHT donors differed significantly between the NE vs. W (p = 0.013), MW vs. NE (p = 0.002), MW vs. W (p < 0.0001) and MW vs. S (p < 0.0001); no differences were noted between the S and the NE or the S and the W; the MW had the lowest rates. Of repeat donors, 36 were HTLV pos; 22 had nonreactive donations >3 yrs prior and 14 within 3 yrs. For these 14, an overall ID of 0.18 PHT PY was calculated (95% CI, 0.10, 0.30). Female donors accounted for nearly all of the calculable Incident donors; the 13 female donors had an ID of 0.34 PHT PY (95% CI, 0.18, 0.58) with 0.03 PHT PY (95% CI, 0.001, 0.141) for the 1 male. Of the 14, 7 were typed as HTLV-II, 5 HTLV-I/II and 2 HTLV-I; 11/14 were IFA pos (1:64-1:1024 endpoint) with the remaining 3 confirmed by WB/RIPA. **Conclusion:** Significant differences in HTLV prevalence rates and gender were observed. With 14 incident infections identified, it is not feasible to screen donors only once for anti-HTLV. These data are consistent with those of earlier time periods.

Disclosure of Commercial Conflict of Interest

J. Brodsky: Nothing to disclose; R. Y. Dodd: Abbott Laboratories, Stocks or Bonds; Abbott Laboratories, Grants or Research Support; Novartis, Travel Support or Honorarium; Ortho Diagnostics, Travel Support or Honorarium; E. P. Notari: Nothing to disclose; W. R. Steele: Nothing to disclose; S. L Stramer: Nothing to disclose

Disclosure of Grants Conflict of Interest

J. Brodsky: Nothing to disclose; R. Y. Dodd: Abbott Laboratories, Grants or Research Support; E. P. Notari: Nothing to disclose; W. R. Steele: Nothing to disclose; S. L. Stramer: Nothing to disclose

Donor Based anti-HTLV Prevalence Rates per 100,000 (PHT)

US Census Region	Total Number of Donors	Number of anti-HTLV Positive	Prevalence Rate	Lower 95% CI	Upper 95% CI
Northeast	1,592,757	98	6.1	5.1	7.5
Midwest	2,238,414	77	3.4	2.9	4.3
South	1,793,341	161	9.0	7.9	10.5
West	1,113,994	107	9.6	7.9	11.6

P3-030A

Age of Red Blood Cells in Premature Infants (ARIPI)

D A Fergusson[1,4] (dafergusson@ohri.ca), P C Hebert[1,4], L LeBel[1], M A Blajchman[2], L Kovacs[3,4], C Lachance[3,4]. [1]Clinical Epidemiology, Ottawa Hospital Research Institute, Ottawa, ON, Canada; [2]Division of Neonatology, The Ottawa Hospital, Ottawa, ON, Canada; [3]Division of Neonatology, Children's and Women's Health Centre of British Columbia, Vancouver, BC, Canada; [4]Division of Neonatology, Royal University Hospital, Saskatoon, SK, Canada; [5]Faculty of Health Sciences, McMaster University, Hamilton, ON, Canada; [6]Division of Neonatology, Jewish General Hospital, Montreal, QC, Canada; [7]Division of Neonatology, Ste Justine Hospital, Montreal, QC, Canada; [8]Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada; [9]College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada; [10]Faculty of Medicine, McGill University, Montreal, QC, Canada; [11]Faculty of Medicine, University of Montreal, Montreal, QC, Canada; [12]Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada

Background/Case Studies: Despite recent trends in decreasing transfusion thresholds and the development of technologies designed to avoid allogeneic exposure, allogeneic red blood cell (RBC) transfusions remain an important supportive and life-saving measure for neonatal intensive care patients experiencing illness and anemia of prematurity. However, a number

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No. 10

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
			2013. 1. 17	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	Francis RO, Strauss D, Williams JD, Whaley S, Shaz BH. Transfusion. 2012 Dec;52(12):2664-70. doi: 10.1111/j.1537-2995.2012.03639.x. Epub 2012 Apr 9.	公表国 米国	
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)				

研究報告の概要

○2010年季節的流行期間中の、ニューヨークにおける供血者のウエストナイルウイルス感染症

背景: 供血者のウエストナイルウイルス(WNV)急性感染について、ミニプール(MP)NATから個別(ID)NATへの転換に関する一律の開始戦略は現在存在しない。1999年以降最大の流行となった2010年のWNV季節的流行期間中の、ニューヨーク血液センターにおけるWNVスクリーニングの結果について報告する。

研究デザインと方法: 2010年7月1日～10月31日の期間中、MP-NATまたはID-NATを用いて供血者スクリーニングを行い、NAT陽性の血液についてWNV-IgM及びIgG抗体の有無を調べた。

結果: ウイルス血症であるとみられる血液20本(0.0129%、1/7752)が確認された。MP-NATでは検出されなかった可能性がある9本が同定され、これらのうち2本はIgM、IgG抗体がいずれも陰性であり、うち1本はID-NATの開始に2本以上の陽性血液が必要であるという条件下では検出できなかったと考えられる。遡及的なID-NATでは2本の陽性血液が確認された。ニューヨーク州におけるNAT陽性血液の多く(16/19本)は、州の中でもヒトWNV症例が多い郡(ナッソー郡及びサフォーク郡)の居住者から採取されたものであった。

結論: 陽性血液が1件検出された後のID-NATの開始、遡及的な検査によって、感染性が想定される血液の出荷が防止できた。しかし、遡及的な検査によりNAT陽性血液が検出されたことは、ID-NATの開始基準の変更が必要であることを示唆している可能性がある。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血
新鮮凍結血漿-LR「日赤」120
新鮮凍結血漿-LR「日赤」240
新鮮凍結血漿-LR「日赤」480

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
2010年のウエストナイルウイルス(WNV)季節的流行期間中、ニューヨーク血液センターで行ったNATスクリーニングの結果である。MP-NATとID-NATを適切に切り替えることにより、WNVの感染性を有する血液の出荷を効果的に防ぐ事ができたが、さらに改善の余地があるとの報告である。	日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、ウエストナイルウイルス感染の国内発生に備え、平成17年10月25日付血液対策課発事務連絡に基づく緊急対応(献血制限、NAT検査)のほか、厚生労働科学研究「血液製剤の安全性確保と安定供給のための新興・再興感染症の研究」班と共同して対応について検討している。今後も引き続き情報の収集に努める。

MedDRA/J Ver.15.1J

West Nile virus infection in blood donors in the New York City area during the 2010 seasonal epidemic

Richard O. Francis, Donna Strauss, Joan Dunn Williams, Shavonne Whaley, and Beth H. Shaz

BACKGROUND: A uniform threshold strategy for converting from minipool (MP)-nucleic acid testing (NAT) to individual donation (ID)-NAT screening for acute West Nile virus (WNV) infection among blood donors is lacking. We report on WNV screening at the New York Blood Center during the 2010 seasonal WNV epidemic, the most severe epidemic in that state since the original outbreak in 1999.

STUDY DESIGN AND METHODS: Between July 1 and October 31, 2010, blood donations were screened by MP-NAT or ID-NAT and the presence of anti-WNV immunoglobulin (Ig)M and IgG was evaluated among NAT-positive donations.

RESULTS: Twenty presumed viremic donations were identified for a frequency of 0.0129% (1 in 7752 donations). Nine donations that could have been missed by MP-NAT were identified. Two of these donations were both IgM and IgG negative, one of which would have been missed if more than one positive donation was required for initiating ID-NAT. Retrospective ID-NAT revealed two positive donations. The majority of the NAT-positive donations in New York (16/19) were from donors who lived in counties that had the highest incidence of human WNV cases in the state.

CONCLUSION: Our data details the identification of WNV NAT-positive blood donations during a severe seasonal epidemic in the New York area. By initiating ID-NAT after one positive donation, using retrospective testing, and triggering ID-NAT regionally, we were able to prevent the release of presumably infectious donations. The detection of NAT-positive donations with retrospective testing, however, may indicate the need for changes in our trigger criteria.

West Nile virus (WNV) is a single-stranded RNA virus that is transmitted by the *Culex* mosquito and usually infects birds. Mammals such as humans and horses are incidental hosts and several human outbreaks have been reported around the world in Romania, Russia, Israel, and most recently in the United States. Approximately 20% of WNV infections lead to a febrile illness, West Nile fever, and less than 1% of infected individuals have neurologic disease (meningoencephalitis).[1] The large proportion of asymptomatic infections, 80%, poses the threat that acutely infected persons may present for blood donation without symptoms of illness. As such, the risk of transfusion-transmitted WNV (TT-WNV) infection was predicted.[2,3] In addition, it has been shown that the virus is stable in stored blood for 42 days under refrigerated conditions.[4]

Twenty-three cases of TT-WNV infection from the 2002 season were retrospectively confirmed in 2003.[5] The outcomes of these cases included asymptomatic infection, febrile illness, meningoencephalitis, and death. Since viral nucleic acid can be detected before the generation of IgM and IgG antibodies against WNV,[6] the Food and Drug Administration, private industry, and blood collection agencies partnered to begin nucleic acid testing (NAT) of blood donations in minipools (MPs) to detect presumed viremic donations (PVDs). MP-NAT for WNV was implemented across the United States in July 2003. It was soon apparent, however, that MP-NAT was not sufficiently sensitive to detect all PVDs as additional cases of TT-WNV infection occurred in 2003.[7]

Because of the prohibitive cost of individual-donation (ID)-NAT for all donations, several strategies were developed for determining when it would be appropriate to transition from MP- to ID-NAT to detect and remove as many PVDs as possible from the blood supply.[8,9] Taken into account in these screening strategies are the number of positive donations after which ID-NAT should be initiated, the size of the geographic area for which the threshold criteria applies, the use of retrospective testing after a PVD is found, and the appropriate interval during which no positive donations are identified to revert to MP-NAT. The effectiveness of these strategies in detecting PVDs as well as the cost associated with them have been evaluated by several authors.[10-12]

The 2010 WNV season in New York had the greatest number of clinical cases of WNV infection since the original outbreak in 1999 in that state.[13] The collection area of our blood center includes New York City, neighboring counties in New York State, and portions of central New Jersey. We report on the detection of PVDs during the 2010 WNV seasonal epidemic in the New York City area using our regional triggering strategy.

ABBREVIATIONS: ID = individual donation; MP(s) = minipool(s); PVD(s) = presumed viremic donation(s); TT-WNV = transfusion-transmitted West Nile virus; WNV = West Nile virus.

From the New York Blood Center, New York, New York; the Department of Pathology and Cell Biology, Columbia University Medical Center–New York Presbyterian Hospital, New York, New York; Creative Testing Solutions, Tempe, Arizona; and the Department of Pathology and Laboratory Medicine, Emory University, Atlanta, Georgia.

Address reprint requests to: Beth H. Shaz, New York Blood Center, 310 East 67 Street, New York, NY 10065, e-mail: BShaz@NYBloodCenter.org.

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MATERIALS AND METHODS

NAT

Blood donations were screened during all months of the year by NAT using the Procleix WNV transcription-mediated amplification assay (Gen-Probe, San Diego, CA). A signal-to-cutoff ratio (S/CO) of 1 or greater defined a positive result. MP-NAT was performed in pools of 16 samples and positive MPs were resolved by testing the individual samples to identify the positive donation(s). ID-NAT was performed on all hematopoietic cellular therapy products. To determine if a sample initially detected by ID-NAT would have been detected in a MP, NAT-positive samples were diluted 1:16 in WNV-negative plasma and NAT was performed. A PVD was defined as an initially reactive donation that repeated as reactive on the original sample from the donation or one that had a signal-to-cutoff ratio of 17 or greater.[14]

Detection of anti-WNV

Anti-WNV were detected in a sample from the index NAT-positive donation. An IgM capture enzyme-linked-immunosorbent assay (MAC-ELISA) and IgG ELISA were performed by a reference laboratory (Sonora Quest Laboratories, Phoenix, AZ). For the IgM MAC-ELISA, an index value of less than 0.90 was negative, an index value of 0.90 to 1.10 was equivocal, and an index value of greater than 1.10 was positive. For the IgG ELISA, an index value of less than 1.30 was negative, an index value of 1.30 to 1.49 was equivocal, and an index value of 1.50 or greater was positive.

Criteria for conversion between MP-NAT and ID-NAT

Triggering to ID-NAT for the collection area was from July 1, 2010 to October 31, 2010. The trigger to ID-NAT was one NAT-positive donation. The algorithm for conversion between MP- and ID-NAT is shown in Fig. 1. Upon identification of a NAT-positive donation the zip code and county of residence of the donor were obtained. ID-NAT was then initiated in the county of residence of the donor from whom the positive donation originated. Retrospec-

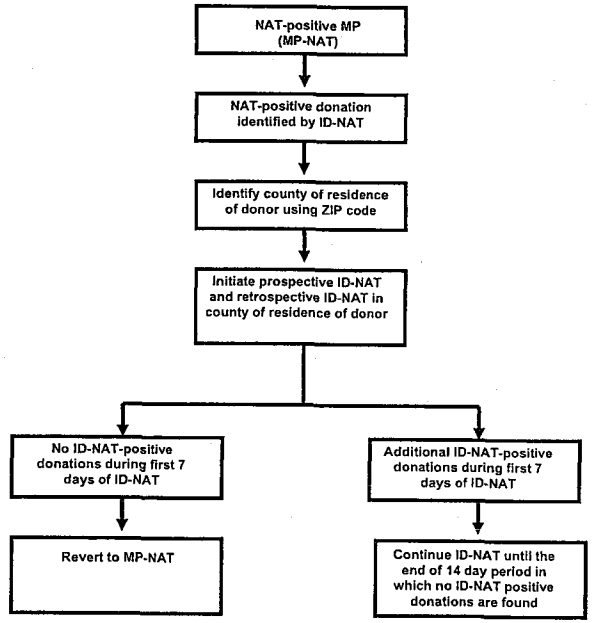

Fig. 1. Algorithm for conversion between MP and ID-NAT.

tive ID-NAT of donations from the affected county was performed beginning on the day the positive donation was identified back to the date of collection of the positive donation. Reversion to MP-NAT in the affected county occurred after 7 days if no other positives were detected or after 14 days from the last date with a NAT-positive donation, if additional positive samples were found. Conversion between MP- and ID-NAT testing for the adjacent counties of Long Island (Nassau and Suffolk Counties) was coordinated such that MP- and ID-NAT were used in both counties at the same time.

Collection of public health data

Public health data for WNV cases and PVDs were obtained from the websites for the various governmental health agencies. Information about WNV cases and PVDs in the 50 states were from the Centers for Disease Control and Prevention at http://www.cdc.gov/ncidod/dvbid/westnile/surv&control_archive.htm. Data for case counts by week for New York and New Jersey were obtained from the United States Geological Survey at http://diseasemaps.usgs.gov/2010/wnv_us_human.html. Case counts and distribution of WNV throughout counties in New York State were found at http://www.health.state.ny.us/nysdoh/westnile/update/update.htm.

RESULTS

The collection area for the New York Blood Center is shown in Fig. 2 and encompasses the five boroughs of New York City (Bronx, Kings, Manhattan, Queens, and Richmond Counties), Long Island (Nassau and Suffolk Counties), the Hudson Valley region of New York (Dutchess, Orange, Putnam, Rockland, Ulster, and Westchester Counties), and central New Jersey (Hunterdon, Middlesex, Somerset, and Union Counties). Between July 1, 2010, and October 31, 2010, a total of 155,280 donations were screened by NAT with 133,306 (86%) donations tested in MPs. The remaining 21,974 (14%) donations were screened by ID-NAT either due to the ID-NAT trigger being activated (21,129/21,974) or because they were hematopoietic cellular therapy products (845/21,974).

Twenty PVDs were detected for a frequency of 0.0129% or 1 in 7752 donations. As shown in Table 1, 19 of 20 PVDs were from New York and one was from New Jersey. Eight PVDs (40%) were detected by MP-NAT and 12 (60%) were detected by ID-NAT due to the ID-NAT trigger being activated. All reactive MPs were resolved by ID-NAT. The two PVDs from August 13 were detected retrospectively after conversion to ID-NAT in response to the two positive donations collected on August 12. In all, 1636 donations were tested retrospectively, for a frequency of 0.12% or 1 in 833 PVDs detected among retrospectively tested donations. In addition, there was one false-positive sample that was reactive on initial testing, but was nonreactive when repeated and was negative for anti-WNV IgM and IgG. There were no reports of TT-WNV infection.

Figure 3 depicts the number of PVDs at our blood center by week, during the WNV season as well as the incidence of reported human WNV clinical cases in New York and New Jersey. The greatest number of PVDs detected in a single week, five, occurred during the week of August 8 to August 14. This week of peak detection of PVDs was also the week during which the greatest number of reported clinical cases in New York occurred. In addition, the PVD collected in New Jersey on September 8 occurred during 1 of 2 weeks (September 5-September 11) in which six WNV cases were reported in New Jersey, the highest during the season for that state. Therefore, the peak detection of PVDs correlated with the period of peak incidence of reported WNV cases in both New York and New Jersey.

Plasma samples from the 20 NAT-positive donations were tested in replicates of eight at a dilution of 1:16 to simulate MP-NAT to determine the likelihood of detecting WNV in these specimens in MPs. The results of this testing are shown in Table 2. Eight of these donations were positive in only none of eight (n = 4), one of eight (n = 3), and two of eight (n = 1) replicates and would be expected to be detected in ID-NAT and not MP-NAT (yield cases). These eight yield cases were all originally detected by ID-NAT. A donation that was originally detected by ID-NAT was positive in four of eight replicates, suggesting that there was only a 50% chance of detection by MP-NAT. The remaining 11 donations were positive in eight of eight replicates and therefore would be detected by MP-NAT.

The plasma samples from the 20 PVDs were tested for the presence of anti-WNV IgM and IgG to assess the WNV immunity status of the donors. The antibody testing results were not used for making decisions about convert-

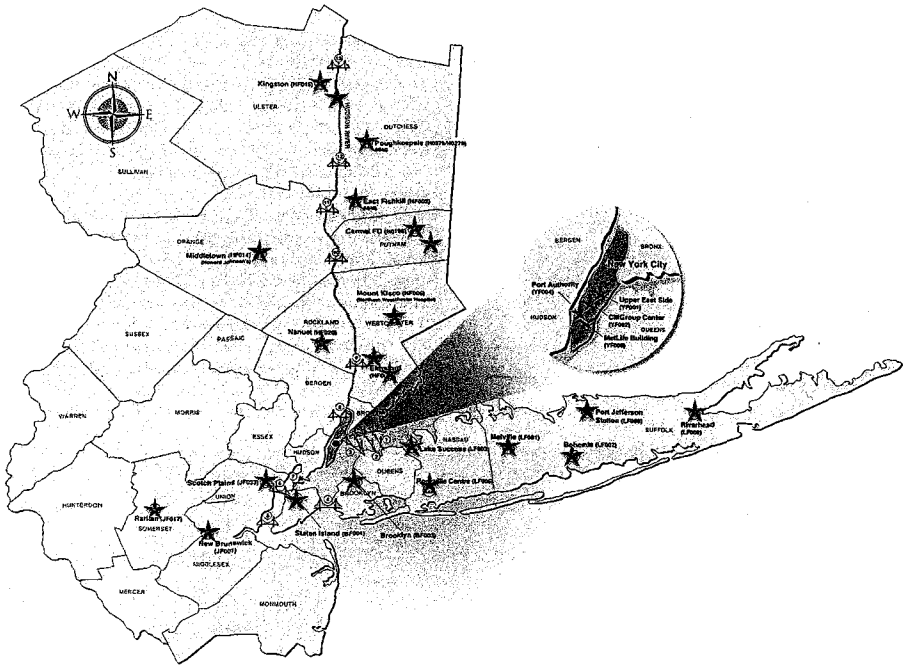

Fig. 2. Blood center collection area. The collection area for the New York Blood Center includes areas of New York and New Jersey. (★) Fixed donation site. Counties in New York City: Bronx, Kings (Brooklyn), Manhattan, Queens, and Richmond (Staten Island). Counties in the Hudson Valley region of New York: Dutchess, Orange, Putnam, Rockland, Ulster, and Westchester. Counties in Long Island: Nassau and Suffolk. Counties in New Jersey: Hunterdon, Middlesex, Somerset, and Union.

TABLE 1. WNV-PVDs by collection date, collection county, and original method of NAT detection (MP vs. ID)

Collection date	County (state)	NAT detection method
July 14	Nassau (NY)	MP
July 22	Suffolk (NY)	MP
July 23	Nassau (NY)	MP
July 26	Suffolk (NY)	ID
July 28	Suffolk (NY)	ID
August 6	Bronx (NY)	MP
August 12	Suffolk (NY)	MP
August 12	Suffolk (NY)	MP
August 13*	Nassau (NY)	ID
August 13*	Nassau (NY)	ID
August 14	Kings (NY)	MP
August 16	Suffolk (NY)	ID
August 17	Nassau (NY)	ID
August 19	Kings (NY)	ID
August 24	Suffolk (NY)	ID
August 25	Suffolk (NY)	ID
August 30	Suffolk (NY)	ID
September 5	Suffolk (NY)	ID
September 7	Suffolk (NY)	ID
September 8	Middlesex (NJ)	MP

* Donations detected with retrospective testing.

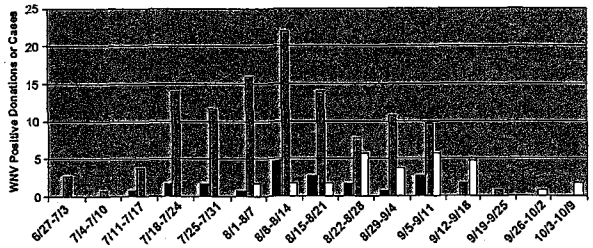

Fig. 3. WNV-positive donations and WNV cases in New York and New Jersey by week during the 2010 season. Data for WNV cases for New York and New Jersey are from the United States Geological Survey website at: http://diseasemaps.usgs.gov/2010/wnv_us_human.html. (■) WNV-PVDs; (▩) clinical WNV cases in New York; (□) clinical WNV cases in New Jersey.

TABLE 2. WNV-PVDs: dilution studies and detection of anti-WNV

Collection date	1:16 dilution testing	Expect to detect by MP or ID	IgM	IgG
July 14	8/8 positive	MP	Negative	Negative
July 22	8/8 positive	MP	Negative	Positive
July 23	8/8 positive	MP	Negative	Negative
July 26	8/8 positive	MP	Negative	Negative
July 28	8/8 positive	MP	Negative	Negative
August 6	8/8 positive	MP	Negative	Negative
August 12	8/8 positive	MP	Negative	Negative
August 12	8/8 positive	MP	Negative	Negative
August 13*	1/8 positive	ID	Positive	Negative
August 13*	2/8 positive	ID	Positive	Positive
August 14	8/8 positive	MP	Negative	Equivocal
August 16	0/8 positive	ID	Positive	Positive
August 17	0/8 positive	ID	Positive	Positive
August 19†	1/8 positive	ID	Negative	Negative
August 24	0/8 positive	ID	Positive	Positive
August 25	1/8 positive	ID	Positive	Negative
August 30	0/8 positive	ID	Positive	Positive
September 5†	4/8 positive	MP/ID	Negative	Negative
September 7	8/8 positive	MP	Negative	Negative
September 8	8/8 positive	MP	Negative	Negative

* Donations detected with retrospective testing.
† Donations possibly not detected by MP-NAT and IgM and IgG negative.

ing between MP- and ID-NAT. PVDs detected in the earlier part of the season from July 14 to August 12 tended to be detectable by MP-NAT and IgM negative (none of eight IgM-positive, one of eight IgG-positive) while NAT-positive donations in the middle to latter part of the season from August 13 to August 30 typically required detection by ID-NAT and were IgM positive (7/12 IgM positive, 5/12 IgG positive). Considering the eight yield cases that would not be detectable by MP-NAT, five were IgM and IgG positive, two were IgM positive and IgG negative, and one was IgM and IgG negative. In addition, the donation that was positive on four of eight replicates in dilution testing was IgM and IgG negative. Therefore, 2 of the 20 PVDs were WNV antibody negative and may not have been detected by MP-NAT.

The majority of the 19 PVDs from New York, 16, were collected from residents of Nassau and Suffolk Counties (Long Island). The remaining three PVDs were from New York City residents (one in Bronx County and two in Kings County). The greater proportion of PVDs collected in Long Island correlated with the majority of clinical cases of WNV being reported in residents of Long Island (82 cases) compared to residents of New York City (42 cases). These results demonstrate that regions of our collection area that had the highest incidence of WNV cases also had the highest incidence of PVDs.

DISCUSSION

We report on the incidence of WNV PVDs in the New York City area during the most active WNV season in that state since the original outbreak in 1999. In the New York Blood Center's collection area that included New York City, Long Island, the Hudson Valley region, and central New Jersey, the frequency of PVDs was 0.0129% (1 in 7752 donations). Of the 20 PVDs that were collected, eight (40%) were yield cases that would not have been detected by MP-NAT and one donation would have had a 50% chance of being detected by MP-NAT. Two PVDs were identified upon retrospective testing in Long Island, the portion of our collection area that had the greatest proportion of PVDs and clinical cases in New York. In addition, two donations that may have been missed by MP-NAT were detected by ID-NAT due to activation of the ID-NAT trigger and were both anti-WNV IgM and IgG negative. Historically, PVDs that were negative by MP-NAT and anti-WNV IgM negative have been associated with TT-WNV infection.[7,15,16] The frequency of 0.0129% for WNV NAT-positive blood donations is comparable to what has been reported in other areas of the United States in which seasonal WNV-epidemics occur.[17-19]

In this study conversion from MP-NAT to ID-NAT within a county occurred after detecting one PVD from a resident of that county. Investigations of triggering strategies have demonstrated that switching from MP-NAT to ID-NAT after detecting one PVD, without a rate requirement, is the most sensitive method for detecting PVDs.[10,20] Among the PVDs in this study, the donation collected on August 19 in Kings County would have only been detected by ID-NAT as demonstrated by dilution testing, was IgM and IgG negative, and was initially tested with ID-NAT because of one prior MP-NAT–positive sample that was detected in the same county on August 14. Therefore, by initiating ID-NAT on one instead of two positive donations, the release of this presumably infectious blood product was prevented.

Reverting to MP-NAT is typically done after either 7 or 14 days of not detecting additional PVDs during ID-NAT. It has been demonstrated that continuing ID testing for 14 instead of 7 days increases the number of low-viremic donations that are detected,[10] albeit at the cost of prolonging ID-NAT. AABB recommends considering continuing ID-NAT for 14 days in areas with ongoing WNV activity.[14] Our strategy entailed ID-NAT for 7 days if no other positive donations were found or 14 days if any additional positive samples were encountered.

Retrospective ID-NAT was performed when converting from MP-NAT to ID-NAT by testing donations from the day of reporting of a NAT-positive donation back to the day of collection of that donation. The frequency of detecting a NAT-positive donation among retrospectively tested donations was almost 10 times that of detecting positive donations among the general donor population (0.12% vs. 0.0129%). While these results demonstrate the utility of retrospective testing for identifying PVDs during periods of high WNV activity, they also indicate that ID-NAT perhaps should have been used for all donations during this epidemic period.

Conversion between MP- and ID-NAT was done for individual counties, except for Nassau and Suffolk Counties in Long Island, which were converted together. As demonstrated by data from Table 1 and Fig. 3, this strategy resulted in MP-NAT being used during peak periods of clinical cases and detection of PVDs. For example, a PVD from Bronx County was collected on August 6, detected by MP-NAT. ID-NAT was initiated only in that county while within the next 7 days four additional PVDs were collected in Suffolk and Nassau Counties. Of these four PVDs, two were collected on August 12, detected by MP-NAT, and two were collected on August 13, detected by retrospective ID-NAT. If conversion to ID-NAT was done for the entire New York City area on August 6, retrospective testing would not have been necessary to detect the August 13 donations. These data suggest that perhaps a wider geographic area than individual counties should be considered for conversion between MP- and ID-NAT in our collection region.

This study has several limitations. First, data about the donors are not available to investigate relationships between the presence of symptoms before, at the time of, and after donation and viremia. Viral loads were not determined, an additional NAT method was not used to confirm transcription-mediated amplification results, and donor follow-up was not performed. Second, our estimate of the frequency of viremic blood donors may be an underestimate because we did not perform ID-NAT throughout the entire season and therefore may have missed cases with levels of viremia that were below the level of detection of MP-NAT during a time that ID-NAT was not triggered. In addition, donors with low levels of viremia that could not be detected by ID-NAT would also not be represented. Third, we are unable to estimate the frequency of WNV infection among blood donors in our collection area because prospective donors who were symptomatic may have not gone to donate or may have been deferred from donation because they reported not feeling well when they presented. Fourth, county of residence of the blood donors from our study may not, in all cases, reflect the location where they became infected. It is expected that a person's exposure to mosquitoes most likely occurs during the evening or early morning hours (when mosquitoes are most active) when the individual is at home. This may not hold true, however, for a person who works an evening or night shift in a different county, outdoors, where he or she could come in contact with mosquitoes. Thus, the expectation that the incidence of PVDs will correlate with the same areas that have high disease activity may not always be the case. Finally, our study does not address the question of whether WNV testing should be performed at all during the parts of the year in which there is no mosquito activity and no WNV cases are being reported.

In conclusion, our results demonstrate the importance of weighing the many variables involved in selecting a strategy for conversion between MP- and ID-NAT for detecting WNV among blood donors. Using our current strategy we were able to prevent 20 PVDs from being released, nine of which may not have been detectable by MP-NAT and two of which may have led to TT-WNV infection. The high rate of detection of PVDs among retrospectively tested samples, however, indicates that improvements in our triggering strategy may be warranted. Initiation of ID-NAT in a single county based on detection of one PVD proved advantageous for detecting subsequent PVDs that would have been missed by MP-NAT. The ability to detect PVDs may be increased even more by considering a larger geographic area for conversion between MP- and ID-NAT, as well as increasing the minimum period of ID-NAT to 14 days as suggested by some.[10] By considering these factors as well as continuing to evaluate seasonal WNV activity as information becomes available, we will improve our ability to protect our blood supply while managing the increased costs of increased use of ID-NAT.

CONFLICT OF INTEREST

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

REFERENCES

1. Petersen LR, Marfin AA. West Nile virus: a primer for the clinician. Ann Intern Med 2002;137:173-9.
2. Biggerstaff BJ, Petersen LR. Estimated risk of West Nile virus transmission through blood transfusion during an epidemic in Queens, New York City. Transfusion 2002;42:1019-26.

3. Biggerstaff BJ, Petersen LR. Estimated risk of transmission of the West Nile virus through blood transfusion in the US, 2002. Transfusion 2003;43:1007-17.
4. Mather T, Takeda T, Tassello J, Ohagen A, Serebryanik D, Kramer E, Brown F, Tesh R, Alford B, Chapman J, Lazo A. West Nile virus in blood: stability, distribution, and susceptibility to PEN110 inactivation. Transfusion 2003;43:1029-37.
5. Pealer LN, Marfin AA, Petersen LR, Lanciotti RS, Page PL, Stramer SL, Stobierski MG, Signs K, Newman B, Kapoor H, Goodman JL, Chamberland ME. Transmission of West Nile virus through blood transfusion in the United States in 2002. N Engl J Med 2003;349:1236-45.
6. Busch MP, Kleinman SH, Tobler LH, Kamel HT, Norris PJ, Walsh I, Matud JL, Prince HE, Lanciotti RS, Wright DJ, Linnen JM, Caglioti S. Virus and antibody dynamics in acute West Nile virus infection. J Infect Dis 2008;198:984-93.
7. Montgomery SP, Brown JA, Kuehnert M, Smith TL, Crall N, Lanciotti RS, Macedo de Oliveira A, Boo T, Marfin AA. Transfusion-associated transmission of West Nile virus, United States 2003 through 2005. Transfusion 2006;46:2038-46.
8. Custer B, Tomasulo PA, Murphy EL, Caglioti S, Harpool D, McEvoy P, Busch MP. Triggers for switching from minipool testing by nucleic acid technology to individual-donation nucleic acid testing for West Nile virus: analysis of 2003 data to inform 2004 decision making. Transfusion 2004;44:1547-54.
9. Kleinman SH, Williams JD, Robertson G, Caglioti S, Williams RC, Spizman R, Morgan L, Tomasulo P, Busch MP. West Nile virus testing experience in 2007: evaluation of different criteria for triggering individual-donation nucleic acid testing. Transfusion 2009;49:1160-70.
10. Biggerstaff BJ, Petersen LR. A modeling framework for evaluation and comparison of trigger strategies for switching from minipool to individual-donation testing for West Nile virus. Transfusion 2009;49:1151-9.
11. Custer B, Busch MP, Marfin AA, Petersen LR. The cost-effectiveness of screening the U.S. blood supply for West Nile virus. Ann Intern Med 2005;143:486-92.
12. Korves CT, Goldie SJ, Murray MB. Cost-effectiveness of alternative blood-screening strategies for West Nile virus in the United States. PLoS Med 2006;3:e21.
13. New York State Department of Health. West Nile virus update [monograph on the internet]. 2011. [cited 2011 Jun 30]. Available from: URL: http://www.health.state.ny.us/nysdoh/westnile/update/update.htm
14. Connor DJ, Lipton KS. West-Nile virus—revised recommendations for triggering individual donation nucleic acid testing and use of communication plans. Association Bulletin No. 08-03. Bethesda (MD): American Association of Blood Banks; 2008.
15. Harrington T, Kuehnert MJ, Kamel H, Lanciotti RS, Hand S, Currier M, Chamberland ME, Petersen LR, Marfin AA. West Nile virus infection transmitted by blood transfusion. Transfusion 2003;43:1018-22.
16. Macedo de Oliveira A, Beecham BD, Montgomery SP, Lanciotti RS, Linnen JM, Giachetti C, Pietrelli LA, Stramer SL, Safranek TJ. West Nile virus blood transfusion-related infection despite nucleic acid testing. Transfusion 2004;44:1695-9.
17. Busch MP, Caglioti S, Robertson EF, McAuley JD, Tobler LH, Kamel H, Linnen JM, Shyamala V, Tomasulo P, Kleinman SH. Screening the blood supply for West Nile virus RNA by nucleic acid amplification testing. N Engl J Med 2005;353:460-7.
18. Kleinman S, Glynn SA, Busch M, Todd D, Powell L, Pietrelli L, Nemo G, Schreiber G, Bianco C, Katz L. The 2003 West Nile virus United States epidemic: the America's Blood Centers experience. Transfusion 2005;45:469-79.
19. Stramer SL, Fang CT, Foster GA, Wagner AG, Brodsky JP, Dodd RY. West Nile virus among blood donors in the United States, 2003 and 2004. N Engl J Med 2005;353:451-9.
20. Stramer SL, Foster G, Krysztof D, Dodd R. Evaluation of triggering strategies for single unit NAT West Nile virus. Transfusion 2008;48:23A. ◘

別紙様式第2-1

No. 17

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 2012. 10. 20	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	新鮮凍結人血漿	研究報告の公表状況	ProMED 20121007.1328469	公表国 カンボジアほか	
販売名（企業名）	新鮮凍結血漿-LR「日赤」（日本赤十字社） 新鮮凍結血漿-LR「日赤」成分採血（日本赤十字社） 新鮮凍結血漿-LR「日赤」120（日本赤十字社） 新鮮凍結血漿-LR「日赤」240（日本赤十字社） 新鮮凍結血漿-LR「日赤」480（日本赤十字社）				使用上の注意記載状況・その他参考事項等 新鮮凍結血漿-LR「日赤」 新鮮凍結血漿-LR「日赤」成分採血 新鮮凍結血漿-LR「日赤」120 新鮮凍結血漿-LR「日赤」240 新鮮凍結血漿-LR「日赤」480

研究報告の概要

○デング熱／デング出血熱　更新情報（抜粋）
カンボジア
今年（2012年）9月第1週までに少なくとも34,483人のデング熱症例が報告され、昨年の同時期の12,972人と比較して2.66倍に増加した。また、今年9月までに146人の子どもがデング熱で死亡し、昨年の同期間の死亡者数59人と比較して2.47倍に増加した。デング熱により1週間に3～5人の子どもが死亡し続けていると言う。保護者が患児をまず最初に個人医院に連れて行き、治療が無効で疾患がより重篤になってから公立病院を訪れるため、その時には既に手遅れとなっているので死亡者数が増加したと当局の専門官は述べた。

報告企業の意見
カンボジアでは2012年9月第1週までのデング熱報告数が前年の同時期と比べて2.66倍に増加したとの報告である。

今後の対応
日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、帰国（入国）後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

7

JRC2012T-041

Published Date: 2012-10-07 11:56:41
Subject: PRO> Dengue/DHF update 2012 (49): Asia
Archive Number: 20121007.1328469

DENGUE/DHF UPDATE 2012 (49) ASIA
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A ProMED-mail post
http://www.promedmail.org
ProMED-mail is a program of the
International Society for Infectious Diseases
http://www.isid.org

In this update:
Cases in various countries:
[1] Cambodia
[2] Cases in various countries
Thailand, Maha Sarakham province
India, Delhi area
India, Chandigarh, Harayana/Punjab states
Pakistan, Karachi, Sindh province

******
[1] Cambodia
Date: Wed 3 Oct 2012
Source: Xinhua News Agency [edited]
http://news.xinhuanet.com/english/health/2012-10/03/c_131886401.htm

At least 34 483 dengue fever cases were reported in Cambodia in the 1st 9 months of this year [2012], a 166 percent increase compared with 12 972 cases in the same period last year [2011], a report of the National Center for Parasitology, Entomology and Malaria Control showed Wednesday [3 Oct 2012].

From January to September this year [2012], the disease had killed 146 Cambodian children, up 147 percent compared with 59 deaths during the same period last year [2011]. "The disease continues to kill between 3 and 5 children a week," said Dr Char Meng Chuor, director of the center.

He explained that there were more deaths this year [2012] because parents had sent their ill children to private clinics 1st, and when the treatment was ineffective and the disease became more severe, they would send them to public hospitals, but it was too late for them to be cured.

Dengue fever is a viral disease transmitted by _Aedes_ mosquitoes. The disease causes an acute illness of sudden onset that usually follows symptoms such as headache, fever, exhaustion, severe muscle and joint pain, swollen glands, vomiting, and rash.

In Cambodia, outbreaks of dengue fever usually begin at the onset of the rainy season in May and last until October.

Char Meng Chuor said that to prevent outbreaks, the center has distributed some 270 tones of Abate (a chemical substance used to kill larvae in water pots) to households this year [2012].

Last year [2011], the country reported 15 980 dengue fever cases, and 73 children died.

-- Communicated by: PRO/MBDS <promed-mbds@promedmail.org>

[According to the newswire above, a total of 34 483 cases and 146 fatalities due to dengue infection were reported in Cambodia during the 1st 9 months of 2012. According to the WHO Western Pacific Regional Office (WPRO) report on the dengue situation, dated 20 Sep 2012, a total of 31 061 cases and 127 deaths due to dengue infection, with a CFR of 0.4 percent, were reported in Cambodia during the 1st 8 months of 2012.

The trend of dengue activity in Cambodia is declining. However, the activity remains above the historic seasonal baseline; 11 017 cases and 48 deaths, with a CFR of 0.4 percent for the same period in 2011 (see http://www.wpro.who.int/emerging_diseases/Dengue.Biweekly.20Sep2012.pdf).

For a map of Cambodia with provinces, see http://ephotopix.com/image/asia/cambodia_province_map.gif. For the interactive HealthMap/ProMED-mail map with links to other recent PRO/MBDS and ProMED-mail postings on Cambodia and neighboring countries, see http://healthmap.org/r/1iGB. - Mod.SCM]

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[2]
Cases in various countries:

- Thailand, Maha Sarakham province. 5 Oct 2012. Dengue 713 cases; Deaths 1; Increasing.
http://www.pattayadailynews.com/en/2012/10/06/dengue-fever-outbreak-kills-isan-teen-girl/

[A map showing the location of Maha Sarakham province can be accessed at
http://www.lib.utexas.edu/maps/middle_east_and_asia/thailand_admin_2005.jpg. - Mod.TY]

India, Delhi area. 3 Oct 2012. Dengue 98 cases; Deaths 2; Increasing. http://www.hindustantimes.com/India-news/NewDelhi/10-new-dengue-cases-in-city/Article1-939488.aspx

[Maps of India can be seen at http://www.mapsofindia.com/maps/india/india-political-map.htm and
http://healthmap.org/r/1pSH. - Mod.TY]

- India, Chandigarh, Harayana/Punjab states. 3 Oct 2012. Dengue, September 2012 only (conf.) 105 cases; Deaths 1. http://timesofindia.indiatimes.com/city/chandigarh/105-dengue-cases-in-Chandigarh/articleshow/16648380.cms

- Pakistan, Karachi, Sindh province. 2 Oct 2012. Dengue for 1-2 Oct 2012 (conf.) 16 cases, (susp.) 243 cases; Increasing. http://www.brecorder.com/pakistan/general-news/83134-16-confirmed-cases-of-dengue-fever-reported-from-different-hospitals-.html

[A HealthMap/ProMED-mail interactive map showing the location of Karachi in Sindh province can be accessed at
http://healthmap.org/r/3DHW. - Mod.TY]

See Also

Dengue/DHF update 2012 (47): Asia 20120930.1316993
Chikungunya & dengue - Cambodia (04): comment 20120925.1308762
Dengue/DHF update 2012 (43): Asia 20120917.1297396
.....sb/dk/ty/msp

http://www.promedmail.org/direct.php?id=20121007.1328469[2012/11/20 18:53:17]

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
				2013. 1. 17	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	Trottier H, Buteau C, Robitaille N, Duval M, Tucci M, Lacroix J, Alfieri C. Transfusion. 2012 Dec;52(12):2653-63. doi: 10.1111/j.1537-2995.2012.03611.x. Epub 2012 Mar 15.	公表国 カナダ	
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)					

研究報告の概要

○幹細胞移植レシピエントにおける、輸血関連エプスタイン・バーウイルス感染:小児後方視的コホート研究

背景: 血液の安全性において、現在スクリーニング検査が行われていない病原体の輸血感染は懸念事項である。このような未検査の病原体のひとつが、リンパ増殖性疾患と関連するエプスタイン・バーウイルス(EBV)である。本研究で、造血幹細胞(HSC)移植を受ける小児における移植後のEBV感染率及び血液製剤の投与と感染との関連性についての分析を行った。

研究デザインと方法: HSC移植小児レシピエントの移植前の血清及びHSCドナーの血清中のEBV抗体の存在について評価し、移植後のEBV感染率、患者の輸血歴について調査した。EBVの移植後累積感染率は、移植前の血清学的データに従い、カプラン・マイヤー法で推定した。血液製剤とEBV感染の関連性についてはコックス回帰モデルを用いて評価した。

結果: 移植前のEBV抗体陽性率は、レシピエントで77.9%、ドナーで61.8%であった。レシピエントの全員が移植前後の期間に血液製剤の投与を受けていた。抗体陰性患者における30日及び60日のEBVの移植後累積感染率は、それぞれ4.6%(95%CI、1.2−17.3%)、13.4%(95%CI、5.8−29.4%)であった。分析を臍帯血移植を受けた抗体陰性患者のみに限定した場合、60日の累積感染率は8.3%(95%CI、2.2−29.4%)であった。重要なことに、EBV感染と輸血量の関連性を肯定する傾向が明確にみられた。

結論: 本研究は、HSC移植レシピエントにおける輸血と移植後EBV感染の関連性を示唆している。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血
新鮮凍結血漿-LR「日赤」120
新鮮凍結血漿-LR「日赤」240
新鮮凍結血漿-LR「日赤」480

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
造血幹細胞(HSC)移植を受ける小児における血液製剤の投与と移植後エプスタイン・バーウイルス(EBV)感染率の関連性について調査したところ、HSC移植レシピエントにおける輸血と移植後EBV感染の関連性が示唆されたとの報告である。	今後も引き続き情報の収集に努める。

MedDRA/J Ver.15.1J

TRANSFUSION COMPLICATIONS

JRC2013T-002

Transfusion-related Epstein-Barr virus infection among stem cell transplant recipients: a retrospective cohort study in children

Helen Trottier, Chantal Buteau, Nancy Robitaille, Michel Duval, Marisa Tucci, Jacques Lacroix, and Caroline Alfieri

BACKGROUND: Blood safety warrants strict screening measures to minimize the risk of transmitting blood-borne pathogens. However, transfusion-transmitted infections for which testing is not currently performed continue to be a concern. Among these untested agents is Epstein-Barr virus (EBV) which, in the transplant setting, is associated with lymphoproliferative disease, a potentially fatal cancer. The aim of this study was to analyze the incidence of posttransplant EBV infection and its association with administration of blood products in children receiving a hematopoietic stem cell (HSC) graft.

STUDY DESIGN AND METHODS: This retrospective cohort study sought to review charts of pediatric recipients of HSC grafts to collect information on the presence of EBV antibodies in the recipients' pretransplant sera and in HSC donor sera, incidence of posttransplant EBV infection, and patients' transfusion history. Cumulative incidence of posttransplant EBV infection was estimated by Kaplan-Meier methods according to pretransplant serology. The association between blood products and EBV infection was measured by Cox regression models.

RESULTS: The pretransplant EBV seroprevalence was 77.9% for recipients and 61.8% for graft donors. Virtually, all recipients received blood products during the peritransplant period. Among seronegative recipients, the 30- and 60-day cumulative incidences of posttransplant EBV infection were 4.6 (95% confidence interval [CI], 1.2-17.3) and 13.4% (95% CI, 5.8%-29.4%), respectively. The 60-day cumulative incidence was 8.3% (95% CI, 2.2%-29.4%) when restricting the analysis to seronegative recipients of cord blood grafts. Importantly, there was a clear positive trend associating EBV infection to transfusion volume.

CONCLUSION: This study suggests an association between transfusions and posttransplant EBV infection in HSC transplant recipients.

In many countries, numerous steps are taken to minimize the risk of infection from transfused blood products. Typically, blood banking organizations will screen for an array of infectious pathogens as part of their quality control protocol. These include hepatitis B and C viruses, human immunodeficiency virus, human T-cell leukemia virus, syphilis, West Nile virus, Chagas disease (*Trypanosoma cruzi*), and on selected units, cytomegalovirus (CMV)/human herpesvirus-5).[1-3] Thus, while transmission of these infections via transfusion has become exceedingly rare, the risk of transfusion-transmitted infections for which testing is not currently performed continues to be a concern.[4-8] Among these untested infectious agents is Epstein-Barr virus (EBV, also known as human herpesvirus-4), which in

ABBREVIATIONS: CSA = cyclosporine A; EBNA = Epstein-Barr nuclear antigen; HSC = hematopoietic stem cell; HSCT = hematopoietic stem cell transplantation; IQR = interquartile range; PTLD = posttransplant lymphoproliferative disease; RR(s) = relative risk(s); VCA = virus capsid antigen.

From the Department of Social and Preventive Medicine; the Department of Pediatrics, Division of Infectious Diseases; the Department of Pediatrics, Division of Hematology-Oncology; the Department of Pediatrics, Division of Pediatric Intensive Care Medicine; and the Department of Microbiology and Immunology, University of Montreal, Sainte-Justine Hospital Research Centre, Montreal, Canada.

Address reprint requests to: Helen Trottier, Sainte-Justine Hospital Research Center, Department of Social and Preventive Medicine, University of Montreal, 3175 Côte Ste-Catherine, Room A-830, Montreal, QC, Canada, H3T 1C5; e-mail: helen.trottier@umontreal.ca.

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TRANSFUSION 2012;52:2653-2663.

immunocompromised patients, can induce lymphoproliferative disease, a potentially fatal cancer.[9-12]

In countries with stringent hygiene practices, EBV seroprevalence tends to increase gradually with age, typically showing two seroconversion peaks: at 2 to 4 years and at 14 to 18 years.[13,14] Hence the mean seroprevalence in children is approximately 50%, which increases steadily to values of 90% to 99% in adults.[11,14] Healthy EBV-seropositive individuals harbor approximately 0.1 to 5 infected B lymphocytes per 10^6 peripheral blood mononuclear cells (MNCs).[15,16] This explains the transmissibility of EBV via the white blood cell (WBC) component of blood. In the United States the prevalence of EBV, detected by polymerase chain reaction (PCR) testing in blood from 100 randomly selected blood donors, has been reported to be 72%.[17] While leukoreduction can significantly reduce the number of EBV genomes in red blood cells (RBCs), it does not completely eliminate EBV-carrying cells.[18]

Nonetheless, with only a handful of documented cases, transfusion-transmitted EBV infection is apparently a relatively rare event. The first report was published by Gerber and colleagues,[19] who showed seroconversion in four of five EBV-seronegative patients undergoing open heart surgery. This was confirmed by another early study that reported that six of 18 EBV-seronegative patients acquired EBV infection following transfusions received after open heart surgery.[20] The risk of primary infection via transfusion in patients without existing antibodies to EBV has been estimated to be 33% to 46% or higher.[20] Several more case reports of EBV-induced postperfusion syndrome can be found in the literature.[21-25] Early studies estimated that EBV infection could be detected 2.5 to 9 weeks after transfusion.[26,27] Other more recent examples of EBV transfusion-transmitted infections have been published. One such case was that of a 19-year-old immune-competent man presenting with infectious mononucleosis 13 days after transfusion with blood that had not been leukoreduced.[28] In the transplant setting, Alfieri and colleagues[29] described the occurrence of a transfusion-related EBV transmission event from a blood donor to a 16-year-old liver transplant recipient who developed protracted infectious mononucleosis 6 weeks after liver transplant surgery. Another situation that provides evidence for the transmissibility of EBV via transfusion is the high prevalence of multiple EBV strains in hemophiliacs.[30]

Because EBV infection is linked to posttransplant lymphoproliferative disease (PTLD), a life-threatening complication occurring after hematopoietic stem cell transplantation (HSCT) and solid organ transplantation,[31] it is important to eliminate the source of infection when possible. For pediatric HSCT patients receiving an EBV-negative graft, transfused EBV-positive blood products may represent an important source of infection. This study was initiated, therefore, to document the risk of acquiring posttransplant EBV infection after blood product transfusion in a pediatric cohort of HSCT patients. Within this cohort we focused more specifically on the umbilical cord blood transplant group as these patients were most likely to have received EBV-negative grafts and EBV-positive blood products. The level of transfusion-related risk for this particular patient group to acquire EBV infection has, to our knowledge, not been reported, and is the focus of this study.

MATERIALS AND METHODS

Study design

A retrospective cohort study was initiated through chart review of all pediatric patients who received hematopoietic stem cell (HSC) grafts at Sainte-Justine Hospital from 1993 to 2009. Existing conditions for HSC transplant in these patients included acute myeloid leukemia, acute lymphoblastic leukemia, juvenile myelomonocytic leukemia, chronic myeloid leukemia, familial erythrophagocytic lymphohistiocytosis, Fanconi anemia, metachromatic leukodystrophy, and lymphoma. Patient charts were reviewed to retrieve information on: 1) the presence of EBV antibodies in pretransplant sera from recipients and HSC donors, 2) the incidence of posttransplant EBV infection in recipients until 1 year posttransplantation, 3) the transfusion history of recipients, and 4) the general characteristics of recipients. This study was approved by the research ethics committee of Sainte-Justine Hospital.

HSCT procedures

HSCT procedures were performed as previously reported.[32] Briefly, children with leukemia were treated with total body irradiation (12 Gy in eight fractions), 120 mg/kg cyclophosphamide over 2 days, and 40 mg/kg etoposide. Alternatively, intravenous (IV) busulfan, adjusted by therapeutic dosing to target a steady-state concentration of 1200 ng/mL (roughly equivalent to 0.8 mg/kg/dose), was given in 16 fractions over 4 days and with 200 mg/kg cyclophosphamide given over 4 days. After transplant, all acute leukemia patients received four to 12 monthly intrathecal methotrexate treatments followed by oral leucovorin rescue. Variations of this protocol for patients with Fanconi anemia, hemophagocytic syndrome, and other congenital or inherited conditions were described previously.[32] Graft-versus-host disease prophylaxis in all patients consisted of 2 mg/kg/day rabbit antithymocyte globulin (Thymoglobulin, Genzyme Corp., Cambridge, MA) administered on Days −2, −1, +1, and +2, along with cyclosporine A (CSA) given IV from Day −3 to Day +21 and orally thereafter. Dose adjustments were made to obtain CSA levels of 250 to 400 ng/mL. In the absence of graft-versus-host disease, CSA was tapered off weekly by 5% starting at Day +100. In addition, cord blood recipients were treated with IV methylprednisolone, followed by 2 mg/kg/day oral prednisolone (tapered by 10% weekly, starting on Day +30). Supportive care was performed as previously reported.[32] This included weekly IV immunoglobulin (500 mg/kg) administered from transplant to Day +100, followed by monthly administration for 6 months. In addition, until 2006, during the months of October to April, monthly treatments of RSV hyperimmune globulin (400 mg/kg Respigam, Medimmune, Boston, MA) were given. Weekly monitoring for EBV and CMV was performed for at least the first 100 days posttransplant. Patients received irradiated, CMV-negative, and leukoreduced blood products to maintain platelet (PLT) counts higher than 20×10^9/L and hemoglobin levels above 70 g/L. Appropriate anti-CMV treatment was provided if two consecutive clinical samples were positive at the required threshold or if there was evidence of clinical disease. Our institution's protocol for diagnosis and treatment of EBV lymphoproliferative disease in allogeneic graft recipients specifies that PCR monitoring for EBV viral load be performed at regular intervals of at least 2 weeks or sooner posttransplant for approximately 4 months or as long as the patient remains immune suppressed. During their hospital stay, patients were isolated to prevent infection and were worked up for PTLD if the EBV PCR test attained the high-risk threshold. Patients were discharged approximately 6 weeks after transplantation.

Chart review for EBV serology, EBV viral load, and transfusion history

Pretransplant sera from recipients and HSC donors were tested for IgG antibodies to the EBV capsid antigen (VCA) using a standard indirect immune fluorescence assay and for antibodies to the Epstein-Barr nuclear antigen (EBNA) by anticomplement immune fluorescence assay. IgG antibody titers to EBV early antigen were also determined by immune fluorescence assay. Donors and recipients were classified according to their pretransplant serologic status as: 1) having past infection (VCA and EBNA IgG titers > 10), 2) having recent infection or being immune suppressed (VCA IgG titers > 10 and EBNA titers < 10), 3) having reactivated infection (VCA, EBNA, and early antigen IgG titers > 10), or 4) being seronegative or naïve (no serologic sign of prior infection). The incidence of posttransplant EBV infection was measured during the first year posttransplantation by semi- and quantitative PCR testing on samples of the recipient's blood taken regularly after transplantation (see above-mentioned protocol). The PCR test was scored as positive if the viral load surpassed the minimum threshold value. All blood products (measured in milliliters) received by the recipients were documented.

Statistical analysis

Descriptive statistics and Kaplan-Meier curves were used to analyze the cumulative incidence (and 95% confidence intervals [CIs]) of infection until 1 year of follow-up according to each recipient's pretransplant serologic status. This was also done for the group of recipients receiving only cord-blood (EBV-negative) grafts. Time zero was defined as the date of transplantation. Patients contributed to follow-up time until documentation of a positive EBV PCR test or until the last recorded visit date up to 1 year posttransplantation for censored observations. Cox regression was used to measure the association (relative risks [RRs] and 95% CI) between posttransplant EBV infection and 1) transfusion of blood products and 2) volume of blood products transfused. Tertile or quartile was used for the categorization of the variable "volume of transfusion." For the analysis regarding the risk conferred by the volume of transfusion, we also tested for trend by fitting models using the volume variable treated as ordinal based on the median value for each quartile or tertile of volume transfused. Types of blood products analyzed were those with potential for viral transmission, such as RBCs, plasma, and PLT concentrates (labile blood products).[33,34] Blood products manufactured with pasteurization (heat inactivation) and solvent/detergent viral inactivation procedures, such as albumin, were not considered in this analysis. Confusion was controlled for using the 5% change in estimate method considering variables such as type of transplantation (autologous, allogeneic cord blood, allogeneic other, or haploidentical), age (linear), sex (male or female), and year of diagnosis (before or after 2000, seeing that universal leukoreduction was instituted in Canada in 1999). All analyses were performed with computer software (Stata 11.1, StataCorp, College Station, TX).

RESULTS

EBV seroprevalence and infection in the cohort

A total of 487 charts were reviewed for HSC grafts performed on 422 pediatric patients between 1993 and 2009. All 422 pediatric recipients were included in this analysis, but only the first transplant was considered for patients receiving more than one graft. The majority of HSC grafts (317 of 422 [75%]) were performed after the implementation of universal leukoreduction. The mean and median ages at transplantation were 8.9 (standard deviation [SD], 5.2) and 8.5 (interquartile range [IQR], 3.6-14.1) years, respectively. There were 177 (42%) females and 245 (58%) males. Grafts were subdivided into three categories, namely 150 autologous (36%), 111 allogeneic cord blood

(26%), and 161 allogeneic other (38%; Table 1). The pretransplant EBV seroprevalence was 77.9% in this recipient cohort; thus 22.1% of our pediatric patients were EBV seronegative before transplantation. EBV seroprevalence data in HSC donors were calculated after excluding autologous and cord blood grafts and were available for 68% of the allogeneic HSC donors (110 of 161). EBV seropositivity among these donors was 61.8%, as determined by the presence of antibodies to VCA IgG. The median time between pretransplant serologic testing and the date of transplantation was 28 days (IQR, 17-54 days) for both recipients and HSC donors.

Table 2 and Fig. 1 show, for HSC recipients with EBV PCR testing, the cumulative incidence of posttransplant EBV infection at different time points stratified according to their pretransplant EBV serostatus. Only patients with EBV PCR results have been included in these analyses (238 patients). Patients with missing PCR results (most of whom are autologous transplant recipients) have been excluded. Among seronegative patients (EBV seronegative before transplantation), the 1-year cumulative incidence of EBV infection was 28.5% (95% CI, 14.2%-51.9%). A total of eight seronegative recipients developed EBV DNAemia within 1 year posttransplant. By 30 and 60 days, 4.6 (95% CI, 1.2-17.3) and 13.4% (95% CI, 5.8%-29.4%), respectively, had evidence of EBV DNAemia as revealed by positive PCR testing in blood. Among the group of seronegative recipients with posttransplant EBV DNAemia, one probable PTLD case was diagnosed and was fatal (data not shown).

TABLE 1. Graft category in recipient population*

Type of transplantation	Number* (%)
Autologous	
Peripheral blood stem cells	132 (31.3)
Marrow	18 (4.3)
Allogeneic	
Related marrow	115 (27.3)
Unrelated marrow	42 (10.0)
Related peripheral blood stem cells	3 (0.6)
Unrelated peripheral blood stem cells	1 (0.2)
Unrelated cord blood	109 (25.8)
Related cord blood	2 (0.5)
Total	422 (100)

* Includes information for first transplantation only in the case of patients who received more than one graft.

This deceased patient had been transfused with 6825 mL of RBCs and 9790 mL of PLTs, respectively, during the peritransplant period and had received a graft from a partially mismatched related donor who was EBV seropositive. The graft was not T depleted. The first positive EBV PCR test occurred on Day 48 posttransplant and progressed with unexplained fever, pleural effusion, digestive symptoms, increasing EBV DNAemia, and high EBV DNA viral load on biopsy specimen (antrum, duodenum, and sygmoid colon) on Day 68. Rituximab was administered, but the patient died on Day 88 posttransplant. This patient had not received immunosuppressive therapy before transplantation, apart from the conditioning regimen, which was begun after serologic testing.

We also noted a case of hemophagocytic syndrome probably related to EBV. This patient was seronegative before transplantation. However, due to a diagnosis of immune deficiency (Griscelli disease), the patient was classified among the group with unknown pretransplant serostatus (even though the serology result was probably valid). This recipient received 657 mL of RBCs and 1430 mL of PLTs during the peritransplant period and had received a marrow graft from a seropositive donor. The patient was first positive by EBV PCR testing at Day 98 posttransplant and did not receive immunosuppressive therapy before transplantation, apart from the conditioning therapy that was begun after serologic testing.

EBV seroprevalence and infection in the cord blood recipient subgroup

With rare exceptions, umbilical cord blood is typically negative for EBV.[35] This allowed us to consider the subgroup of EBV-negative recipients of cord blood who become EBV positive posttransplant as the ideal population to examine to resolve the question of whether EBV might infect HSC transplant patients through EBV-positive transfused blood products. Table 3 and Fig. 2 show the incidence of posttransplant EBV infection in the subgroup of children who received cord blood grafts. Interestingly, the 30-day cumulative incidence of EBV was 8.3% (95% CI, 2.2%-29.4%; two recipients of 24 had EBV DNAemia), whereas the 1-year cumulative incidence was 27.1% (95% CI, 10.1%-60.8%). All of these recipients received RBC and PLT transfusions during the peritransplant period (mean of 805 mL RBCs [SD, 531 mL] and 1178 mL PLTs [SD, 697 mL]).

TABLE 2. Cumulative incidence of posttransplant EBV infection* after HSCT according to recipient pretransplant EBV serostatus†

Cumulative incidence % (95% CI)	At 30 days	At 60 days	At 100 days	At 200 days	At 1 year
Seronegative recipients (n = 42)	4.6 (1.2-17.3)	13.4 (5.8-29.4)	16.4 (7.7-33.1)	16.4 (7.7-33.1)	28.5 (14.2-51.9)
Recipients with prior EBV antibodies (n = 185)	7.2 (4.2-12.1)	24.3 (18.6-31.5)	33.1 (26.4-40.9)	38.5 (31.2-46.9)	48.6 (39.5-58.5)
Unknown pretransplant serostatus (n = 11)	19.9 (5.2-57.7)	29.3 (10.5-66.3)	39.4 (16.9-74.2)	67.8 (36.2-94.1)	67.8 (36.2-94.1)

* EBV infection measured by PCR testing in blood. n = number of subjects (excludes patients who did not have EBV-PCR testing [missing EBV PCR] most of whom are autologous transplant recipients).
† Data are reported as percentage (95% CI).

Classification and volume of blood products received by recipients and RR calculation

Table 4 provides a description of the total volume of blood products received by the recipients during the peritransplant period. The proportion of recipients who received at least one RBC transfusion was 93.3%. Nearly all recipients (99%) received PLTs. Thus only a few recipients (less than 1%) were free of transfused products.

Table 5 provides the adjusted RR for the association between transfusion of blood products (as well as for volume of transfusion) and posttransplant EBV infection. The adjusted RRs between posttransplant EBV infection and transfusion of RBCs and fresh-frozen plasma (FFP) were 2.36 (95% CI, 0.58-9.70) and 1.34 (95% CI, 0.62-2.93), respectively. It was not possible to study the association between EBV infection and the reception of PLTs because 99% of stem cell transplant recipients received PLTs. It was also not possible to analyze the association with cryoprecipitate and granulocyte transfusions because less than 2% of recipients had been transfused with these blood products. However, it was possible to analyze the association between the volume of blood products transfused and the posttransplant EBV infection. For all these labile blood products, a clear positive trend was shown. The risk of EBV infection increased with the augmentation of the volume transfused (most of the RR in the highest quartile or tertile as well as the p values for trend were significant). Also, as it is impossible to analyze the risk for RBCs independently from PLTs (seeing that virtually all recipients received PLTs), we analyzed the risk for RBCs restricted to the group of patients who received the lowest volume (less than 2000 mL) of PLTs (data not shown) and found similar results with a significant p value for trend ($p = 0.035$). We also ran the analysis after exclusion of recipients who received a graft before year 2000 (thus eliminating patients grafted before implementation of the universal leukoreduction system) and we obtained similar results (data not shown). For example, even with the use of leukoreduced products, the adjusted RR for patients who received over 200 mL of FFP was 3.80 (95% CI, 1.13-12.80) compared to those who did not receive FFP and the adjusted RR for patients receiving more than 2530 mL of PLTs was 2.54 (95% CI, 1.32-4.87) compared to those who received less than 1260 mL.

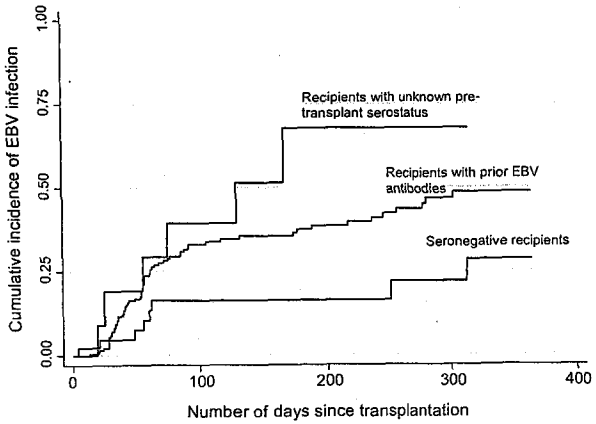

Fig. 1. Kaplan-Meier curve for the cumulative incidence of posttransplant EBV infection among all HSCT recipients according to their pretransplant EBV serostatus. EBV infection measured by PCR testing in blood. The difference between survival curves was not significant (p value = 0.08 by log-rank test).

DISCUSSION

Globally, the results of this study suggest that transfused leukoreduced blood is a vehicle for EBV transmission in immunosuppressed HSCT patients. In support of this we noted a significant and clear positive trend associating EBV infection to transfusion volume. Also, two cases of EBV DNAemia occurred in seronegative recipients of cord blood grafts within 30 days posttransplant;

TABLE 3. Posttransplant EBV infection* in recipients of cord blood grafts†

Cumulative incidence % (95% CI)	At 30 days	At 60 days	At 100 days	At 200 days	At 1 year
Seronegative recipients (n = 24)	8.3 (2.2-29.4)	8.3 (2.2-29.4)	8.3 (2.2-29.4)	8.3 (2.1-29.4)	27.1 (10.1-60.8)
Recipients with prior EBV antibodies (n = 70)	1.4 (0.2-9.7)	10.5 (5.2-20.8)	15.9 (8.8-27.7)	18.6 (8.8-27.7)	32.1 (19.7-49.4)
Unknown pretransplant serostatus (n = 6)	0	20.0 (3.1-79.6)	20.0 (3.1-79.6)	46.7 (13.7-93.2)	46.7 (13.7-93.2)

* EBV infection measured by PCR testing in blood. n = number of subjects (excludes patients who did not have EBV PCR testing [missing EBV PCR] most of whom are autologous transplant recipients).
† Data are reported as percentage (95% CI).

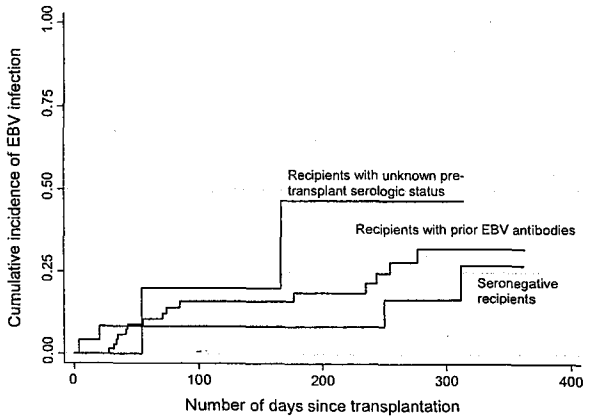

Fig. 2. Kaplan-Meier curve for the cumulative incidence of posttransplant EBV infection* among cord blood transplant recipients. *EBV infection measured by PCR testing in blood. The difference between survival curves was not significant (p value = 0.61 by log-rank test).

TABLE 4. Type of transfused blood product and quantity received by recipients

Type	Number of patients* (%)	Total volume (mL) Median (IQR)	Total volume (mL) Mean (SD)
RBCs			
No	28 (6.7)		
Yes	389 (93.3)	1050 (600-2150)	1806 (2342)
FFP or frozen plasma			
No	383 (91.9)		
Yes	34 (8.1)	300 (200-1065)	2056 (6044)
PLT concentrates			
No	4 (1.0)		
Yes	413 (99.0)	1806 (825-3677)	3373 (5289)
Cryoprecipitates			
No	413 (99.0)		
Yes	4 (1.0)	90 (23-188)	105 (100)
Albumin			
5%			
No	369 (88.5)		
Yes	48 (11.5)	250 (250-500)	427 (383)
25%			
No	375 (89.9)		
Yes	42 (10.1)	250 (200-518)	500 (717)
Granulocytes			
No	414 (98.1)		
Yes	8 (1.9)	1682 (1568-3434)	2707 (2156)
Any product			
No	5 (1)		
Yes	417 (99)		

* Total frequencies may differ slightly from the total number of recipients because missing data are not listed. Includes data related to the first transplantation only.

these cannot be attributed to a reactivation event of endogenous recipient virus nor can they implicate the graft as a source of infection.

EBV pretransplant seroprevalence for our pediatric recipient cohort was 77.9%, a proportion substantially higher than the 50% documented in the solid organ pediatric transplant population.[36,37] It is possible that the higher seroprevalence in our cohort was due to passive antibody transfer given that children with leukemia and other malignant hematologic disorders are more likely to receive blood products before transplantation. On the other hand, we might be less confident about serology for the detection of a recent infection in patients receiving cancer chemotherapy. While we cannot completely rule out the possibility that EBV infection might have been missed in these patients before transplant, the patient charts show that our cohort had a higher prevalence of EBV antibodies before transplant than the expected EBV seroprevalence rate in children. Although our protocol does not include pretransplant PCR testing, serologic testing at our institution always includes both VCA IgG and EBNA antibody testing. We are therefore confident that the EBV serologic data for patients with a seronegative profile are accurate. Seroprevalence among HSC donors was 61.8%, which is within the range usually found in children. This was not surprising considering that donors are often the patients' siblings.

Our data indicate that 13.4% (95% CI, 5.8%-29.4%) of seronegative recipients developed EBV DNAemia within 60 days posttransplant. Moreover, among the group of eight seronegative recipients who developed EBV DNAemia, one case of probable PTLD was diagnosed and was fatal. Interestingly, this patient had received substantial amounts of RBCs (6825 mL) and PLTs (9790 mL) during the peritransplant period, a transfusion volume much greater than the average for this group of recipients (Table 4). Furthermore, it is noteworthy that this case of probable PTLD in seronegative patients, as well as most of the incidences of EBV posttransplant infection in our seronegative patients, occurred after the year 2000, and were subsequent to the implementation of universal prestorage leukoreduction in Canada.

Transmission of EBV in seronegative recipients may have occurred through virus contained either in the donor graft or in the transfused blood products. If EBV DNAemia were to occur in seronegative patients receiving an EBV-negative graft, then blood products could be suspected as the vector for transmission. Numerous cases of EBV DNAemia occurred in recipients for which the pretransplant serostatus of the donor was unknown (missing data). However, many children were transplanted with cord blood, which is normally negative for EBV.[35] Interestingly, within 30 days, EBV infection had occurred in 8.3% (95% CI, 2.1%-29.4%) of seronegative recipients after cord blood transplantation. Barring natural infection, which is possible but unlikely in such a short time period—more so because recipients were isolated in hospital in a HEPA air-filtered room—this strongly points to blood products as the vehicle for transmission. It is noteworthy that EBV seroconversion also occurred after 3 months, but these "late" cases are difficult to attribute to transfusion as most transfusions would have been expected in the first 3 months posttransplant. EBV is a ubiquitous virus transmitted by saliva; therefore, we cannot rule out the possibility that patients were exposed naturally to the virus after the isolation period. Natural infection may explain the cases of DNAemia especially those that occurred long after the transplant. One might also argue on the validity of the pretransplant serology of children with leukemia receiving immunosuppressive therapy. However, this cannot explain the seronegative status of our EBV-negative recipients of cord blood who developed EBV DNAemia, as none of these patients (except for one case which occurred within 30 days posttransplant) received pretransplant immunosuppressive therapy (apart from the pretransplant conditioning therapy, which always begins after testing for EBV serology).

Finally, it is not possible to completely rule out the possibility that EBV originated from the cord blood graft. Although such an event would be exceedingly rare, such unusual cases of EBV-infected cord blood have been documented. For example, Weinberg and colleagues[35] reported no case of positive EBV PCR among 362 cord blood samples. However, in 1973, Chang and Blakenship[38] showed that one of the 696 cord blood samples tested was EBV positive. One such case has also been documented by Haut and coworkers.[39]

The results of this study indicate that recipients who received RBC transfusions were 2.37 times more at risk of developing EBV DNAemia than those who did not receive RBCs (although this was not significant). We also showed a clear positive association between the volume of RBCs, plasma, and PLTs transfused and the incidence of posttransplant EBV infection. The RRs for the highest quartile or tertile of volume transfused was 2.86 (95% CI, 0.68-12.11) for RBCs, 3.16 (95% CI, 1.00-11.17) for plasma, and 2.19 (95% CI, 1.21-3.97) for PLTs and the p value for trend was significant for the volume of RBCs and PLTs transfused. This shows a clear association between transfusion and EBV infection.

TABLE 5. Adjusted RR* for the association between transfusion of blood products and incidence of posttransplant EBV infection

Type of blood product†	Adjusted RR (95% CI)	p value for trend‡
RBC		
Transfusion		
No	1.00 (reference)	
Yes	2.37 (0.58-9.70)	
Volume of transfusion (mL)		
0	1.00 (reference)	0.047§
<850.0	1.99 (0.47-8.44)	
850.0-1890.0	2.40 (0.56-10.24)	
>1890.0	2.86 (0.68-12.11)	
FFP		
Transfusion		
No	1.00 (reference)	
Yes	1.34 (0.62-2.93)	
Volume of transfusion (mL)		
0	1.00 (reference)	0.079
<200.0	0.70 (0.22-2.25)	
>200.0	3.16 (1.00-11.17)§	
PLTs		
Transfusion		
No		
Yes		
Volume of transfusion (mL)		
< 1260	1.00 (reference)	0.012§
1260-2530	1.65 (0.86-3.18)	
>2530	2.19 (1.21-3.97)§	

* RRs are hazard ratio estimated with Cox regression and adjusted for empirical confounders using 5% change in estimate method (for variables such as type of transplantation [autologous, allogeneic cord blood, allogeneic other, or haploidentical], age [linear], year of diagnosis [before or after 2000], and sex [male or female]).
† It was not possible to analyze the risk related to the reception of PLTs because virtually all recipients received PLT concentrates. However, it was possible to analyze the risk associated with different volumes of PLTs transfused. Also, because of too little data, it was not possible to analyze the risk related to cryoprecipitate and granulocyte transfusions. Because albumin has no potential for viral transmission it was not considered in our analysis.
‡ We tested for trend by fitting models using the volume variable treated as ordinal based on the median value for each quartile or tertile of volume transfused.
§ Results significant.

In an effort to prevent transfusion reactions and transfusion-transmitted infectious diseases, Canadian Blood Services and Héma-Québec implemented systematic prestorage RBC unit leukoreduction in the summer of 1999; in addition, prestorage leukoreduction of PLTs had been available since February 1998. Leukoreduction is a process in which WBCs, ordinarily present in collected blood components, are intentionally reduced in number. Typically, the number of WBCs in a RBC unit is decreased from 5×10^9 to less than 5×10^6 WBCs per unit by prestorage leukoreduction.[40] Through this process the number of viral copies associated with WBCs would be expected to be reduced accordingly. It would have been very interesting to perform our analysis by comparing data from specimens taken before with those taken after the implementation of leukoreduction to measure the impact of leukoreduction on the risk of EBV transmission. Unfortunately, too few data were available before year 2000 to allow this stratification. However, our results indicate significant RRs even when the analysis was restricted to patients who received a graft after the implementation of universal leukoreduction. It is important to point out that leukoreduction does not reduce to zero the risk of transmitting certain viruses. For example, it has been shown that CMV-seronegative units may provide greater protection than leukoreduced products in some at-risk population groups such as transplant recipients and immunosuppressed patients.[41,42] This might also be the case for EBV.

Transmission of EBV infection by transfusion is thought to be relatively infrequent for the following reasons: 1) most adult recipients of blood and blood products are already immune to EBV; 2) whole blood and serum from seropositive donors contain EBV-neutralizing antibodies, which may protect the recipient from infection; 3) the viability of B lymphocytes carrying the EBV genome may decline during blood storage; 4) viral load in blood from healthy seropositive donors is normally low ($5/10^6$-$1/10^7$ peripheral blood MNCs); and 5) the risk of EBV transmission from RBC and/or PLT transfusions is significantly reduced by leukoreduction. Thus, in most instances, EBV genomes contained in blood products should not cause severe disease when the transfused recipient is immune competent. In fact, with regard to EBV, blood products are safe for the general adult population since over 90% of adults have immunity to EBV. Occurrence of infectious mononucleosis in EBV-negative recipients receiving EBV-positive blood products has been documented, but is rare.[28]

While immune-competent individuals can control the infection, those with congenital or acquired immunodeficiency are highly vulnerable to developing EBV-associated lymphoproliferative disease.[43-47] The overall incidence of PTLD among allogeneic HSCT adult recipients has been estimated to be approximately 1% (approx. 3% for pediatric HSCT).[12,48,49] This risk increases to more than 8% with the presence of risk factors such as T-cell depletion of the donor marrow.[10,49,50] The occurrence of PTLD is higher during the early posttransplant period due to the ablated state of the immune system. Lack of a robust immune response may lead to high EBV viral load which is a risk factor and prognostic indicator for PTLD.[10] Among allogeneic stem cell recipients who develop PTLD, approximately 25% will die and 25% will incur graft failure.[9] The mortality incidence after PTLD may reach 82%.[12] It stands to reason, therefore, that transfusion of EBV-positive blood products to immune-suppressed stem cell transplant pediatric patients may prove detrimental during the early posttransplant period.

Despite the limitations of this study, which include its retrospective design, missing chart data, and inclusion of only one center, there are nonetheless numerous strengths. One of these is the study's appreciable sample size. Further, the population is diverse and thus representative of a typical transplant population sampling from a large North American city. The results are clinically significant and suggest an association between EBV infection and transfusion of leukoreduced blood product units. The number of patients was too small to draw conclusions on any potential association between blood product transfusion and PTLD, but large enough to yield interesting RRs and to consider designing a prospective study in the pediatric transplant population. Unfortunately, typing of donor-recipient strains is not possible. Legal and ethical norms pertinent to blood donation require anonymity, thus impeding any tracing of donor units for EBV isolation and typing postdonation.

Our patient population included EBV-seronegative patients who showed a surprisingly high rate of EBV infection acquired within a time frame unlikely to be compatible with acquisition through an infected contact. Indeed, our data suggest that transmission of EBV infection occurred through the transfusion of blood products. To our knowledge, this is the first report to document the level of transfusion-related risk of acquiring EBV infection in an immunosuppressed population. Pretransplant EBV-seropositive recipients also showed evidence of EBV DNAemia at various time points posttransplant. For the latter, the source of the DNAemia may be reactivation of their own virus or new infection or reinfection by virus originating from the graft or from transfused blood products. Moreover, because cord blood progenitor cells are increasingly used for transplantation in children and because EBV is not normally found in cord blood,[35] the probability of EBV infection via the donor graft is essentially eliminated. This points to the potential importance of EBV in blood products as a source of infection among the pediatric transplant population. It also suggests a need to consider instituting EBV screening of blood products destined to immunosuppressed pediatric patients and developing appropriate EBV prophylactic measures (vaccine, antibody therapy) for use in such patients. Instituting EBV screening of blood products may not be easy to achieve given the high prevalence of EBV seropositivity in adults, but it would be theoretically possible taking into account that approximately 1% to 10% of blood donors might be called upon to give blood for such a small subgroup of patients.

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CONFLICT OF INTEREST

The authors declare no competing financial interests. The authors were not restricted in experimental design, in data collection and analysis, nor in public disclosure of the findings contained in this manuscript.

REFERENCES

1. Hema-Québec. Safety measures in place. [cited 2011 May 24]. Available from: URL: http://www.hema-quebec.qc.ca/comprendre/mesures-de-securite/securite-en-place.en.html
2. Canadian Blood Services. Safety and testing. [cited 2011 May 3]. Available from: URL: http://www.bloodservices.ca/CentreApps/Internet/UW_V502_MainEngine.nsf/page/Testing?OpenDocument
3. Macdonald N. Transfusion and risk of infection in Canada: update 2005. Can J Infect Dis Med Microbiol 2005; 16:161-5.
4. Chamberland ME, Alter HJ, Busch MP, Nemo G, Ricketts M. Emerging infectious disease issues in blood safety. Emerg Infect Dis 2001;7 Suppl:552-3.
5. Strong DM, Katz L. Blood-bank testing for infectious diseases: how safe is blood transfusion? Trends Mol Med 2002;8:355-8.
6. Vamvakas EC. Is human herpesvirus-8 transmitted by transfusion? Transfus Med Rev 2010;24:1-14.
7. Karimi G, Samiei S, Hatami H, Gharehbaghian A, Vafaiyan V, Namini MT. Detection of Chlamydia pneumoniae in peripheral blood mononuclear cells of healthy blood donors in Tehran Regional Educational Blood Transfusion Centre. Transfus Med 2010;20:237-43.
8. Trimble SR, Parker CS, Grant AM, Soucie JM, Reyes N. Assessing emerging infectious threats to blood safety for the blood disorders community. Am J Prev Med 2010;38 Suppl:S468-74.
9. Smith JM, Corey L, Healey PJ, Davis CL, McDonald RA. Adolescents are more likely to develop posttransplant lymphoproliferative disorder after primary Epstein-Barr virus infection than younger renal transplant recipients. Transplantation 2007;83:1423-8.
10. Aalto SM, Juvonen E, Tarkkanen J, Volin L, Haario H, Ruutu T, Hedman K. Epstein-Barr viral load and disease prediction in a large cohort of allogeneic stem cell transplant recipients. Clin Infect Dis 2007;45:1305-9.
11. Allen U, Alfieri C, Preiksaitis J, Humar A, Moore D, Tapiero B, Tellier R, Green M, Davies D, Hébert D, Weitzman S, Petric M, Jacobson K; Canadian PTLD Workshop Group—1999. Epstein-Barr virus infection in transplant recipients: summary of a workshop on surveillance, prevention and treatment. Can J Infect Dis 2002;3:89-99.
12. Ocheni S, Kroeger N, Zabelina T, Sobottka I, Ayuk F, Wolschke C, Muth A, Lellek H, Petersen L, Erttmann R, Kabisch H, Zander AR, Bacher U. EBV reactivation and post transplant lymphoproliferative disorders following allogeneic SCT. Bone Marrow Transplant 2008;42: 181-6.
13. Higgins CD, Swerdlow AJ, Macsween KF, Harrison N, Williams H, McAulay K, Thomas R, Reid S, Conacher M, Britton K, Crawford DH. A study of risk factors for acquisition of Epstein-Barr virus and its subtypes. J Infect Dis 2007;195:474-82.
14. Pariente M, Bartolome J, Lorente S, Crespo MD. Age distribution of serological profiles of Epstein-Barr virus infection: review of results from a diagnostic laboratory. Enferm Infecc Microbiol Clin 2007;25:108-10.
15. Rocchi G, de Felici A, Ragona G, Heinz A. Quantitative evaluation of Epstein-Barr virus-infected mononuclear peripheral blood leukocytes in infectious mononucleosis. N Engl J Med 1977;296:132-4.
16. Riddler SA, Breinig MC, McKnight JL. Increased levels of circulating Epstein-Barr virus (EBV)-infected lymphocytes and decreased EBV nuclear antigen antibody responses are associated with the development of posttransplant lymphoproliferative disease in solid organ transplant recipients. Blood 1994;84:972-84.
17. Hudnall SD, Chen T, Allison P, Tyring SK, Heath A. Herpesvirus prevalence and viral load in healthy blood donors by quantitative real-time polymerase chain reaction. Transfusion 2008;48:1180-7.

18. Wagner HJ, Kluter H, Kruse A, Bucsky P, Hornef M, Kirchner H. Determination of the number of Epstein-Barr virus genomes in whole blood and red cell concentrates. Transfus Med 1995;5:297-302.
19. Gerber P, Walsh JH, Rosenblum EN, Purcell RH. Association of EB-virus infection with the post-perfusion syndrome. Lancet 1969;1:593-5.
20. Henle W, Henle G, Scriba M, Joyner CR, Harrison FS Jr, *Von Essen R, Paloheimo J, Klemola E. Antibody responses* to the Epstein-Barr virus and cytomegaloviruses after open-heart and other surgery. N Engl J Med 1970;282: 1068-74.
21. Carter JW, Edson RS, Kennedy CC. Infectious mononucleosis in the older patient. Mayo Clin Proc 1978;53:146-50.
22. McMonigal K, Horwitz CA, Henle W, Henle G, Lawton J, Polesky H, Peterson L. Post-perfusion syndrome due to Epstein-Barr virus. Report of two cases and review of the literature. Transfusion 1983;23:331-5.
23. Turner AR, McDonald RN, Cooper BA. Transmission of infectious mononucleosis by transfusion of pre-illness plasma. Ann Int Med 1972;77:751-3.
24. Blacklow NR, Watson BK, Miller G, Jacobson BM. Mononucleosis with heterophil antibodies and EB virus infection. Acquisition by an elderly patient in hospital. Am J Med 1971;51:549-52.
25. Henle W, Henle G. Epstein-Barr virus and blood transfusion. In: Dodd RY, Barker LF, editors. Infection, immunity and blood transfusion. Proceedings of the XVth Annual Scientific Sympoium of the American Red Cross. New York: Liss; 1985. p. 201.
26. Solem JH, Jörgensen W. Accidentally transmitted infectious mononucleosis. Report of a case. Acta Med Scand 1969; 186:433-7.
27. Kapsenberg JG, Langenhuysen MM, Nieweg HO, Deiss H. Posttransfusion mononucleosis with heterophil antibodies. Simultaneous infection with cytomegalovirus and EB virus. Acta Med Scand 1970;187:79-82.
28. Tattevin P, Crémieux AC, Descamps D, Carbon C. Transfusion-related infectious mononucleosis. Scand J Infect Dis 2002;34:777-8.
29. Alfieri C, Tanner J, Carpentier L, Perpête C, Savoie A, Paradis K, Delage G, Joncas J. Epstein-Barr virus transmission from a blood donor to an organ transplant *recipient with recovery of the same virus strain from the* recipient's blood and oropharynx. Blood 1996;87:812-7.
30. Yao QY, Croom-Carter DS, Tierney RJ, Habeshaw G, Wilde JT, Hill FG, Conlon C, Rickinson AB. Epidemiology of infection with Epstein-Barr virus types 1 and 2: lessons from the study of a T-cell-immunocompromised hemophilic cohort. J Virol 1998;72:4352-63.
31. Murdock J, Morris TC, Alexander HD, McNamee P. Red cell aplasia due to erythrovirus parvovirus B19 infection in a Jehovah's witness with post-transplant EBV-induced aggressive lymphoma: management considerations. Leuk Lymphoma 2002;43:211-3.
32. Dalle J, Duval M, Moghrabi A, Wagner E, Vachon MF, Barrette S, Bernstein M, Champagne J, David M, Demers J, Rousseau P, Winikoff R, Champagne MA. Results of an unrelated transplant search strategy using partially HLA-mismatched cord-blood as an immediate alternative to HLA-matched bone marrow. Bone Marrow Transplant 2004;33:605-11.
33. Tabor E. The epidemiology of virus transmission by plasma *derivatives: clinical studies verifying the lack of transmis*sion of hepatitis B and C viruses and HIV type 1. Transfusion 1999;39:1160-8.
34. Hillyer CD, Silberstein LE, Ness PM, Anderson KC, Roback JD, editors. Blood banking and transfusion medicine—basic principles and practice. 2nd ed. Philadelphia (PA): Churchill Livingstone; 2007.
35. Weinberg A, Enomoto L, Li S, Shen D, Coll J, Shpall EJ. Risk of transmission of herpesviruses through cord blood transplantation. Biol Blood Marrow Transplant 2005;11:35-8.
36. Breinig MK, Zitelli B, Starzl TE, Ho M. Epstein-Barr virus, cytomegalovirus, and other viral infections in children after liver transplantation. J Infect Dis 1987;156:273-9.
37. Ho M, Jaffe R, Miller G, Breinig MK, Dummer JS, Makowka L, Atchison RW, Karrer F, Nalesnik MA, Starzl TE. The frequency of Epstein-Barr virus infection and associated lymphoproliferative syndrome after transplantation and its manifestation in children. Transplantation 1988;45:719-27.
38. Chang RS, Blakenship W. Spontaneous in vitro transformation of leukocytes from a neonate. Proc Soc Exp Biol Med 1973;144:337-9.
39. Haut PR, Kovarik P, Shaw PH, Walterhouse D, Jenson HB, Kletzel M. Detection of EBV DNA in the cord blood donor for a patient developing Epstein-Barr virus-associated lymphoproliferative disorder following mismatched unrelated umbilical cord blood transplantation. Bone Marrow Transplant 2001;27:761-5.
40. Gibson BE, Todd A, Roberts I, Pamphilon D, Rodeck C, Bolton-Maggs P, Burbin G, Duguid J, Boulton F, Cohen H, Smith N, McClelland DB, Rowley M, Turner G; British Commitee for Standards in Haematology Transfusion Task Force: Writing group. Transfusion guidelines for neonates and older children. Br J Haematol 2004;124:433-53.
41. Nichols WG, Price TH, Gooley T, Corey L, Boeckh M. Transfusion-transmitted cytomegalovirus infection after receipt of leukoreduced blood products. Blood 2003;101: 4195-200.
42. Hillyer CD, Strauss RG, Luban NL. Handbook of pediatric transfusion medicine. San Diago (CA): Academic; 2004.
43. Joncas JH, Russo P, Brochu P, Simard P, Briseboís J, Dubé J, Marton D, Leclerc JM, Hume H, Rivard GE. Epstein-Barr virus polymorphic B-cell lymphoma associated with leukemia and with congenital immunodeficiencies. J Clin Oncol 1990;8:378-84.
44. Young L, Alfieri C, Hennessy K, Evans H, O'Hara C, Anderson KC, Ritz J, Shapiro RS, Rickinson A, Kieff E, Cohen JI. Expression of Epstein-Barr virus transformation-associated genes in tissues of patients with EBV lymphoproliferative disease. N Engl J Med 1989;321:1080-5.
45. Hanto DW, Gajl-Peczalska KJ, Frizzera G, Arthur DC, Balfour HH Jr, McClain K, Simmons RL, Najarian JS. Epstein-Barr virus (EBV) induced polyclonal and monoclonal B-cell lymphoproliferative diseases occurring after renal transplantation. Clinical, pathologic, and virologic findings and implications for therapy. Ann Surg 1983;198: 356-69.
46. Zutter MM, Martin PJ, Sale GE, Shulman HM, Fisher L, Thomas ED, Durnam DM. Epstein-Barr virus lymphoproliferation after bone marrow transplantation. Blood 1988; 72:520-9.
47. Cohen JI. Epstein-Barr virus lymphoproliferative disease associated with acquired immunodeficiency. Medicine (Baltimore) 1991;70:137-60.
48. Curtis RE, Travis LB, Rowlings PA, Socié G, Kingma DW, Banks PM, Jaffe ES, Sale GE, Horowitz MM, Witherspoon RP, Shriner DA, Weisdorf DJ, Kolb HJ, Sullivan KM, Sobocinski KA, Gale RP, Hoover RN, Fraumeni JF Jr, Deeg HJ. Risk of lymphoproliferative disorders after bone marrow transplantation: a multi-institutional study. Blood 1999;94: 2208-16.
49. Landgren O, Gilbert ES, Rizzo JD, Socié G, Banks PM, Sobocinski KA, Horowitz MM, Jaffe ES, Kingma DW, Travis LB, Flowers ME, Martin PJ, Deeg HJ, Curtis RE. Risk factors for lymphoproliferative disorders after allogeneic hematopoietic cell transplantation. Blood 2009;113:4992-5001.
50. Cesaro S, Pegoraro A, Tridello G, Calore E, Pillon M, Varotto S, Abate D, Barzon L, Mengoli C, Carli M, Messina C. A prospective study on modulation of immunosuppression for Epstein-Barr virus reactivation in pediatric patients who underwent unrelated hematopoietic stem-cell transplantation. Transplantation 2010;89:1533-40.

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研究報告の概要

○西アフリカの流行地域であるガーナにおける、ヒトヘルペスウイルス8の輸血感染

ヒトヘルペスウイルス8(HHV-8)の抗体陽性率は、欧州や北米では5%未満、サハラ以南アフリカでは50～70%と地域によって開きがある。HHV-8の輸血感染の証拠は間接的なものに留まっている。今回、供血者と全血輸血を受けた免疫正常受血者のペア252組から得た検体に対して血清学的検査及び分子生物学的検査を行った。その結果、受血者28人(11%)及び供血者16人(6%)がHHV-8抗体陽性であり、抗体陽性の血液を輸血された抗体陰性受血者12人中1人(8.3% 信頼区間0～23%)に感染の疑いが高いことが確認された。当該供血者の血液にはHHV-8 DNAが含まれており、当該供血者を含む5人のHHV-8 DNAの配列は、ブートストラップ値97%で既知のジェノタイプとは異なるクラスターを形成していた。そのため、新しいジェノタイプ(HHV-8-G)を命名法に加えることを提案する。

今回のHHV-8伝播は、受血者の多くが免疫正常者であったため、臨床的影響はなかった。しかし、サハラ以南アフリカでは、免疫抑制剤の使用の増加に伴い、臨床的なリスクは増大するだろう。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血
新鮮凍結血漿-LR「日赤」120
新鮮凍結血漿-LR「日赤」240
新鮮凍結血漿-LR「日赤」480

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
ヒトヘルペスウイルス8(HHV-8)の流行地域であるガーナにおいて、血液供血者と受血者のペア252組についてHHV-8の検査を行ったところ、抗体陽性血液を輸血した抗体陰性受血者12人中1人に感染の疑いが高いことが示され、また当該供血者を含む5人のHHV-8 DNA配列は既知のジェノタイプとは異なるクラスターを形成していることが分かったとの報告である。	今後も情報の収集に努める。

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TRANSFUSION COMPLICATIONS

Human herpesvirus 8 transfusion transmission in Ghana, an endemic region of West Africa

Francesca Gobbini, Shirley Owusu-Ofori, Anne-Genevieve Marcelin, Daniel Candotti, and Jean-Pierre Allain

BACKGROUND: Human herpesvirus 8 (HHV-8) seroprevalence ranges between less than 5% in Europe and North America and 50% to 70% in sub-Saharan Africa. Evidence of HHV-8 transfusion transmission is only indirect. We conducted a serologic (anti-HHV-8) and molecular (HHV-8 DNA) study of samples from paired donor-immunocompetent recipients transfused with whole blood.

STUDY DESIGN AND METHODS: Samples from 252 donor-recipient pairs were tested. Immunoglobulin G to HHV-8 was detected with enzyme immunoassays and confirmed with an in-house immunofluorescence assay. The cellular fraction from seroreactive donors and their recipients was tested for HHV-8 DNA.

RESULTS: Anti-HHV-8 was positive (reactive in two or more assays) in 28 (11%) patients and 16 (6%) donors. Of 12 seronegative recipients (at risk of transmission) receiving seropositive blood, one very likely transmission was identified (8.3%; confidence interval, 0%-23%). The donor blood contained HHV-8 DNA and his and four other donors' sequences clustered separately from recorded genotypes with a 97% bootstrap constituting a distinct genotype.

CONCLUSIONS: HHV-8 is transmitted in Ghana but does not carry clinical consequences since most patients are immunocompetent. The clinical risk will increase with the availability of immunosuppressive drugs in sub-Saharan Africa. We propose that a new genotype (HHV-8-G for Ghana) be added to the current nomenclature.

Human herpesvirus 8 (HHV-8), also known as Kaposi sarcoma–associated herpes virus, was identified as the etiologic agent of Kaposi's sarcoma and was associated with two B-cell lymphoproliferative disorders: primary effusion lymphoma and multicentric Castelman's disease.[1]

HHV-8 seroprevalence varies geographically. In Africa, up to 50% of the population is seropositive,[2] while in northern Europe and America the seroprevalence is less than 5%, increasing to 10% to 15% in Mediterranean regions.[3] Eight genotypes have been identified so far. Genotypes A/C, J, and K are prevalent in Europe, the United States, North of Asia, and the Middle East; in South Asia and Polynesia Genotype D/F has been found while B, Q, R, and N have been identified in sub-Saharan Africa.[4]

In sub-Saharan Africa, routes of HHV-8 transmission include saliva contact within family members and infection occurs mostly during childhood.[2,5,6] In low-prevalence developed countries, sexual transmission between men appears more frequent than in heterosexual relationships.[3] HHV-8 transmission after transplantation

ABBREVIATIONS: HHV-8 = human herpesvirus 8; IFA = immunofluorescence assay; qPCR = quantitative polymerase chain reaction; S/CO = sample-to-cutoff ratio; SNP(s) = single-polymorphism nucleotide(s).

From the Division of Transfusion Medicine, Department of Haematology, University of Cambridge, Cambridge, UK; the Transfusion Medicine Unit, Komfo Anokye Teaching Hospital, Kumasi, Ghana; the Virology Laboratory Hospital Pitié-Salpêtrière, Paris, France; and the National Blood Service, Cambridge Blood Centre, Cambridge, UK.

Address reprint requests to: J.-P. Allain, Department of Haematology, Cambridge Blood Centre, Long Road, Cambridge CB2 2PT, UK; e-mail: jpa1000@cam.ac.uk.

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of organs (mostly kidney) from HHV-8–seropositive donors was reported as well as viral reactivation in seropositive patients receiving immunosuppressive drugs.[1]

Transmission through blood products remains unclear but is considered low risk.[7] In 2009, Cannon and colleagues[8] conducted a large study including linked donor-recipient samples and found no transmission of HHV-8 through blood transfusion. In Uganda, a high-prevalence country, Hladik and colleagues[9] concluded on the basis of serologic indirect evidence to an HHV-8 transfusion transmission risk of 2.8% in seronegative recipients receiving blood from a seropositive donor.

To estimate the risk of transfusion-transmitted HHV-8 infection in a different sub-Saharan African endemic country (Ghana), we conducted a serologic and molecular study on paired donor-recipient blood samples from immunocompetent recipients transfused with whole blood.

MATERIALS AND METHODS

Samples collection

Whole blood samples from patients transfused at the Komfo Anokye Teaching Hospital (Kumasi, Ghana, West Africa) were obtained from the Blood and Organ Transmissible Infectious Agent repository.[10] None of the units transfused were leukoreduced nor washed. A total of 252 sets of donor (<2 weeks of storage before transfusion) and before and 3-months-posttransfusion recipient samples were tested. Whole blood was collected in 10 mL K3 EDTA tubes, separated by decantation, into plasma and cellular fraction, and both were stored at −40°C or below.

HHV-8 serology

Immunoglobulin (Ig)G antibodies to HHV-8 lytic antigens were tested using a bipeptide enzyme immunoassay (EIA; Biotrin International GmbH, Heidelberg, Germany) following the manufacturer's instructions. Samples with a sample-to-cutoff ratio (S/CO) value of less than 0.8 were considered nonreactive; S/CO values of more than 1.2 were reactive and S/CO values between 0.8 and 1.2 were considered undetermined (gray zone). The manufacturer discontinued distribution of this kit halfway through the study so the commercial Advanced Biotechnologies (Columbia, MD) EIA was used to test the rest of the samples. Sixty-nine samples were tested with both commercial kits and only 43 showed concordant results. Because of the high frequency of discrepancy between the two assays, we decided to use as confirmation method for positive samples an in-house immunofluorescence assay in Prof. Agut's Laboratory at Hospital Pitié-Salpêtrière, Paris, France.[11]

HHV-8 DNA extraction, PCR, and sequencing

Viral DNA was isolated from 200 µL of cell fraction by using a viral nucleic acid kit (High Pure, Roche, Lewes, UK) according to the manufacturer's instructions. HHV-8 DNA was detected by a real-time quantitative PCR (qPCR) assay as previously described.[12] Tenfold dilutions of an in-house plasmid pKS2471 containing the HHV-8 ORF26 were used to construct the standard curve and estimate the viral load. To confirm qPCR results, a 1251-nucleotide region including the minor capsid protein coded by ORF26 was amplified by a more sensitive seminested PCR. The forward primers used were KS26/D[4] in the first round of amplification and Orf26Fwd1[13] in the second round. LGH2574[4] was used as reverse primer in both rounds. The two amplification reactions were performed in identical conditions. Briefly, 5 µL of extracted DNA was amplified in a 50-µL mixture containing 1× HiFi Buffer 3 (Roche), 1.5 mmol/L $MgCl_2$, 0.8 mmol/L dNTPs, 0.6 µmol/L of each primer, 2.6 units of enzyme blend (Expand High-Fidelity, Roche), and 26.25 µL of DNase-free water. After an initial denaturation at 94°C for 5 minutes, a touchdown amplification was carried out as follows: 94°C for 1 minute, 60°C for 1 minute, and 72°C for 2 minutes. The 1-minute annealing temperature was 60°C for five cycles, 58°C for five cycles, 57°C for five cycles, and then 55°C for the last 15 cycles. Nested PCR products were purified from gel and sequenced at the University of Cambridge Biochemistry Department with a DNA analyzer (3730xl, Applied Biosystems) using the primers utilized in the PCR. All the ORF26 sequences obtained were submitted to GenBank under the Accession Numbers JN662003 to JN662017.

RESULTS

HHV-8 seroprevalence and transfusion transmission

The Blood and Organ Transmissible Infectious Agent repository including paired pretransfusion and 3-months-posttransfusion samples from Ghanaian immunocompetent whole blood recipients and corresponding donors were tested to investigate the largely unknown transfusion transmission of HHV-8 in a context of reported high endemicity.

Posttransfusion samples from 252 Ghanaian patients and 248 blood donors were tested for HHV-8 IgG antibodies. Results are summarized in Table 1. After a first enzyme-linked immunosorbent assay (ELISA) screening, 72 (29%) of the 252 patients and 56 (23%) of the 248 donors tested positive for HHV-8 antibodies. However, only 29 (11%) patients and 16 (6%) donors were confirmed reactive. Recipients of 16 transfusions with seropositive blood and donors of 29 seroreactive recipients after transfusion were retrospectively investigated.

TABLE 1. Serologic and molecular results of Ghanaian donor and recipient samples

	Anti-HHV-8 (S/CO)		
	Reactive	Nonreactive	HHV-8 DNA
Whole blood donations	16	232	2 positive
Posttransfusion recipients	29	223	2 positive
Pretransfusion recipients of seropositive blood	2	12	0
Posttransfusion exposed* susceptible recipients	1	11	0
Donor 2027	1 (4.7)		Positive
Recipient 2027			
Before transfusion		1	Negative
After transfusion	1 (9.5)		Negative
Donor 2003	1 (3.0)		Positive
Recipient 2003			
Before transfusion		1	Negative
After transfusion		1	Negative

* Seronegative before transfusion.

Before transfusion, 12 patients exposed to seroreactive blood were anti-HHV-8 seronegative and considered susceptible (at risk) to whole blood transfusion–related infection. One of them was seropositive after transfusion and 11 remained seronegative (Table 1). Donor 2027 and the paired susceptible recipient after transfusion were both anti-HHV-8 reactive with 4.7 and 9.5 S/CO, respectively. Anti-HHV-8 in donation 2027 was confirmed by immunofluorescence. Plasma and cellular fraction samples from this pair were tested for HHV-8 DNA. Donor 2027 blood contained HHV-8 DNA but not the recipient's sample collected 3 months after transfusion. In contrast, the recipient of donation 2003 that contained both antibodies and HHV-8 DNA remained seronegative after transfusion (Table 1). The other 10 susceptible recipients exposed to seropositive but DNA negative whole blood did not show serologic or molecular evidence of HHV-8 infection.

HHV-8 genomic sequence and new subtype

To confirm the qPCR screening results, HHV-8 DNA from two viremic patients and two donors was amplified by nested PCR targeting ORF26. Phylogenetic analysis of the sequences suggested that they formed a cluster different from previously reported African strains. To investigate further this genetic variability, 34 Ghanaian HIV-infected samples identified as anti-HHV-8 positive in a previous study were tested for HHV-8 DNA. The ORF26 region was successfully amplified and sequenced in four samples. In parallel, 84 random blood donors' samples from Guinea, another West African country, were screened for HHV-8 DNA and seven sequences were obtained. The Guinean samples clustered with the Q genotype but five of eight Ghanaian sequences clustered separately from the other GenBank references with a bootstrap value of 97% over 1000 replicates (Fig. 1) constituting a separate and new genotype. The other three Ghanaian sequences (Gh1623, 2003D, 2027D) clustered with other genotypes (two R and one Q; Fig. 1).

DISCUSSION

Transmission of HHV-8 through blood transfusion remains a controversial topic. Despite the seroprevalence in the general population and in blood donors in North America and North Europe ranging between 3.5 and 7.3%, no cases of transfusion transmission were reported.[3] HHV-8 DNA has been found in blood donors and healthy individuals in low- and higher-prevalence endemic areas such as Italy[14] and Africa.[4,12,13] These findings, in addition to the reports of HHV-8 infection associated with injection drug use,[7] raised the concern of HHV-8 transmission through blood products. Two studies conducted in Uganda by Hladik and coworkers[9] and Mbulaiteye and coworkers[15] found a risk of HHV-8 transmission through blood product of 2.8 and 2.6%, respectively. However, the conclusions of these two reports were purely based on serological testing and did not include DNA detection.

In this study the risk of HHV-8 transmission through blood transfusion in Ghana was investigated. A seroprevalence of 6% in blood donors and 11% in immunocompetent patients who received blood transfusion was found (Table 1). The relatively low seroprevalence compared to the study of Ablashi and colleagues[5] might be explained by the fact that we used a very specific immunofluorescence assay (IFA) to confirm initial results obtained by a less laborious, more sensitive, but less specific ELISA kit. Admittedly, however, IFA may present its own problems. Our results are more in line with the lower seroprevalence found in Zimbawe and South Africa.[6] One likely case of HHV-8 transmission was identified. Recipient 2027, negative for IgG to HHV-8 before transfusion, seroconverted after receiving an antibody and DNA positive whole blood transfusion. As demonstrated by Fowlkes and coworkers,[16] passive antibodies are detected immediately after transfusion with a relatively high titer and become undetectable in approximately 3 weeks. Immune response becomes detectable 4 to 10 weeks posttransfusion and is high titer. Our case clearly falls in the second scenario.

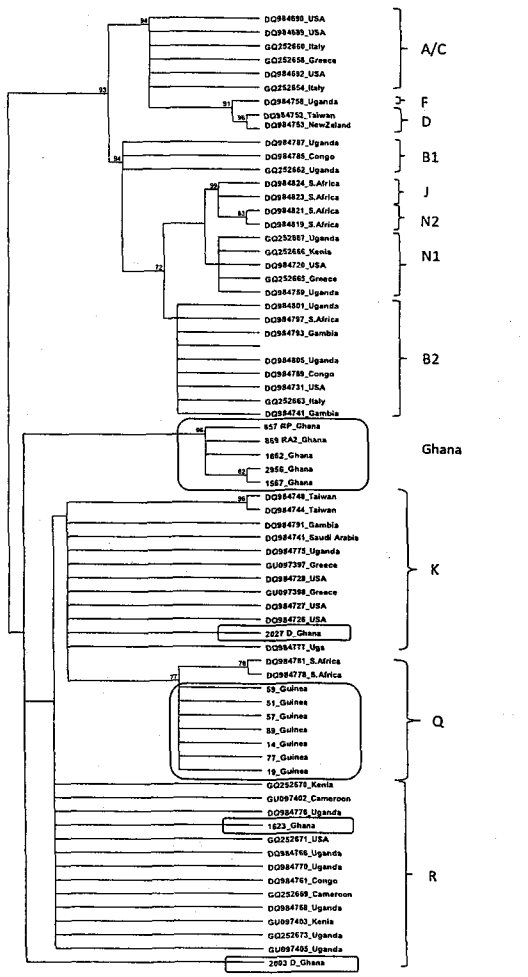

Fig. 1. Neighbor-joining phylogenetic tree of HHV-8 sequences found in Ghana and Guinea. The eight HHV-8 genotypes and their subgenotypes are indicated on the right and highlighted in orange. The red boxes indicate strains from Ghana while the blue box indicates strains from Guinea. Bootstrap values of more than 70% over 1000 replicates are considered significant and are indicated. Five Ghanaian sequences form a subcluster (98% bootstrap value) inside the larger group constituted of K, Q, and R genotypes. The six Guinean samples are part of the Q genotype with a bootstrap of 77%. The last sequence from Ghana (Gh-1623) is clustering with R genotype references.

Indeed, we detected high-titer antibodies (S/CO 9.5 compared to S/CO 4.5 in the donor) 3 months after transfusion, excluding passive transmission (Table 1). Such exclusion was further supported by data from five recipients transfused with seropositive donor blood with S/CO values of at least 9 who were seronegative in the posttransfusion samples. The likelihood of finding viral DNA 3 months postinfection is very low. Therefore, it is not surprising that we did not detect any HHV-8 DNA in the posttransfusion sample. The presence of HHV-8 DNA in the transfused whole blood, the confirmation of antibody to HHV-8 in the posttransfusion sample by an alternative method (immunofluorescence), and the high antibody level strongly suggest transfusion transmission. However, the absence of DNA in the recipient makes impossible to have a direct link with the donor strain. Therefore, community infection cannot be totally ruled out. A second recipient receiving blood from DNA-positive Donor 2003 showed no evidence of seroconversion 3 months after transfusion, a period of time that would be sufficient for the development of an immune response (Table 1). It was assumed that blood donations with detectable DNA contained a higher load of HHV-8 and were presumed more infectious than seropositive DNA-negative blood. Eleven susceptible patients exposed to HHV-8–seropositive blood did not seroconvert (Table 1). This might be related to either seropositive DNA-negative blood containing virus below the infectious dose or to false-positive antibody testing. The latter appears unlikely since both screening EIAs and IFAs were reactive in all 10 HHV-8 DNA–negative donations. Altogether, among 12 patients susceptible to infection and exposed (seronegative individuals receiving seropositive blood) one of two HHV-8 DNA–positive blood transmitted but none of 10 seropositive, HHV-8 DNA–negative blood did. This corresponds to an estimated transmission risk of 8% (95% confidence interval, 0%-23.3%), which is consistent with the 2.8% excess risk reported by

Hladick and colleagues.[9] These data also suggest low infectivity of HHV-8 DNA–negative blood being below the threshold of infectivity in immunocompetent recipients. The lack of HHV-8 transmission found by previous studies might be related to low viral load or to false-positive serologic results due to a lack of serologic confirmation.

HHV-8 may not represent a major issue for blood safety in developed areas but still remains a concern in countries with high seroprevalence among blood donors such as Ghana. Despite the fact that HHV-8 in immunocompetent individuals is usually associated with mild or no symptoms, infections in immunodeficient patients can lead to severe complications and, in some cases, to fatal outcomes. A relatively high incidence of HHV-8 infection has been described in solid organ transplantation patients such as liver and kidney transplant recipients caused by both transmission through the graft and reactivation of a previous infection.[17] Thus, HHV-8 infection in high endemic areas is a growing concern with the emergence of a population of immunodeficient blood recipients in sub-Saharan Africa related to the increasing availability of cancer chemotherapy and immunosuppressive drugs.

New HHV-8 subtype in Ghana

Despite the limited variability of HHV-8 genome, eight genotypes (or clusters) have been identified based on single-polymorphism nucleotides (SNPs) in the ORF26 (minor capsid protein) extended sequence.[4] In this study, we successfully amplified the ORF26 of eight Ghanaian individuals (six patients and two donors) and of seven Guinean blood donors. Five Ghanaian sequences clustered separately from the references with high bootstrap values while three other Ghanaian and seven Guinean strains appeared to belong to Genotype Q (Fig. 1). HHV-8 genotypes were defined by SNPs in an approximately 1-kb region including ORF26 and only three SNPs were necessary to distinguish between the two related Genotypes K and R. These are also the closest genotypes to the Ghanaian sequences. However, six SNPs differentiate five Ghanaian samples from K and R genotypes. For this reason, we propose that a new genotype (G for Ghana) be added. However, this new subgroup is found only in Ghana and does not seem to extend to Guinea, west of Ghana. Indeed, the samples from Guinea have different SNPs and cluster with Q and K whose subclassification into separate subtypes is unconvincing. This study contributes to the understanding of HHV-8 genome variation and distribution that can be used for further studies linking HHV-8 virulence and Kaposi sarcoma incidence.

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CONFLICT OF INTEREST

None.

REFERENCES

1. Laurent C, Meggetto F, Brousset P. Human herpesvirus 8 infections in patients with immunodeficiencies. Hum Pathol 2008;39:983-93.
2. Butler LM, Were WA, Balinandi S, Downing R, Dollard S, Neilands TB, Gupta S, Rutherford GW, Mermin J. Human herpesvirus 8 infection in children and adults in a population-based study in rural Uganda. J Infect Dis 2011; 203:625-34.
3. Soulier J, Grollet L, Oksenhendler E, Cacoub P, Cazals-Hatem D, Babinet P, d'Agay MF, Clauvel JP, Raphael M, Degos L, Sigaux F. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in multicentric Castleman's disease. Blood 1995;86:1276-80.
4. Tornesello ML, Biryahwaho B, Downing R, Hatzakis A, Alessi E, Cusini M, Ruocco V, Katongole-Mbidde E, Loquercio G, Buonaguro L, Buonaguro FM. Human herpesvirus type 8 variants circulating in Europe, Africa and North America in classic, endemic and epidemic Kaposi's sarcoma lesions during pre-AIDS and AIDS era. Virology 2010;398:280-9.
5. Ablashi D, Chatlynne L, Cooper H, Thomas D, Yadav M, Norhanom AW, Chandana AK, Churdboonchart V, Kulpradist SA, Patnaik M, Liegmann K, Masood R, Reitz M, Cleghorn F, Manns A, Levine PH, Rabkin C, Biggar R, Jensen F, Gill P, Jack N, Edwards J, Whitman J, Boshoff C. Seroprevalence of human herpesvirus-8 (HHV-8) in countries of Southeast Asia compared to the USA, the Caribbean and Africa. Br J Cancer 1999;81:893-7.
6. Dollard SC, Butler LM, Jones AM, Mermin JH, Chidzonga M, Chipato T, Shiboski CH, Brander C, Mosam A, Kiepiela P, Hladik W, Martin JN. Substantial regional differences in human herpesvirus 8 seroprevalence in sub-Saharan Africa: insights on the origin of the "Kaposi's sarcoma belt." Int J Cancer 2010;127:2395-401.
7. Vamvakas EC. Is human herpesvirus-8 transmitted by transfusion? Transfus Med Rev 2010;24:1-14.
8. Cannon MJ, Operskalski EA, Mosley JW, Radford K, Dollard SC. Lack of evidence for human herpesvirus-8 transmission via blood transfusion in a historical US cohort. J Infect Dis 2009;199:1592-8.
9. Hladik W, Dollard SC, Mermin J, Fowlkes AL, Downing R, Amin MM, Banage F, Nzaro E, Kataaha P, Dondero TJ, Pellett PE, Lackritz EM. Transmission of human herpesvirus 8 by blood transfusion. New Engl J Med 2006;355:1331-8.
10. Lefrère JJ. [The BOTIA project ("Blood and Organ Trans-

missible Infectious Agents?): a European collection of blood samples and an observatory of agents transmitted by blood transfusion or organ transplantation]. Transfus Clin Biol 2005;12:93-4.
11. Simpson GR, Schulz TF, Whitby D, Cook PM, Boshoff C, Rainbow L, Howard MR, Gao SJ, Bohenzky RA, Simmonds P, Lee C, de Ruiter A, Hatzakis A, Tedder RS, Weller IV, Weiss RA, Moore PS. Prevalence of Kaposi's sarcoma associated herpesvirus infection measured by antibodies to recombinant capsid protein and latent immunofluorescence antigen. Lancet 1996;348:1133-8.
12. Compston LI, Sarkobie F, Li C, Candotti D, Opare-Sem O, Allain JP. Multiplex real-time PCR for the detection and quantification of latent and persistent viral genomes in cellular or plasma blood fractions. J Virol Methods 2008; 151:47-54.
13. Endo T, Miura T, Koibuchi T, Nakamura H, Takahashi T, Odawara T, Goto M, Ajisawa A, Iwamoto A, Nakamura T. Molecular analysis of human herpesvirus 8 by using single nucleotide polymorphisms in open reading frame 26. J Clin Microbiol 2003;41:2492-7.
14. Viviano E, Vitale F, Ajello F, Perna AM, Villafrate MR, Bonura F, Aricò M, Mazzola G, Romano N. Human herpesvirus type 8 DNA sequences in biological samples of HIV-positive and negative individuals in Sicily. AIDS 1997;11: 607-12.
15. Mbulaiteye SM, Biggar RJ, Bakaki PM, Pfeiffer RM, Whitby D, Owor AM, Katongole-Mbidde E, Goedert JJ, Ndugwa CM, Engels EA. Human herpesvirus 8 infection and transfusion history in children with sickle-cell disease in Uganda. J Natl Cancer Inst 2003;95:1330-5.
16. Fowlkes AL, Brown C, Amin MM, Roback JD, Downing R, Nzaro E, Mermin J, Hladik W, Dollard SC. Quantitation of human herpesvirus 8 (HHV-8) antibody in patients transfused with HHV-8-seropositive blood. Transfusion 2009;49: 2208-13.
17. Andreoni M, Goletti D, Pezzotti P, Puzzetto A, Monini P, Sarmati L, Parchi F, Tisone G, Piazza A, Pisani F, Angelico M, Leone P, Citterio F, Ensoli B, Rezza G. Prevalence, incidence and correlates of HHV-8/KSHV infection and Kaposi's sarcoma in renal and liver transplant recipients. J Infect 2001;43:195-9.

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No. 24

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
				2012. 11. 19	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	Hladik W, Pellett PE, Hancock J, Downing R, Gao H, Packel L, Mimbe D, Nzaro E, Mermin J. J Infect Dis. 2012 Nov;206(10):1497-503. doi: 10.1093/infdis/jis543. Epub 2012 Sep 4.	公表国 ウガンダ共和国	
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)					

研究報告の概要

○ヒトヘルペスウイルス8抗体陽性血液の輸血とその後の死亡率の関連
背景: ヒトヘルペスウイルス8(HHV-8)はウガンダで流行しており、血液により伝播する。今回、HHV-8抗体陽性血液の輸血後の死亡率を調査した。
方法: 6カ月の追跡期間を設けた病院ベースの前向きコホート研究で、少なくとも7日間生存した輸血患者にHHV-8抗体陽性血液が及ぼした影響について調べた。
結果: 1092人の受血者中471人(43.1%)にHHV-8抗体陽性血液が輸血された。年齢中央値は1.8歳(0.1～78歳)で、111人(10.2%)が追跡期間中に死亡した。交絡因子(加齢、HIV感染、マラリア以外の疾患、複数回輸血)の調整後、短期(4日以内)保管したHHV-8抗体陽性血液の受血者はHHV-8抗体陰性血液受血者と比較して死亡率が高かった(補正ハザード比[AHR]、1.92;95%CI、1.21～3.05;P=0.01)。一方、長期(5日以上)保管HHV-8陽性血液の輸血は死亡率の増加と有意な関連がなかった(P=0.58)。
結論: 短期保管HHV-8抗体陽性血液は、死亡リスクの増加と関連していた。急性HHV-8感染と若年死亡率の間に観察された関連性を検証するために更なる研究が必要である。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血
新鮮凍結血漿-LR「日赤」120
新鮮凍結血漿-LR「日赤」240
新鮮凍結血漿-LR「日赤」480

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
ヒトヘルペスウイルス8(HHV-8)抗体陽性血液の輸血後の死亡率について調査したところ、短期(4日以内)保管したHHV-8抗体陽性血液の受血者は、HHV-8抗体陰性血液の受血者と比較して死亡率が高かったとの報告である。	今後も情報の収集に努める。

10

MAJOR ARTICLE

Association Between Transfusion With Human Herpesvirus 8 Antibody–Positive Blood and Subsequent Mortality

Wolfgang Hladik,[1,3] Philip E. Pellett,[4] John Hancock,[1] Robert Downing,[1] Hongjiang Gao,[5] Laura Packel,[7] Derrick Mimbe,[1] Esau Nzaro,[2] and Jonathan Mermin[6]

[1]Division of Global HIV/AIDS, Center for Global Health, Centers for Disease Control and Prevention (CDC), Entebbe, and [2]Department of Haematology, Mulago Hospital, Kampala, Uganda; [3]Department of Clinical Epidemiology, Academic Medical Center, University of Amsterdam, The Netherlands; [4]Department of Immunology and Microbiology, Wayne State University, Detroit, Michigan; [5]Division of Global HIV/AIDS, Center for Global Health, and [6]Division of HIV/AIDS Prevention, National Center for Hepatitis, HIV, STD, and Tuberculosis Prevention, Centers for Disease Control and Prevention (CDC), Atlanta, Georgia; and [7]Global Health Sciences, University of California, San Francisco

(See the editorial commentary by Operskalski, on pages 1485–7.)

Background. Human herpesvirus 8 (HHV-8) is endemic in Uganda and transmissible by blood. We evaluated mortality following transfusion of HHV-8 antibody–positive blood.

Methods. In a hospital-based, observational, prospective cohort study with a 6-month follow-up, we examined the effect of HHV-8 antibody–positive blood on transfusion recipients surviving at least 7 days.

Results. Of 1092 recipients, 471 (43.1%) were transfused with HHV-8 antibody–positive blood. Median age was 1.8 years (range, 0.1–78); 111 (10.2%) died during follow-up. After adjusting for confounders (increasing age, human immunodeficiency virus infection, illness other than malaria, receipt of multiple transfusions), recipients of HHV-8 antibody–positive blood stored ≤4 days ("short-stored") were more likely to die than recipients of HHV-8 antibody–negative blood (adjusted hazards ratio [AHR], 1.92; 95% confidence interval [CI], 1.21–3.05; $P = .01$). The AHR of the effect of each additional short-stored HHV-8 antibody–positive transfusion was 1.79 (95% CI, 1.33–2.41; $P = .001$).

Conclusions. Transfusion with short-stored HHV-8 antibody–positive blood was associated with an increased risk of death. Further research is warranted to determine if a causal pathway exists and to verify the observed association between acute HHV-8 infection and premature mortality.

Human herpesvirus 8 (HHV-8 or Kaposi's sarcoma–associated herpes virus) causes Kaposi's sarcoma, multicentric Castleman's disease, and primary effusion lymphoma [1]. In Uganda and other sub-Saharan African countries, Kaposi's sarcoma is frequent [2] and causes substantial morbidity and mortality. However, there is a paucity of literature describing any adverse outcomes following acute HHV-8 infection.

In sub-Saharan Africa, adult HHV-8 seroprevalence can exceed 50%, [1] with similarly high seroprevalence in healthy blood donors. The possibility of HHV-8 infection through blood transfusion has been suggested [3–5] and was demonstrated in a study in Uganda [6]. We analyzed data from the same prospective, observational cohort study to compare the risk of death within 6 months following transfusion of blood that was positive for HHV-8 antibodies with that following transfusion of blood that was negative for HHV-8 antibodies.

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Correspondence: Wolfgang Hladik, MD, Uganda Virus Research Institute, PO Box 49, Entebbe, Uganda (wfh3@cdc.gov).

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METHODS

Transfusion Recipients and Blood Donations

As previously described [6], between December 2000 and July 2001, written informed consent (and assent, as appropriate) was obtained from transfusion recipients or their parents or guardians if participants were aged <18 years at Mulago Hospital, Kampala, Uganda. Transfusion recipients were eligible for enrollment if their pretransfusion specimen after blood typing and cross-matching was available, identifying information for the transfused blood was known, and no other transfusions had taken place in the previous 6 months. Follow-up visits were scheduled at 1, 2, and 4 weeks post-transfusion, then monthly for 5 additional months. Participants were also seen at the study clinic for unscheduled acute care visits free of charge. At enrollment and each follow-up visit, blood was drawn, and a questionnaire was administered to collect information on patient demographics, health, and repeat transfusions. Participants who did not return for scheduled visits were followed up at home, and any deaths were recorded (Figure 1).

From November 2000 to September 2001, all blood donors in central Uganda were offered study participation, and blood specimens from consenting donors were stored for HHV-8 serologic testing. Donations were screened at the Uganda Blood Transfusion Services for human immunodeficiency virus (HIV), hepatitis B surface antigen, and *Treponema pallidum* and stored at 4°–8° C according to routine procedures. Most blood was divided into plasma and several smaller packed red blood cell units for use in young children; some blood units were left undivided for use in adults, although such units were sometimes split at the hospital into smaller units for use in children. Leukoreduction filters were not used; the buffy coat was partially removed from packed cell units.

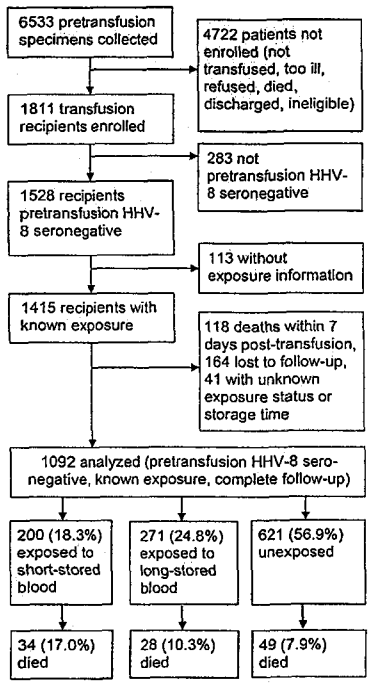

Figure 1. Trial profile of transfusion recipients.

Laboratory Procedures

Recipient plasma collected pretransfusion was tested for hemoglobin levels and HIV antibodies. HIV reactivity was confirmed by polymerase chain reaction if recipients were aged ≤24 months. Pretransfusion recipient blood and linked blood donor specimens were tested for HHV-8 antibodies at the Centers for Disease Control and Prevention (CDC) laboratory in Atlanta, as previously described [6].

Exposure Classification and Transfusion Events

Each transfusion was treated as a discrete event and was counted separately. Each transfusion could comprise ≥1 blood units (depending on patient body weight and degree of anemia as well as blood unit size and availability). Most recipients who received multiple transfusions did so within the first 7 days of their hospital stay. For the purpose of this analysis, an "exposed" person received ≥1 transfusions with HHV-8 antibody–positive blood products whether or not exposure to or infection with the virus occurred. Laboratory testing for antibodies against HHV-8 took place only after completion of follow-up. Recipients transfused with any HHV-8 antibody–positive blood units in the first 7 days were classified as "exposed," whereas recipients transfused exclusively with HHV-8 antibody–negative blood were classified as "unexposed." Because previous analysis of data from the same study found that HHV-8 antibody–positive blood stored ≤4 days was likely responsible for most transfusion-associated HHV-8 infections [6], recipients were grouped into risk categories from high to low as follows: (1) exposed to (any) HHV-8 antibody–positive blood stored ≤4 days (short-stored); (2) exposed to HHV-8 antibody–positive blood stored >4 days (long-stored); or (3) unexposed. Transfusions of blood products with any HHV-8 antibody status occurring after 7 days of the first transfusion (usually following readmission to the hospital) were regarded as "repeat" transfusions.

Data Management and Analysis

Data were entered in duplicate using Epi Info 6.04 (CDC) and analyzed using SAS software (SAS Institute). We excluded participants who were positive for HHV-8 antibodies pretransfusion or who were lost to follow-up. Recipients who received blood of unknown or equivocal HHV-8 serostatus and were not

also transfused with short-stored HHV-8 antibody–positive blood were also excluded. Participants were censored for the first 7 days following their initial transfusion and the first 7 days following the first subsequent transfusion that changed their HHV-8 exposure status to exposed. Transfusion recipients who died within 7 days of their initial transfusion or their first HHV-8 antibody–positive transfusion were removed from analysis, assuming that any effect of receipt of HHV-8 antibody–positive blood on mortality would take longer than 1 week to materialize. Using SAS Tphreg, we performed Cox proportional hazards analysis to estimate the hazard of death for potential risk factors (age, HIV serostatus, pretransfusion anemia, reason for transfusion, number of transfusions, and exposure to HHV-8 antibody–positive blood). We then estimated the adjusted hazard ratio (AHR) for receipt of short-stored HHV-8 antibody–positive blood by controlling simultaneously for these confounders. We repeated the main multivariate analysis with the reference group ("unexposed") restricted to recipients of short-stored HHV-8 antibody–negative blood only. No data anomalies or interactions were noted when we examined multiple-variate results in conjunction with individual-variate results.

Using SAS Proc Logistics, we adjusted for the same confounders and estimated the adjusted odds ratio for death among recipients of short-stored HHV-8 antibody–positive blood within the first 60 days of follow-up. We also estimated the adjusted population-attributable fraction of death due to receipt of short-stored HHV-8 antibody–positive blood as a function of follow-up time under the assumptions of the proportional hazard model and that censoring time is independent of event time [7].

The study was approved by the institutional review boards of the Uganda Virus Research Institute, the Uganda National Council for Science and Technology, and the CDC.

Enrollment and Follow-Up

Pretransfusion blood specimens for 6533 potential transfusion recipients were sent to the hospital's blood bank for typing and cross-matching (Figure 1). Of these, 1811 participants were enrolled; the remaining were not transfused (31%), were ineligible (28%), were too ill to consent (5%), refused to consent (13%), died prior to enrollment (2%), or were discharged prior to enrollment (22%). Of the 1811 enrolled recipients, 1528 (84%) were negative for HHV-8 antibodies pretransfusion. Of these, 436 (29%) were excluded from analysis because of unknown exposure status (10%), early death within 7 days of transfusion (8%), or loss to follow-up (11%).

RESULTS

We included 1092 pretransfusion HHV-8 antibody–negative recipients in the analysis (Table 1). These patients were transfused a total of 1328 times (median, 1; range, 1–8) with 2416 blood units (median, 1; range, 1–16) from 1498 blood donations. Most blood units transfused were packed red blood cells (78%), followed by whole blood (14%), blood of unknown product type (8%), and plasma and/or platelet products (<1%). Most recipients were aged <5 years (median age, 1.8 years; range, 0.1–78 years) and had malaria as a baseline diagnosis. Recipients transfused for malaria were younger than recipients transfused for other reasons (median age, 1.3 vs 17.0 years).

Median follow-up was 167 days (interquartile range [IQR], 116–169 days) and was similar among exposed and unexposed recipients. Among blood donations linked to study participants, HHV-8 antibody positivity was 36.5%. Among study participants, 471 (43.1%) were exposed, and 621 (56.9%) were unexposed. Among the exposed recipients, most (69%) were transfused with a single HHV-8 antibody–positive unit; the remainder received 2 (17%) or >2 (14%) units. Among those exposed to short-stored HHV-8 antibody–positive blood, 67% received 1 such blood product, 19% received 2, and 14% received ≥3. Recipients across the different exposure groups had similar HIV prevalence, pretransfusion anemia status, and reason for transfusion, but they differed by sex, age, and number of transfusions or blood units received (Table 1).

One hundred eleven (10.2%) recipients died during follow-up, with a median time from transfusion to death of 43 days (IQR, 19–73 days). Of the 621 unexposed recipients, 49 (7.9%) died, and of the 271 recipients of long-stored HHV-8 antibody–positive blood, 28 (10.3%) died, compared with 34 (17.0%) of the 200 recipients of short-stored HHV-8 antibody–positive blood. Using person-time as the denominator, unadjusted mortality per 100 person-years was 20.1 for recipients transfused with HHV-8 antibody–negative blood, 26.0 for recipients transfused with long-stored HHV-8 antibody–positive blood, and 44.2 for recipients transfused with short-stored HHV-8 antibody–positive blood.

In bivariate analysis, significant risk factors for death included age, HIV infection, illness other than malaria, receipt of multiple transfusions, and receipt of short-stored HHV-8 antibody–positive blood (Table 2). In multivariate analysis, transfusion with short-stored HHV-8 antibody–positive blood remained significantly associated with mortality during follow-up (AHR, 1.92; $P = .01$) (Table 2). When we restricted the multivariate analysis to the first 60 days of follow-up, the risk of death remained significant (adjusted odds ratio 2.29; 95% confidence interval [CI], 1.29–4.09, $P = .005$). Receipt of long-stored HHV-8 antibody–positive blood was not significantly associated with an excess risk of death ($P = .58$). When the reference group for the multivariate analysis was restricted to recipients of short-stored HHV-8 antibody–negative blood, the AHR due to receipt of short-stored HHV-8 antibody–positive blood remained statistically significant (AHR, 2.39; $P = .005$) and no significant risk of death was associated with transfusion of either long-stored HHV-8 antibody–positive or long-stored HHV-8 antibody–negative positive blood (Table 3).

With the multivariate model restricted to recipients of a single transfusion (n = 937), the hazard for death due to receipt of short-stored HHV-8 antibody–positive blood remained (AHR, 1.95; 95% CI, 1.10–3.45; $P = .02$). With the model restricted to recipients of a single blood unit (n = 868), the hazard for death upon receipt of short-stored HHV-8 antibody–positive blood was similar but not statistically significant (AHR, 1.70; 95% CI, .94–3.09; $P = .08$).

In a separate analysis, we restricted the risk set to recipients of a single blood unit and kept the reference group defined as recipients of a single short-stored HHV-8 antibody–negative blood unit. In this model, recipients of a single short-stored HHV-8 antibody–positive blood unit had a significantly higher mortality than reference group recipients (AHR, 2.19; 95% CI, 1.06–4.53; $P = .03$), whereas there was no excess risk of death among recipients of a single long-stored HHV-8 antibody–negative or HHV-8 antibody–positive blood unit. We also analyzed the data in a separate multivariate model similar to that shown in Table 2 except that exposure to HHV-8 antibody–positive blood was expressed as the continuous number of short- or long-stored HHV-8 antibody–positive or HHV-8 antibody–negative blood units. In this model, additional short-stored HHV-8 antibody–positive blood units transfused provided no survival benefit (AHR, 0.94; 95% CI, .61–1.43); whereas for all other blood units, each additional transfused unit had a protective effect on survival (long-stored HHV-8 antibody–positive: AHR, 0.67; 95% CI, .49–.93; short-stored HHV-8 antibody–negative: AHR, 0.53, 95% CI, .33–.83; compared with transfusion with long-stored HHV-8 antibody–negative units).

We also altered the main model (as shown in Table 2) such that the number of transfusions (by HHV-8 serostatus and storage time) replaced the categorical exposure variables and

Table 1. Characteristics of Study Participants by Human Herpesvirus 8 (HHV-8) Antibody Exposure Status

Characteristic	All (N = 1092)	Exposure Status (transfusion with HHV-8 antibody–positive blood, by storage time)			P Value
		Stored >4 days (n = 271)	Stored ≤4 days (n = 200)	Unexposed (n = 621)	
Age, years					
Median (range)	1.80 (0.1–78)	1.50 (0.2–59)	1.85 (0.1–78)	1.50 (0.1–78)	.03[a]
Sex, female	575 (52.7)	140 (51.7)	123 (61.5)	312 (50.2)	.02
HIV status					
Negative	948 (86.8)	233 (86.0)	177 (88.5)	538 (86.6)	.24
Positive	112 (10.3)	25 (9.2)	18 (9.0)	69 (11.1)	
Missing	32 (2.9)	13 (4.8)	5 (2.5)	14 (2.3)	
Pretransfusion anemia status					
Anemic	791 (72.4)	197 (72.7)	140 (70.0)	454 (73.1)	.67
Not anemic	17 (1.6)	6 (2.2)	4 (2.0)	7 (1.1)	
Unknown	284 (26.0)	68 (25.1)	56 (28.0)	160 (25.8)	
No. transfusions received					
1	937 (85.8)	210 (77.5)	147 (73.5)	580 (93.4)	<.0001
≥2	155 (14.2)	61 (22.5)	53 (26.5)	41 (6.6)	
No. blood units received					
1	868 (79.5)	191 (70.5)	134 (67.0)	543 (87.4)	<.0001
2	135 (12.4)	43 (15.9)	38 (19.0)	54 (8.7)	
≥3	89 (8.1)	37 (13.6)	28 (14.0)	24 (3.9)	
Reason for transfusion					
Malaria	912 (83.5)	220 (81.2)	163 (81.5)	529 (85.2)	.23
Other/unknown	180 (16.5)	51 (18.8)	37 (18.5)	92 (14.8)	
Survival status					
Alive	981 (89.8)	243 (89.7)	166 (83.0)	572 (92.1)	.001
Dead	111 (10.2)	28 (10.3)	34 (17.0)	49 (7.9)	
Time to death, days					
Median	43	50	35	37	<.0001[a]

Data are no. (%) unless otherwise noted.

Abbreviation: HIV, human immunodeficiency virus.

[a] P value based on difference in mean values.

Table 2. Risk Factors for Post-Transfusion Mortality, N = 1092 (human herpesvirus 8 [HHV-8] reference group: transfused with short- or long-stored HHV-8 antibody–negative blood)

	Recipients			Unadjusted Hazard Ratio			Adjusted Hazard Ratio		
Risk Factor	Person-time	No. of Recipients	Mortality	Point Estimate	95% CI	*P* Value	Point Estimate	95% CI	*P* Value
Age, years, continuous	428.0	1092	25.9	1.02	1.01–1.03	.04	1.00	.98–1.02	.78
HIV uninfected	377.3	948	17.5	Ref	...	...	Ref	...	...
HIV infected	37.4	112	109.5	5.96	4.03–8.80	.01	6.50	4.33–9.76	<.0001
HIV unknown	13.3	32	30.8	1.80	.67–4.94	.25	2.13	.77–5.91	.14
Not pretransfusion anemic	6.6	17	30.1	Ref	...	...	Ref	...	...
Pretransfusion anemic	304.3	791	28.0	0.89	.22–3.60	.87	1.43	.33–6.16	.63
Unknown anemia status	117.1	284	20.6	0.65	.16–2.81	.58	1.17	.26–5.22	.84
Transfused for malaria	359.3	912	22.5	Ref	...	.01	Ref	...	.06
Transfused for other reasons	68.8	180	43.6	1.92	1.26–2.97		1.64	.97–2.78	
Number of transfusions (continuous)	428.0	1328[a]	25.9	1.55	1.35–1.79	.01	1.60	1.36–1.88	<.0001
Transfused with									
HHV-8-seronegative blood	243.6	621	20.1	Ref	...	...	Ref	...	...
HHV-8-seropositive blood stored >4 days	107.2	271	26.0	1.30	.82–2.69	.27	1.15	.71–1.86	.58
HHV-8-seropositive blood stored ≤4 days	77.6	200	44.2	2.18	1.41–3.37	.01	1.92	1.21–3.05	.01

Person-time in years. Mortality expressed as number of deaths per 100 person-years. Hazard ratios: the hazard of death among patients by differing characteristic. *P* values apply to differences in the hazards observed.

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus.

[a] Refers to number of transfusions (rather than recipients).

the overall number of transfusions. In this model, too, short-stored HHV-8 antibody–positive blood was associated with an increased risk for death (AHR for each additional transfusion, 1.79; 95% CI, 1.33–2.41; $P = .0001$), whereas the AHR for each additional long-stored HHV-8 antibody–positive transfusion (1.23), short-stored HHV-8 antibody–negative transfusion (1.11), and long-stored HHV-8 antibody–negative transfusion (1.18) was nonsignificant. When replacing the number of transfusions with the number of blood units transfused in the model shown in Table 2, the AHR for exposure to short-stored HHV-8 antibody–positive blood remained significant (AHR, 2.25; 95% CI, 1.44–3.53; $P < .001$), and each additional blood unit transfused carried a significant risk for death (AHR, 1.19; 95% CI, 1.06–1.34; $P = .004$), similar to the number of transfusions. When including both the number of blood units and the number of transfusions in the same main model, HIV infection, additional transfusions during follow-up (AHR, 2.76), and exposure to short-stored HHV-8 antibody–positive blood (AHR, 1.76) remained significant predictors for death, whereas each additional blood unit (of any HHV-8 antibody status) transfused was associated with a decreased risk of death (AHR, 0.67; 95% CI, .48–.92; $P = .014$).

Stratifying the analysis by the major reason for transfusion did not alter the point estimate of association but led to wide confidence intervals for the AHR in each strata (data not shown). We detected no significant effect related to age, illness leading to transfusion, or blood product type transfused on the association between exposure and death. We estimated the median adjusted population attributable fraction of mortality due to short-stored HHV-8 antibody–positive blood to be 13.7% (95% CI, 2.9%–23.4%), which decreased from 16.9% at the beginning of follow-up to 11.0% at the end of follow-up.

We excluded deaths occurring within the first week following transfusion. During this time period, a total of 104 deaths occurred, with a median time to death of 2 days (IQR, 1–4 days). Transfusion of HHV-8 antibody–positive blood was not associated with an increased risk of death within these 7 days (overall: AHR, 0.95; $P = .83$; for short-stored HHV-8 antibody–positive blood: AHR, 0.61; $P = .23$; and for long-stored HHV-8 antibody–positive blood: AHR, 1.14; $P = .61$). Confounding by passive antibody transfer made it difficult to identify active HHV-8 seroconversions among the deceased. Three active HHV-8 seroconvertors were identified (2 recipients of short-stored HHV-8 antibody–positive blood, 1 recipient of long-stored HHV-8 antibody–positive blood, none in the unexposed group), which was insufficient for further analysis.

Table 3. Risk Factors for Post-Transfusion Mortality, N = 1074 (human herpesvirus 8 [HHV-8] reference group: transfused with short-stored HHV-8 antibody–negative blood)

	Adjusted Hazard Ratio		
Risk Factor	Point Estimate	95% CI	*P* Value
Age, years, continuous	1.00	.98–1.02	.90
HIV uninfected	Ref	...	...
HIV infected	6.61	4.40–9.93	<.0001
HIV unknown	2.29	.83–6.35	.11
Not pretransfusion anemic	Ref	...	...
Pretransfusion anemic	1.55	.36–6.71	.56
Unknown anemia status	1.31	.29–5.89	.72
Transfused for malaria	Ref	...	.06
Transfused for other reasons	1.67	.98–2.83	
Number of transfusions (continuous)	1.57	1.33–1.86	<.0001
Transfused with			
HHV-8 Ab–negative blood stored ≤4 d	Ref	...	...
HHV-8 Ab–negative blood stored >4 d	1.51	.83–2.75	.18
HHV-8 Ab–positive blood stored >4 d	1.45	.78–2.72	.24
HHV-8 Ab–positive blood stored ≤4 d	2.39	1.30–4.42	.005

Hazard ratios: the hazard of death among patients by differing characteristic. *P* values apply to differences in the hazards observed.

Abbreviations: Ab, antibody; CI, confidence interval; HIV, human immunodeficiency virus.

DISCUSSION

In this study, recipients of HHV-8 antibody–positive blood stored ≤4 days had a 1.9-fold greater risk of death than recipients of HHV-8 antibody–negative blood. The risk of death increased with each additional unit of short-stored HHV-8 antibody–positive blood transfused; in contrast, unexposed recipients experienced no additional risk from receipt of additional HHV-8 antibody–negative units regardless of their storage time.

We note several study limitations. We were unable to collect extensive information on the causes of death. Due to the observational study design, study participants were not truly randomized to the different exposure categories. However, this was unlikely to have biased our results because we adjusted for the number of transfusions received throughout the observation period. Also, the mortality risk remained when we restricted analysis to recipients without repeat transfusions during follow-up, and it remained when we right-censored both exposed and unexposed in the same fashion (ie, upon receipt of an HHV-8 antibody–positive transfusion during follow-up).

Our adjusted analysis accounted for several confounders, some of which remained significant in our model. However, several observations support the hypothesis of an exposure-related risk of death. First, the mortality risk was significant only for transfusion with short-stored blood. This is consistent with our earlier finding that most transfusion-associated HHV-8 infections were likely due to short-stored HHV-8 antibody–positive blood [6] and a similar infection risk differential is known for other infectious agents (eg, cytomegalovirus) [8, 9]. Further, the increased mortality risk for each additional short-stored HHV-8 antibody–positive blood unit transfused suggests a dose-response relationship between exposure and subsequent death that was not observed for HHV-8 antibody–negative units and remained after controlling for the total number of transfusions. Lastly, the absence of an exposure-related risk of death during the first 7 days following transfusion indirectly supports our hypothesis because a causal association between transfusion of HHV-8 antibody–positive blood and post-transfusion death would likely take time to manifest itself and suggests that at the time of the baseline transfusion recipients of HHV-8 antibody–positive blood were not more acutely ill than others.

The adjusted estimated attributable risk of death due to transfusion with short-stored HHV-8 antibody-positive blood implies that approximately 5 (95% CI, 1.0–8.0) of the 34 deaths among recipients of short-stored HHV-8-antibody-positive blood or 4.2% of all 111 deaths may have been due to transfusion of short-stored HHV-8 antibody-positive blood. The association with mortality could be due to transfusion-associated HHV-8 being rapidly and highly pathogenic in some patients or to a different infectious agent or other hazard associated with HHV-8 seropositivity. We previously estimated the excess HHV-8 infection risk due to transfusion of short-stored HHV-8-antibody-positive blood alone as 4.2% (95% CI, .1–8.3) [6], or approximately 13 excess HHV-8 infections in this cohort. Among exposed patients who completed >4 weeks of follow-up before dying, there was no serological evidence of HHV-8 infection. However, some individuals may have died of acute illness before seroconversion would have been detected in the context of our sampling intervals. Acute disease has been associated with HHV-8 infection in both immunocompetent [10, 11] and immunocompromised persons, including well-documented severe disease in HIV-infected patients and organ transplant recipients [12–15]. All of our study participants were sufficiently ill to require transfusion; their immune status may have been further compromised by the immunosuppressive effects of transfused blood [16], especially if it

contained allogeneic leukocytes [17, 18]. Thus, it is plausible that HHV-8 itself directly contributed to the observed mortality. Additional research that considers cause of death, HHV-8 DNA in donors and recipients, or the effect of leukoreduction or irradiation on the outcome of transfused short-stored HHV-8 antibody–positive blood in transfusion recipients may clarify the association of HHV-8 with mortality among transfusion recipients.

In conclusion, transfusion of short-stored HHV-8 antibody–positive blood was associated with increased risk of death during the 2–28 weeks following transfusion. If this association is confirmed, blood transfusion systems in HHV-8 endemic areas will face a dilemma. Donated blood is a scarce resource in most countries, particularly in sub-Saharan Africa; removal of HHV-8 antibody–positive blood would further exacerbate existing shortages. The benefits of transfused blood will need to be weighed against its known and potential adverse effects.

Notes

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W. H. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy the data analysis. P. E. P., W. H., R. D., and J. M. were responsible for the study concept and design. D. M., W. H., and E. N. were responsible for the acquisition of data. L. P., J. H., H. G., D. M., and W. H. analysed and interpreted the data. W. H., J. M., and P. E. P. drafted the manuscript. W. H., J. M., P. E. P., E. N., R. D., and J. H. critically revised the manuscript for important intellectual content. J. H., L. P., and H. G. provided statistical analysis. W. H. obtained funding. J. M. and W. H. provided administrative, technical, or material support.

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

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Henke-Gendo C, Schulz TF. Transmission and disease association of Kaposi's sarcoma-associated herpesvirus: recent developments. Curr Opin Infect Dis 2004; 17:53–7.
2. Dedicoat M, Newton R. Review of the distribution of Kaposi's sarcoma-associated herpesvirus (KSHV) in Africa in relation to the incidence of Kaposi's sarcoma. Br J Cancer 2003; 88:1–3.
3. Blackbourn DJ, Ambroziak J, Lennette E, Adams M, Ramachandran B, Levy JA. Infectious human herpesvirus 8 in a healthy North American blood donor. Lancet 1997; 349:609–11.
4. Dollard SC, Nelson KE, Ness PM, et al. Possible transmission of human herpesvirus-8 by blood transfusion in a historical United States cohort. Transfusion 2005; 45:500–3.
5. Enbom M, Urassa W, Massambu C, Thorstensson R, Mhalu F, Linde A. Detection of human herpesvirus 8 DNA in serum from blood donors with HHV-8 antibodies indicates possible bloodborne virus transmission. J Med Virol 2002; 68:264–7.
6. Hladik W, Dollard SC, Mermin J, et al. Transmission of human herpesvirus 8 by blood transfusion. N Engl J Med 2006; 355:1331–8.
7. Chen L, Lin DY, Zeng D. Attributable fraction functions for censored event times. Biometrika 2010; 97:713–26.
8. Roberts TC, Buller RS, Gaudreault-Keener M, et al. Effects of storage temperature and time on qualitative and quantitative detection of cytomegalovirus in blood specimens by shell vial culture and PCR. J Clin Microbiol 1997; 35:2224–8.
9. Roback JD. CMV and blood transfusions. Rev Med Virol 2002; 12:211–9.
10. Andreoni M, Sarmati L, Nicastri E, et al. Primary human herpesvirus 8 infection in immunocompetent children. JAMA 2002; 287:1295–300.
11. Wang QJ, Jenkins FJ, Jacobson LP, et al. Primary human herpesvirus 8 infection generates a broadly specific CD8(+) T-cell response to viral lytic cycle proteins. Blood 2001; 97:2366–73.
12. Oksenhendler E, Cazals-Hatem D, Schulz TF, et al. Transient angiolymphoid hyperplasia and Kaposi's sarcoma after primary infection with human herpesvirus 8 in a patient with human immunodeficiency virus infection. N Engl J Med 1998; 338:1585–90.
13. Thaunat O, Mamzer-Bruneel MF, Agbalika F, et al. Severe human herpesvirus-8 primary infection in a renal transplant patient successfully treated with anti-CD20 monoclonal antibody. Blood 2006; 107:3009–10.
14. Luppi M, Barozzi P, Schulz TF, et al. *Bone marrow failure associated with human herpesvirus 8 infection after transplantation.* N Engl J Med 2000; 343:1378–85.
15. Pietrosi G, Vizzini G, Pipitone L, et al. Primary and reactivated HHV8 infection and disease after liver transplantation: a prospective study. Am J Transplant 2011; 11:2715–23.
16. Vanvakas EC, Blajchman MA. Deleterious clinical effects of transfusion-associated immunomodulation: fact or fiction? Blood 2001; 97:1180–95.
17. Blajchman MA. Immunomodulation and blood transfusion. Am J Ther 2002; 9:389–95.
18. Kao KJ. Mechanisms and new approaches for the allogeneic blood transfusion-induced immunomodulatory effects. Transfus Med Rev 2000; 14:12–22.

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No. 22

医薬品 研究報告 調査報告書

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研究報告の概要

○白血球除去赤血球による*Anaplasma phagocytophilum*伝播

背景:ヒト顆粒球アナプラズマ症(HGA)はマダニの一種によって伝播されるリケッチア感染症である。輸血伝播によるアナプラズマ症(TTA)は過去に4例が報告されているのみで、全例が米国中西部で発生している。

症例・所見:64歳男性患者は、3日間続く倦怠感、労作時呼吸困難、下血により入院し、慢性閉塞性肺疾患の既往歴及び鉄欠乏性貧血を伴う再発性胃炎を有していた。保存前白血球除去赤血球製剤5ユニットが輸血され、その後容態は安定し退院した。その2日後、頭痛、発熱、悪寒により再入院した。末梢血スメアによりHGAと一致する桑実胚を持つ多形核白血球が確認された。受血者及び全5ユニットの白血球除去赤血球製剤の供血者セグメント検体について*Anaplasma phagocytophilum*の検査を行ったところ、1名の供血者に感染が確認された。当該供血者は媒介ダニIxodesの多発地帯であるロードアイランド州在住の81歳健常男性で、屋外活動は行うがダニ刺咬歴はなかった。

結論:白血球除去はHGA伝播の予防とはならず、赤血球製剤がHGA多発地帯から非多発地帯へ供給されて輸血される例は多いため、受血者が予期せず発熱した場合は地域に関係なくTTAの可能性を考慮すべきである。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血
新鮮凍結血漿-LR「日赤」120
新鮮凍結血漿-LR「日赤」240
新鮮凍結血漿-LR「日赤」480

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
白血球除去赤血球の輸血により*Anaplasma phagocytophilum*が伝播したとの報告である。	今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

compared with donors without Chagas cardiomyopathy (178/340 [52%]; mean 7.1 parasites/20 mL). In the univariate model PCR positivity was associated with pro-BNP level ($p < 0.001$), left ventricular ejection fraction ($p = 0.024$), Troponin ($p < 0.001$) and CKMB ($p = 0.043$). Conclusion: *T. cruzi* PCR positivity correlates with presence of cardiomyopathy and with markers of severity of disease indicating a direct role of parasite persistence in disease pathogenesis. These findings support guidelines for treatment of chronically infected patients and potential utility of *T. cruzi* PCR for prognosis and therapeutic monitoring.

Disclosure of Commercial Conflict of Interest

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Disclosure of Grants Conflict of Interest

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S64-030U

Increasing Rate of Babesiosis in Transfused Patients at a New York City Hospital

C. Goss[1] (chg9006@med.cornell.edu), P. Giardina[2], M. S. Simon[3], D. A. Kessler[4], B. H. Shaz[5], M. Cushing[1]; [1]Transfusion Medicine and Cellular Therapy, Weill Cornell Medical College, New York, NY, United States; [2]Pediatrics, Weill Cornell Medical College, New York, NY, United States; [3]Internal Medicine, Weill Cornell Medical College, New York, NY, United States; [4]New York Blood Center, New York, NY, United States

Background/Case Studies: Babesiosis, a tick-borne infection primarily due to the intra-erythrocytic protozoa, *Babesia microti*, results in a wide-spectrum of clinical illness that ranges from asymptomatic to fatal. Asymptomatic donors in endemic areas are difficult to identify, and transfusion-transmitted babesiosis (TTB) has been increasingly identified in certain regions particularly the Northeastern U.S. We describe a 10-month experience at our institution, during which 7 cases of suspected TTB were reported to the transfusion service (TS). **Study Design/Methods:** All suspected cases of TTB are reported to the TS by clinicians, microbiologists, or epidemiologists. TS physicians investigate the possibility of TTB, and if RBC or granulocyte units were transfused within three months prior to the babesiosis diagnosis, suspected units are reported to the blood supplier and New York State Department of Health. The patient's chart is reviewed for demographics, comorbidities, specific risk factors for babesiosis, and the clinical course. **Results/Findings:** Seven cases of suspected TTB were reported from 8/2011 to 5/2012 (1 reported case for 2857 RBC units transfused). In the prior 6 years, 8 cases had been reported, 3 of which occurred in 2010. Four patients (57%) were asplenic. The non-thalassemic patients were all older than 60 years of age. The parasitemia at diagnosis ranged from 0.3% to 7.9%. Five of 7 experienced laboratory evidence of hemolysis. Two patients required hospitalization and a third required transfer to the ICU for management of babesiosis. Four were treated, as outpatients with a prolonged course of antibiotics. Eight of 15 (53%) diagnosed with babesiosis in the last 8 years had warm RBC autoantibodies and in 5 cases the autoantibody was identified within 1 month of the babesiosis diagnosis. For 2 patients, review of pre-transfusion specimens revealed evidence of babesia infection prior to transfusion. Three patients were considered presumed to have TTB (1 in 6668 RBC transfused) with donor titers ranging from 64 to 256. The two remaining patients received blood solely at outside hospitals and remain unconfirmed for TTB. No fatalities due to TTB occurred during this time; 1 patient died within 42 days of diagnosis from complications of their underlying condition. **Conclusion:** In the past year, our institution experienced an alarming increase in frequency of TTB associated with significant morbidity and financial cost. These results are consistent with recent publications documenting the parasite's expanding geographic range in New York State and highlight the urgent need for donor screening assays. Until such a test is licensed, clinicians must be aware of this risk in transfused patients particularly in vulnerable populations such as the elderly and neonates, and patients with asplenia, hemoglobinopathies or hematologic malignancies.

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S65-030U

Anaplasma phagocytophilum Transmission by Leukoreduced Red Blood Cells

H. Alhumaidan[1] (alhumaidan@me.com), B. Westley[2], C. Esteva[1], V. Berardi[1], C. Young[3], J. Sweeney[1]; [1]Blood Bank and Transfusion Medicine, Miriam Hospital, Providence, RI, United States; [2]Division of Infectious Disease, Miriam Hospital, Providence, RI, United States; [3]Rhode Island Blood Center, Providence, RI, United States; [4]Imugen Inc, Norwood, MA, United States

Background/Case Studies: Human Granulocytic Anaplasmosis (HGA) is a tickborne rickettsial infectious disease caused by gram-negative, obligate intracellular bacteria infecting neutrophils and transmitted to humans by species of the Ixodes tick. *Ixodes scapularis* is endemic in the Northeast and upper Midwest regions of the US. Only four previous cases of transfusion transmitted anaplasmosis (TTA) have been described, all from the Midwest and only one was proven to be from a leukoreduced red blood cell (RBC). **Study Design/Methods:** A 64 year old male patient was admitted to hospital with a 3 day history of fatigue, dyspnea on exertion and melena for several days. The Patient had a history of chronic obstructive pulmonary disease (COPD) and was on oral prednisone. There was a history of recurrent gastritis with iron deficiency anemia. His hemoglobin on admission was 6.2 g/dL. He received five units of prestorage leukoreduced RBCs, subsequently stabilized and was discharged. Two days after discharge, he developed headache, fever and chills and was readmitted. He was started on antibiotics and IV methylprednisolone for exacerbation of his COPD. The patient's symptoms did not improve and on day 5 of his second admission, the temperature was 101.4 F, and the WBC decreased to 2.3 x10^9/L. Polymorphonuclear leukocytes containing morulae consistent with HGA were reported in the peripheral smear. Samples from the recipient and donor segments from all 5 leukoreduced RBC units were retrieved and tested for *Anaplasma phagocytophilum*. **Results/Findings:** All five donor segments were evaluated by polymerase chain reaction (PCR). One donor tested PCR positive for HGA. This unit had been stored for 12 days prior to transfusion. The donor was a healthy 61 year old male from an Ixodes endemic area in RI with outdoor activities who reported no tick bites. Laboratory data are shown in the Table. **Conclusion:** Leukoreduction does not interdict the transmission of HGA. TTA requires consideration in recipients of red cell transfusion with unexplained fever, regardless of the geographic location of the transfusion since red cells are commonly exported from HGA endemic to HGA nonendemic areas.

Anaplasma phagocytophilum		
Donor testing (segment)	Serology PCR	ELISA positive IgM > 17.4 IgG > 12.1 Positive
Recipient testing (WB and serum)	Serology PCR	ELISA IgG negative IgM not reported Positive

Disclosure of Commercial Conflict of Interest

H. Alhumaidan: PALL Medical, Grants or Research Support; V. Berardi: IMUGEN Inc, Ownership or Partnership; C. Esteva: No Answer; J. Sweeney: Nothing to disclose; B. Westley: No Answer; C. Young: No Answer

Disclosure of Grants Conflict of Interest

H. Alhumaidan: PALL Medical, Grants or Research Support; V. Berardi: IMUGEN Inc, Grants or Research Support; C. Esteva: No Answer; J. Sweeney: Nothing to disclose; B. Westley: No Answer; C. Young: No Answer

医薬品 研究報告 調査報告書

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一般的名称	新鮮凍結人血漿		研究報告の公表状況	Jimenez-Marco T, Fisa R, Riera C, Girona-Llobera E, Sedeño M, Saura A, Iniesta L, Guillen C, Muncunill J. Vox Sang. 2012 Nov;103(4):356-8. doi: 10.1111/j.1423-0410.2012.01622.x. Epub 2012 Jun 19.	公表国 スペイン	
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)					

研究報告の概要

○*Leishmania infantum*の無症候性キャリア由来の血液成分に適用される病原体不活化技術

無症候性リーシュマニア感染症は、流行地域における輸血感染の主な原因となっている。バレアレス諸島では、供血者の無症候性*L.infantum*の感染率は極めて高い(調査対象供血者の5.9%)。現在、血液銀行の基準を満たす供血者のためのリーシュマニアスクリーニング検査は存在しないため、血液製剤中のリーシュマニアの除去のために数種類の方法が用いられている。今回、アモトサレンとUVA照射(INTERCEPT)を用いて無症候性*L.infantum*感染供血者から採取した血液製剤を用いて病原体除去技術の能力を調査した。病原体不活化処理実施前の血小板製剤6例中5例で、RT-PCR結果が陽性であった。INTERCEPTでの不活化後、これらの血小板製剤はRT-PCRで陰性となり、in vitro培養において6カ月後も全て陰性であった。これは供血者の血液成分から原虫を除去する目的でINTERCEPTが用いられた初の報告である。この所見を確認するためには更なる研究が必要である。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血
新鮮凍結血漿-LR「日赤」120
新鮮凍結血漿-LR「日赤」240
新鮮凍結血漿-LR「日赤」480

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
無症候性*Leishmania infantum*感染供血者からの血小板製剤中の原虫を除去するためにINTERCEPTが用いられ、有効であったとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

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CASE REPORT

Pathogen inactivation technology applied to a blood component collected from an asymptomatic carrier of Leishmania infantum: a case report

T. Jimenez-Marco,[1,*] R. Fisa,[2,*] C. Riera,[2] E. Girona-Llobera,[1] M. Sedeño,[1] A. Saura,[2] L. Iniesta,[2] C. Guillen[2] & J. Muncunill[1]
[1]*Fundació Banc de Sang i Teixits de les Illes Balears, Balearic Islands, Mallorca, Spain*
[2]*Laboratori de Parasitologia, Facultat de Farmàcia, Universitat de Barcelona, Barcelona, Spain*

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Asymptomatic *Leishmania* infections have been the main cause of transfusion transmission in endemic areas. Polymerase chain reaction has been used to detect *L. infantum* DNA in the peripheral blood of asymptomatic *Leishmania* carriers. In our region, the prevalence of asymptomatic *L. infantum* infection in donors is markedly high (5·9% of donors studied). We investigated the ability of pathogen inactivation technology, using amotosalen and UVA illumination, to eliminate *L. infantum* in a blood component collected from an asymptomatic *L. infantum* infected donor. This is the first report of the INTERCEPT system being used to eliminate a parasite from a component collected from a donor.

Key words: donors, malaria and protozoal infections, pathogen inactivation.

Introduction

Visceral leishmaniasis caused by *Leishmania infantum* is endemic in the Mediterranean basin. Most *L. infantum* infections are asymptomatic and resolve spontaneously in immunocompetent individuals. A minority progress to classic visceral leishmaniasis [1]. *Leishmania* infection is naturally transmitted through the bite of phlebotomine sand flies, but transmission of *Leishmania* by transfusion has also been reported [2]. The existence of asymptomatic *L. infantum* carriers, associated with intermittent low-density circulation of the parasite [1,3], has been proposed as the main cause of transmission by blood.

In the Balearic Islands, the prevalence of asymptomatic *L. infantum* infection in blood donors is substantially high (*L. infantum* DNA in blood was detected in 5·9% of blood donors studied) [4], which is consistent with other findings regarding asymptomatic carriers from the Mediterranean region [1,5].

Although some research studies have investigated *Leishmania* infection in blood donors [1,3–5], at present there are no *Leishmania* donor screening tests that are capable of meeting Blood Bank criteria with respect to speed, standardization and automation.

As no suitable donor screening tests are currently available, several methods have been used to eliminate *Leishmania* in blood products [6,7]. As an approach to reducing the risk of transfusion transmission in our area, we investigated the ability of a pathogen inactivation technology using amotosalen HCl and ultraviolet-A (320–400 nm) light [INTERCEPT Blood System for Platelets; Cerus, BV, Amersfoot, the Netherlands] to eliminate *L. infantum* in apheresis platelet units obtained from an asymptomatic infected blood donor.

Materials and methods

This study was conducted under a protocol approved by the Balearic Island Ethic Committee after written informed consent was obtained from the participating donor. A 53-year-old male donor, previously known to be asymptomatically infected with *L. infantum* by detecting *L. infantum* DNA in his peripheral blood, was enrolled in this study. He gave a platelet apheresis donation using the Amicus device (Fenwal, Lake Zurich, IL, USA). According to the Spanish specifications for platelet products, the targeted platelet content of each unit suitable for transfusion had to be $\geq 3{\cdot}0 \times 10^{11}$/component. Apheresis products were suspended in approximately 35% plasma and 65% platelet additive solution (Intersol, Fenwal) and treated according to the INTERCEPT manufacturer's instructions for use. Western blot (WB) and real-time polymerase chain reaction (RT-PCR) analyses were carried out on peripheral blood samples on the day of the donation. RT-PCR was also carried out on the platelet unit both before and after inactivation with the INTERCEPT.

Anti-*Leishmania* antibodies were tested by WB using a whole *L. infantum* antigen (MHOM/FR/78/LEM75) as previously described [8]. We considered a serum positive when immunoreactivity against the 14 and/or 16 kDa *L. infantum* antigen fraction was observed.

The presence of *Leishmania* DNA was analysed by amplification of kinetoplast DNA sequence by RT-PCR as previously described [9]. DNA was extracted in duplicate, and each amplification was performed in triplicate using the ABI Prism 7700 system. In total, six DNA amplifications were performed for each platelet unit. RT-PCR was considered positive for *Leishmania* spp. when the threshold cycle (tC) was <45. The tC for a given sample is the first cycle of the PCR reaction where fluorescence is detected above the baseline. '*In vitro*' culture was performed as previously described [4]. All samples were cultured regardless of the RT-PCR results.

Results

The *Leishmania*-specific antibodies were revealed by WB in the sera of this donor and showed the characteristic 16-kDa band. Both WB and RT-PCR analyses on peripheral blood samples were positive on the day of the donation. The preinactivation platelet units were detected positive by RT-PCR at 0·01 parasites/ml in five of six DNA amplifications performed. After inactivation, the platelet unit was RT-PCRs negative. All '*in vitro*' cultures were negative after 6-month follow-up.

Discussion

Asymptomatic carriers of *L. infantum* are a major cause of transfusion transmission in endemic areas [2]. There is a reasonable possibility that blood products from infected persons, if parasitemic at the time of blood donation, may transmit leishmaniasis to the recipient. Several methods based on pathogen inactivation technology have been used to prevent transfusion-acquired leishmaniasis [6,7], including the INTERCEPT Blood System [7]. Peripheral blood PCR is a good, noninvasive alternative to traditional diagnosis methods, such as microscopic examination and/or bone marrow and spleen aspirate cultures, for detecting *L. infantum* asymptomatic carriers [1,3,4]. PCR testing can be considered as a true direct method for detecting parasite presence as DNA is rapidly degraded following parasite death [10]. Culture methods, in reality, have a low sensitivity compared with the high sensitivity of PCR when these methods are used for the detection of asymptomatic carriers [3,4]. This may be due to the low level of circulating parasites in asymptomatic individuals, ranging from 0·001 parasites/ml to 1 parasite/ml [11], which it is sufficient to render a positive PCR but not a positive culture result [12].

Therefore, culture techniques do not seem to be the best method to detect asymptomatic carriers due to the low level of circulating parasites found in these individuals.

We studied a blood donor with detectable *Leishmania* DNA in peripheral blood but who was otherwise a healthy individual. WB and RT-PCR analyses performed on the donor's peripheral blood samples taken on the day of the donation were positive. The preinactivation platelet unit RT-PCR was positive at 0·01 parasites/ml. However, the '*in vitro*' culture results were negative. This result is not surprising given the small size of the 'inoculum' (0·01 parasites/ml), which is far below the dose that is considered necessary to produce cell growth (over 10^4 parasites/ml) [12]. However, RT-PCRs were negative after platelet unit inactivation, thereby guaranteeing the absence of viable parasites since *Leishmania* nucleic acids are rapidly degraded following parasite death [10].

Until now, studies into pathogen inactivation technologies applied to the reduction in *Leishmania* risks have been based on '*in vitro*' studies. Basically, *Leishmania*-infected monocytes and/or promastigotes were deliberately added at high doses to blood components collected from healthy, noninfected donors. The presence of viable postinactivation parasites in these studies was evaluated by culture methods [6,7]. In reality, it is more likely that the levels of parasites in blood donations from asymptomatic individuals, and which need to be inactivated, are much lower than those used for '*in vitro*' spiking studies. Essentially, if pathogen inactivation technology is able to inactivate the high doses used for '*in vitro*' spiking studies, this gives more weight to the indication that it will be able to inactivate the low doses presented in asymptomatic blood donors. The application of this technology should, therefore, provide a wide margin of safety.

The INTERCEPT Blood System may represent an interesting approach to prevent transfusion-transmitted leishmaniasis. However, these findings need to be confirmed through additional studies.

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Correspondence: Teresa Jimenez-Marco, Fundació Banc de Sang i Teixits de les Illes Balears, C/Rosselló i Caçador, 20 07004, Balearic Islands, Palma de Mallorca, Spain
E-mail: matejimenez@hotmail.com; tjimenez@fbstib.org
*Both authors contributed equally to this work.

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Conflict of interest

The authors declare that there are no conflicts of interest.

References

1 le Fichoux Y, Quaranta JF, Aufeuvre JP, *et al.* Occurrence of *Leishmania infantum* parasitemia in asymptomatic blood donors living in an area of endemicity in southern France. *J Clin Microbiol* 1999; 37: 153–157

2 Singh S. Transfusion transmitted leishmaniasis: a case report and review of literature. *Indian J of Med Microbiol* 2006; 24: 165–170

3 Riera C, Fisa R, Udina M, *et al.* Detection of *Leishmania infantum* cryptic infection in asymptomatic blood donors living in an endemic area (Eivissa, Balearic Islands, Spain) by different diagnostic methods. *Trans R Soc Trop Med Hyg* 2004; 98: 102–110

4 Riera C, Fisa R, López-Chejade P, *et al.* Asymptomatic infection by *Leishmania infantum* in blood donors from the Balearic Islands (Spain). *Transfusion* 2008; 48: 1383–1389

5 Biglio A, Bolla C, Concialdi E, *et al.* Asymptomatic *Leishmania infantum* infection in an area of north-western Italy (Piedmont region) where such infections are traditionally endemic. *J Clin Microbiol* 2010; 48: 131–136

6 Cardo LJ, Rentas FJ, Ketchum L, *et al.* Pathogen inactivation of Leishmania donovani infantum in plasma and platelet concentrate using riboflavin and ultraviolet light. *Vox Sang* 2006; 90: 85–91

7 Eastman RT, Barrett LK, Dupuis K, *et al.* Leishmania inactivation in human apheresis platelets by a psoralen (amotosalen HCl) and long-wavelength ultraviolet irradiation. *Transfusion* 2005; 45: 1459–1463

8 Riera C, Valladares JE, Gállego M, *et al.* Serological and parasitological follow-up in dogs experimentally infected with *Leishmania infantum* and treated with meglumine antimoniate. *Vet Parasitol* 1999; 2: 33–47

9 Martín-Ezquerra G, Fisa R, Riera C, *et al.* Role of Leishmania spp. infestation in nondiagnostic cutaneous granulomatous lesions: report of a series of patients from a Western Mediterranean area. *Br J Dermatol* 2009; 161: 320–325

10 Prina E, Roux E, Matte D, *et al.* *Leishmania* DNA is rapidly degraded following parasite death: an analysis by microscopy and real-time PCR. *Microbes Infect* 2007; 9: 1307–1315

11 Mary C, Faraut F, Drogoul MP, *et al.* Reference values for *Leishmania infantum* parasitemia in different clinical presentations: quantitative polymerase chain reaction for therapeutic monitoring and patient follow-up. *Am J Trop Med Hyg* 2006; 75: 858–863

12 Lemesre JL, Darcy F, Kweider M, *et al.* Requirements of defined cultivation conditions for standard growth of *Leishmania* promastigotes in vitro. *Acta Trop* 1988; 45: 99–108

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No. 27

医薬品 研究報告 調査報告書

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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-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)			ProMED 20130217.1546451	スペイン	

研究報告の概要

○リーシュマニア症－スペイン、マドリッド

スペイン、マドリッド南部の市町村(Bosquesurの緑地帯周辺のFuenlabrada、Leganes、Getafe、Humanes等)で発生しているリーシュマニア症のアウトブレイクは、2012年の150症例を含めて過去3年間に500症例が報告されており、まだ継続しているとみられる。

リーシュマニア症は感染動物を刺咬したサシチョウバエに刺咬されることにより伝播し、内臓リーシュマニア症を発症した場合、治療を行わなければ死亡することもある。Bosquesurで増加しているウサギが保有宿主となっている可能性がある。2012年、Fuenlabradaは緊急事態地域と宣言され、事態を制御するためにウサギの駆除が許可された。厚生大臣は、2012年末までに報告された内臓リーシュマニア症は3例で、2011年の47例と比べて改善したと述べたが、2013年の現時点で既に3例の新規症例が報告されており、アウトブレイクはまだ終了していないとみられる。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血
新鮮凍結血漿-LR「日赤」120
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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
スペイン、マドリッド南部の市町村におけるリーシュマニア症のアウトブレイクは過去3年間で500症例が報告され、未だ終息していないとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

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LEISHMANIASIS - SPAIN: MADRID
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A ProMED-mail post
http://www.promedmail.org
ProMED-mail is a program of the
International Society for Infectious Diseases
http://www.isid.org

Date: Wed 13 Feb 2013
Source: SER Madrid Sur [in Spanish, trans. Corr.SB, edited]
http://www.sermadridsur.com/noticias/el-brote-de-leishmaniasisque-afecta-a-municipios-del-sur-no-remitio-en-2012-con-150-casos_31239/

The outbreak of leishmaniasis that has affected the southern municipalities (of Madrid) has not decreased in 2012, with 150 cases reported.

According to the "Report on the Health Status of the Population of Madrid, 2012," the outbreak of leishmaniasis is not over, as evidenced by the 150 cases detected last year [2012], including nearly 500 cases which have emerged in the last 3 years. These cases have occurred in southern municipalities of the region, such as Fuenlabrada, Leganes, Getafe or Humanes, near the green zones of Bosquesur.

Leishmaniasis is spread by the bite of an insect (a _Phlebotominae_ female sand fly that has previously bitten an infected animal) and can lead to death if the visceral disease occurs and if it is left untreated.

The strong increase occurred from 2009, with 471 cases, compared to 15 or 25 that had been reported in previous years. The cases have been found in municipalities in southern Madrid, such as Fuenlabrada with 322 cases, Leganes with 37, Getafe with 20 affected, and Humanes, which had 5 patients. All of them were near the green area of Bosquesur, which officials believe may be the focus of leishmaniasis.

This disease is transmitted by a sandfly that has previously bitten an infected animal. It is believed that hares and rabbits, which proliferate in Bosquesur, can act as reservoirs or carriers of the parasite.

Leishmaniasis causes 2 types of disease: cutaneous, and visceral, which can affect organs such as the spleen and liver and is fatal if not treated properly.

Last year [2012], the community declared Fuenlabrada an "Emergency Area," allowing the free hunting of rabbits to try to control the extent of the problem. The Ministry of Health said that by the end of 2012, there were 3 cases of visceral leishmaniasis, while in 2011, there had been 47, which was good progress. Still, the outbreak is not considered to be over. So far this year [2013], there have been 3 new cases.

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Communicated by:
ProMED-mail <promed@promedmail.org>

[Dogs are considered the main reservoir of Leishmania in Spain (M.G. et al. Current situation of _Leishmania infantum_ infection in shelter dogs in northern Spain. Parasit. Vectors. 2012;5:60). It has been hypothesized that rabbits, hares, and squirrels may also be hosts, but this remains to be demonstrated. - Mod.EP

A HealthMap/ProMED-mail map can be accessed at: http://healthmap.org/r/1zJm.]

See Also

2012

Leishmaniasis - Spain: Madrid: 20120504.1123085
Leishmaniasis, human, canine - Spain (02): background 20120329.1084736
Leishmaniasis, human, canine - Spain: (MD) 20120328.1083656
Leishmaniasis, canine - Singapore ex Spain: OIE 20120125.1022003
2010

Leishmaniasis, human - Spain 20100612.1969
2004

Leishmaniasis, dog reservoir - Spain 20040524.1388
2000

Leishmaniasis - Germany ex Spain (02) 20000729.1254
Leishmaniasis, Germany ex Spain: background 20000727.1248
Leishmaniasis - Germany ex Spain 20000725.1236
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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	新医薬品等の区分	総合機構処理欄
一般的名称	－	研究報告の公表状況	Transfusion. 2012 Nov;52(11):2285-93.	公表国：アイルランド	
販売名（企業名）	－				

研究報告の概要

変異型クロイツフェルトヤコブ病(vCJD)は、まれで進行性の致命的な非炎症性の神経変性疾患である。アイルランドは輸血を通してのvCJD伝播率が英国(176/6200万)に次いで、世界中で2番目に高い(2/4500万)。
vCJD伝播の危険を減らすためプリオンを取り除くフィルター(the P-Capt filter)が開発された。
この研究は、アイルランド共和国で赤血球のプリオンろ過を実行することについての費用効果を評価することを目的とした。
費用対効果モデルは、受血者が感染した赤血球を輸血された結果、臨床的変異型CJDを発症する可能性をシミュレートするために開発された。
モデル変数は公表文献や専門家の意見を収集し、費用はプリオンろ過を実装するために必要な処理の変更に基づいて推定した。
プリオン濾過を行わなければ、10年間に2人が赤血球輸注によるvCJD発症すると推定され、失われる寿命は18.5年となるがプリオン濾過を行えば、寿命は失われない。
普遍的なプリオン濾過の費用は68.2百万ユーロで、寿命1年当たりのコストは3.7百万ユーロである。
シミュレーションの25.3%においては、プリオン濾過の有無にかかわらず、感染血液の輸血を介したvCJD感染の死亡例はない。
そのため、プリオン濾過導入の費用対効果は高くないと考えられた。
多くの非費用対効果の高い血液の安全戦略が過去に実施されてきたが、輸血医療における有限な資源の最も効率的な使用を考慮するべきである。

使用上の注意記載状況・その他参考事項等

重要な基本的注意
現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

報告企業の意見	今後の対応
赤血球製剤に対するプリオンろ過フィルターの費用対効果に関する情報である。 現時点まで血友病以外で血漿分画製剤からvCJD伝播が疑われた報告はなく、血漿分画製剤の製造工程でプリオンが除去できるとの情報もある。	今後ともvCJDに関する安全性情報等に留意していく。

14

TRANSFUSION COMPLICATIONS

Cost-effectiveness of prion filtration of red blood cells to reduce the risk of transfusion-transmitted variant Creutzfeldt-Jakob disease in the Republic of Ireland

Conor Teljeur, Martin Flattery, Patricia Harrington, Michelle O'Neill, Patrick S. Moran, Linda Murphy, and Máirín Ryan

BACKGROUND: Variant Creutzfeldt-Jakob disease (vCJD) is a rare, progressive fatal noninflammatory neurodegenerative disease. Ireland has the second highest rate of vCJD in the world with an ongoing risk of vCJD transmission through blood transfusion. Prion-removing filters have been developed to reduce the risk of vCJD transmission. This study aimed to evaluate the cost-effectiveness of implementing a policy of prion filtration of red blood cells (RBCs) in the Republic of Ireland.

STUDY DESIGN AND METHODS: A cost-effectiveness model was developed to simulate the likelihood of RBC recipients developing clinical vCJD as a result of being transfused with infected RBCs. Model variables were collected from published literature and expert opinion. Costs were estimated based on the processing changes required to implement prion filtration.

RESULTS: In the absence of prion filtration, it is estimated that two individuals will develop clinical vCJD arising from RBC transfusions over a 10-year time horizon. The discounted life-years lost will be 18.5 years. With prion filtration, there will be no deaths or life-years lost. The discounted cost of universal prion filtration is €68.2 million over 10 years with a corresponding incremental cost-effectiveness ratio of €3.7 million per life-year gained. In 25.3% of simulations there were no deaths from vCJD infection through infected blood transfusions, irrespective of prion filtration.

CONCLUSION: Prion filtration is considered not cost-effective by traditional measures. Although numerous non-cost-effective blood safety strategies have been implemented in the past, consideration should be given to the most efficient use of finite resources in transfusion medicine.

Variant Creutzfeldt-Jakob disease (vCJD) is one of a group of rare, progressive fatal noninflammatory neurodegenerative diseases known as transmissible spongiform encephalopathies (TSEs).[1] CJD is the most common TSE affecting humans; a new variant form, termed vCJD, was first described in 1996. This variant form is characterized by having a different neuropathologic profile, a younger age of onset, different clinical findings, and the absence of abnormal electroencephalogram findings typical of other CJD forms.[2] The origin of vCJD is linked to the consumption of beef from cattle infected with a bovine form of the disease, bovine spongiform encephalitis (BSE),[3] which was prevalent in the United Kingdom and elsewhere in the 1980s and 1990s. Of the 224 cases of vCJD worldwide to date, 175 have occurred in the United Kingdom,[4] where the incidence peaked in 2000, declining since. Ireland has the second highest rate of vCJD in the world behind the United Kingdom, with four cases reported to date in a population of 4.5 million.[4]

There is an ongoing risk of vCJD transmission from transfusion of blood or blood products originating from *subclinical carriers of the disease*. Measures have been taken by transfusion services worldwide in accordance

ABBREVIATIONS: BSE = bovine spongiform encephalitis; IBTS = Irish Blood Transfusion Service; ICER = incremental cost-effectiveness ratio; QALY = quality-adjusted life-year; TSE(s) = transmissible spongiform encephalopathy (-ies); VAT = value-added tax; vCJD = variant Creutzfeldt-Jakob disease.

From the Health Information and Quality Authority, Dublin, Ireland.

Address correspondence to Conor Teljeur, Health Information and Quality Authority (HIQA), George's Court, George's Lane, Dublin 7, Ireland; e-mail: cteljeur@hiqa.ie.

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with best available evidence to minimize this risk. These measures include donor deferral policies, importation of plasma from countries with a low incidence of vCJD and BSE, and universal leukoreduction of red blood cells (RBCs). Worldwide there have been four documented cases of vCJD infection arising from transfusion of RBCs, resulting in three deaths from clinical vCJD.[5] All of these cases occurred in the United Kingdom before the introduction of universal leukoreduction of RBCs. The high incidence of vCJD in Ireland has raised concerns that there may be a significant risk of secondary transmission through infected blood products.[6]

Prototype blood tests have been developed for detection of vCJD in symptomatic individuals, but as yet there are no tests sufficiently sensitive to screen blood from individuals who are asymptomatic carriers of the infectious agent.[7,8] Prion-removing filters have been developed to further reduce the risk of vCJD transmission from transfusion of RBCs by, it is claimed, substantially reducing any residual prion protein present in donated blood.[9] The use of such filters would complement existing measures adopted to contain the risk of transmission. Although prion-removing filters may alter the composition of RBCs that are passed through them, the resulting RBCs are considered safe for transfusion.[6,10] The aim of this study was to evaluate the cost-effectiveness of implementing a policy of prion filtration of RBCs in the Republic of Ireland.

MATERIALS AND METHODS

Literature review on efficacy and safety of prion filters

Published literature was obtained by searching MEDLINE, CINAHL, the Cochrane Library, Database of Abstracts of Reviews of Effects, NHS Economic Evaluation Database, and EBSCO Psychology and Behavioural Sciences Collection and Health Business on the PubMed and EBSCO systems. Regular alerts were established on PubMed and EBSCO and relevant information retrieved via alerts was current to December 16, 2010. Given the limited data available on the topic, a broad search was used to capture as many potentially relevant results as possible. No date restrictions or other filters were applied to limit the retrieval to specific study designs or document types.

Economic model

A cost-utility analysis is the preferred type of economic evaluation for assessing health care interventions in Ireland.[11] As no published quality-of-life data were found for vCJD, or for TSEs generally, a cost-effectiveness analysis was performed as an alternative in this study. Prion filtration of all RBCs was compared to a policy of no filtration. The perspective was that of the publicly funded health and social care system in Ireland with only direct costs to the Irish Blood Transfusion Service (IBTS) and Health Service Executive included. The target population included all individuals receiving a transfusion of RBCs. It was assumed that no additional transfusion-related adverse events would arise due to filter-related changes in the composition of RBCs. Discounting is a technique that allows comparison between costs and benefits that occur at different times. It reflects a societal preference for benefits to be realized in the present and costs to be experienced in the future. Costs and benefits were discounted at the rate of 4% as prescribed in Irish guidelines.[11] A 10-year time horizon was used for evaluating costs. Benefits were determined as the life-years gained based on infections prevented from blood transfusions in the 10-year horizon. The benefits could therefore extend beyond the 10-year horizon as long as the original transfusion took place during the 10-year interval. No utility data associated with vCJD or CJD were available to develop a suitable quality-adjusted life-year (QALY) measure, hence a cost-utility analysis was not possible. Variable values were determined using peer-reviewed literature, with gray literature and expert opinion used in the absence of peer-reviewed evidence.

The model comprised two distinct components: the transmission model and the costs model. The former simulated the transfusion of infected units of RBCs into recipients; the latter estimated the costs associated with prion filtration. The transmission model was adapted from a model originally developed by the UK Department of Health. The UK model was deterministic and estimated cost-effectiveness for a number of best- and worst-case scenarios. This was converted into a fully probabilistic model that allowed the inherent uncertainty around variable estimates to be incorporated.

The transmission model simulated the likelihood of RBC recipients developing vCJD as a result of being transfused with blood donated by individuals who were subclinical carriers of vCJD. There are approximately 96,000 donors and 32,000 recipients of units of RBCs each year in Ireland. The median age of recipients is 69 and on average 41% of recipients are alive 5 years after transfusion.[12] The model relied on a number of key variables (see Table 1): the prevalence of subclinical vCJD in the donor population; the infectivity of blood sourced from an infected donor; the susceptibility of the recipient to developing clinical vCJD; once infected, the incubation period before developing clinical vCJD; and the efficacy of the filter. The prevalence of subclinical vCJD in Ireland was estimated using the results of the UK Hilton study.[13] To account for the difference in observed clinical cases, the prevalence was multiplied by the ratio of indigenous clinical prevalence in Ireland (2 in 4.5 million) to the UK (176 in 62 million). The infectivity of human blood has been inferred from animal studies. All confirmed clinical cases

TABLE 1. Transmission model variable distributions and values

Variable	Median (95% CI)	Distribution
National prevalence of preclinical vCJD*	153 (35-413)†	Beta (3, 12,671) × 0.158[13]
Susceptibility to developing clinical vCJD (%)	9.7 (5.0-16.6)	Beta (10, 90)[14]
Donations per infected donor (units per annum)	1 (1-4)	Sampled[15]
Percentage of collected units used (%)	89 (86-91)	Beta (512, 63)[13]
Infectivity of vCJD infected blood (ID/mL)	9.3 (0.9-35.6)	Gamma (1.57, 0.135)[16]
Infectivity removed by leukoreduction (%)	50.0 (32.4-67.5)	Beta (14.9, 14.9)[9,17]
Residual plasma (mL)		
Top and top (TT)	20.2 (13.7-26.7)	Normal (20.2, 3.3)[18]
Top and bottom (TB)	9.3 (5.2-13.4)	Normal (9.3, 2.1)[18]
Incubation (years)		
MM homozygous	7.6 (5.6-9.6)	Normal (7.60, 1.02)[19]
Non-MM homozygous‡	21.3 (15.9-28.6)	Log normal (3.06, 0.15)
Percentage population MM homozygous	39.2 (34.5-44.0)	Beta (156, 242)[20]
Probability of infectious doses after prion filtration	6.9×10^{-6} (2.5×10^{-7}-3.7×10^{-5})	Beta (1, 9,999)[21]

* A factor of 0.158 is applied to the prevalence to reflect the relative difference in observed indigenous clinical cases between Ireland and the United Kingdom.
† Prevalence has been multiplied by national population of 4.5 million to give number of infected individuals nationally.
‡ As there are no observed cases of clinical vCJD in non–MM-homozygous individuals, this distribution is based on expert opinion alone.
Beta = α and β in parentheses; Gamma = shape and rate in parentheses; normal and log normal = mean and SD in parentheses.

of vCJD to date have been methionine-homozygous (MM) at Codon 129 of the prion protein expressing gene (*PRNP*). However, it cannot be ruled out that clinical vCJD could occur in individuals with other polymorphisms.[22] A Poisson dose–response model was used that entails a high probability of infection even at minimal exposure levels. Under this model, there is a 99% probability of transmission with exposure to only five infectious doses. Susceptibility may also be linked to the age of the recipient, but there are insufficient data to support a parameterization.[23] Values for susceptibility and incubation period in non–MM-homozygous individuals are unknown and were based on published modeling exercises.[21,24] It was assumed that all individuals can be infected by exposure to infected RBCs but that only those who are susceptible may go on to develop clinical vCJD. To generate plausible results, susceptibility and incubation length must be negatively correlated.[24] There is no biologic rationale for a negative correlation and this relationship may simply reflect a shortcoming of the model rather than the characteristics of vCJD transmission. Susceptibility was set at a lower range and a corresponding short incubation period was used for this study. The same values for susceptibility to clinical vCJD were applied to MM and non–MM-homozygous individuals. The efficacy of the filter is derived from a single study that determined that 1 in 10,000 infectious doses would not be retained by the filter.[25] The filters therefore appear to be very efficacious, but no subsequent studies are yet available to either confirm or contradict those findings.

There are two methods for extracting RBCs from whole blood: top and top and top and bottom. The method used impacts on the amount of residual plasma in the processed RBCs and therefore the model takes into account the proportions processed by each method in the IBTS. It is thought that vCJD infectivity in human blood is distributed such that half is in the plasma and the remaining 50% split between the white blood cells (WBCs) and buffy coat; hence the amount of residual plasma in RBCs affects the amount of residual infectivity.[26] Leukoreduction is assumed to remove approximately 50% of infectivity.

The wide distributions around variable values reflect the uncertainty in the underlying scientific evidence (see Table 1). There were no data to support the choice of incubation period in non–MM-homozygous individuals, so expert opinion was used to select a plausible value. The age–sex profile of recipients was sampled from national hospital inpatient data and posttransfusion survival data were based on a UK study.[12]

Costs model

Two models of prion-removing filter were considered in this assessment: the P-Capt filter (MacoPharma Ltd, Mouvaux, France) and the Leukotrap Affinity Plus combined WBC and prion-removing filter system (PRF2BE; Pall Medical, Portsmouth, UK). Unlike the PRF2BE, the P-Capt has been independently validated and could be adopted immediately. Such studies typically take 3 to 4 years to complete and it is therefore assumed the PRF2BE filter system could only be adopted after 4 to 5 years under the assumption that it will be shown to have equivalent efficacy to the P-Capt filter.

Costs related to the introduction of prion filtration of RBCs were identified (see Table 2). The costs inputs for the cost-effectiveness model relate to the incremental cost of prion filtration. Where appropriate, cost savings related to the reduced consumption of existing resources were included. Costs considered in estimating the marginal

TABLE 2. Cost data included in the model (2010 costs)*

Item	Cost (€)
P-Capt filter	55.00 (46.20-63.80)
PRF2BE filter†	43.70 (36.71-50.69)
Processing cost per unit (excluding prion filtration)	248.71 (208.92-288.50)
Wafer	2.73 (2.29-3.17)
Macopharma bag	9.87 (8.29-11.45)
Pall bag	8.75 (7.35-10.15)
Classic bag	6.95 (5.84-8.06)
F IX assay	6.20 (5.21-7.19)
Waste bin (per unit)	0.12 (0.10-0.14)
Incineration (per unit)	0.26 (0.22-0.30)
Mean annual staffing costs‡	295,074.00 (247,862.16-342,285.84)

* Data are reported as median (95% CI).
† Filter price estimated based on quoted price in sterling for purchase of 150,000 units.
‡ Comprises three medical laboratory aides and one senior medical scientist. Salary cost includes social insurance, pension costs, and IBTS overheads.

unit cost for the intervention included the cost of procurement, processing, storage, and distribution of prion-filtered RBCs. Consistent with national guidelines, value-added tax (VAT) was not applied to costs.[11] Filter costs were supplied by the manufacturers. Prices for the PRF2BE filter system were quoted in sterling and converted to Euro using the exchange rate at the time of the analysis (€1.14 to GBP£1.00, October 2010). Variation in the exchange rate is assumed to follow a normal distribution (mean, 1; standard deviation [SD], 0.025) around the previous years' exchange rate. Prices for processing equipment were supplied by the IBTS. Salaries were derived from IBTS pay scales and subsequently adjusted for pay-related costs. All costs were based on 2010 prices. All costs were varied by ±20% according to a beta distribution ($\alpha = 2, \beta = 2$).

The costs are based on the total number of units processed rather than transfused, as not all processed units are transfused. Some units are not used within the allowable time or else are brought to the operating room and then not transfused. Approximately 148,000 units are processed per annum with 137,000 used. Prion filtration has been associated with a reduction in the hemoglobin (Hb) content of filtered RBCs. The reduction in Hb content per unit may have clinical consequences for transfusion-dependent patients with a percentage of these patients requiring additional units of RBCs annually. It was assumed that this would equate to an increase of 0.5% in the number of units required nationally per annum.

The P-Capt filter represents an additional processing step after leukoreduction whereas the PRF2BE is an integrated WBC and prion removal filter. The use of the P-Capt filter would require an additional sterile connector device (wafer) and associated waste bin and incineration charges. With use of the PRF2BE filter, no leukoreduced plasma (for issue as fresh-frozen plasma) would be generated. In Ireland, approximately 500 units are generated locally per annum with the remaining units required imported from abroad. The cost of an additional processing step to produce leukoreduced plasma was also included. A Factor (F)IX assay is used as a process control measure to indicate filter exposure. It is assumed that 1% of prion filtered units will be selected for testing using F IX assays.

Two-year supply contracts are expected to apply to the purchasing of prion-removing filters. For the cost-effectiveness model, the cost of filters only changes every second year while all consumables vary from year to year. Every 2 years, at the point of filter costs changing, it is assumed that the IBTS will select from the two filter models based on lowest price. Although the PRF2BE filter is less expensive, applying the price fluctuation of ±20% results in the P-Capt filter being less expensive in some simulations.

The budget impact analysis was also determined for a 5-year time horizon using the same perspective as the economic analysis. The data for the budget impact analysis are the same as those used in the cost-effectiveness analysis with the difference being that prices are inclusive of VAT, and no discounting is applied. All items are subject to VAT at 21% apart from staff and the cost per unit of the processed RBCs, which is classified as VAT exempt.

The model was developed and run in the open-source statistics program R.[27] The model was run for 25,000 simulations. Discounting was applied to the results from each year and the results were then aggregated to generate a simulation-level result. The incremental cost-effectiveness ratio (ICER) was computed as the additional cost of prion filtration divided by the additional benefits of prion filtration, in this case calculated as life-years gained. The median, 2.5th percentile, and 97.5th percentile were computed for each outcome across all simulations. These values represent the point estimate, lower and upper bounds, respectively, for each outcome. A univariate sensitivity analysis was used to determine the impact of setting each individual variable in turn to the upper and lower bounds, respectively, while varying all other variables as per the standard model. The results of the sensitivity analysis are presented using a tornado plot, which ranks the variables by their impact on the results. Although the discount rate was not varied in the main model, it was varied between 0 and 6% in the univariate sensitivity analysis. The same discount rate was applied to costs and benefits.

RESULTS

Over 10 years an estimated 45 donors (95% confidence interval [CI], 2-142) infected with subclinical vCJD will donate a total of 70 units (95% CI, 3-224) of RBCs. In the absence of prion filtration, the infected units of blood will be transfused to six recipients (95% CI, 0-26) that are susceptible to clinical vCJD. Of those six, two (95% CI, 0-11) will survive to 5 years posttransfusion and two individuals (95% CI, 0-8) will develop clinical vCJD and die from this disease (Table 3). In the absence of prion filtration, the life-years lost will be 18.5 (95% CI, 0-102.5). With prion filtration, there will be no deaths or life-years lost. The discounted cost of universal prion filtration will be €68.2 million over 10 years. The corresponding ICER is €3.7 million (95% CI, €0.7 m-∞) per life-year gained.

TABLE 3. Outcomes with and without prion filtration*

Outcome	Without prion filtration	With prion filtration
Deaths from clinical vCJD	2 (0-8)	0 (0-0)
Discounted life-years lost	18.5 (0-102.5)	0 (0-0)
Discounted life-years gained		18.4 (0-101.3)
Discounted cost (€m)		*68.2 (61.7-75.0)*
ICER (€m/LYG)		3.7 (0.7-∞)
Five-year budget impact (€m)		51.6 (46.4-57.5)

* Data are reported as median (95% CI).

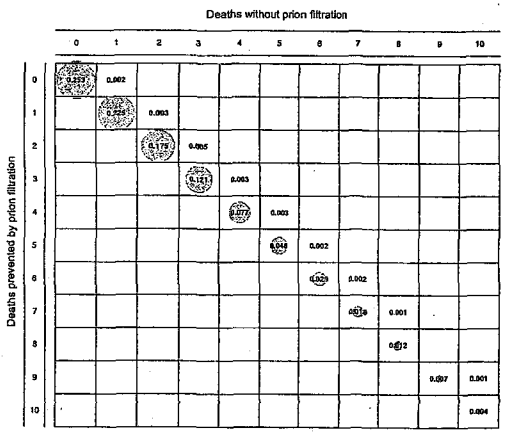

Fig. 1. Balloon plot showing probability of different outcomes with and without prion filtration. Notes: 1) The value in a cell represents the probability of that particular outcome. For example, there is a probability of 0.005 that there will be three deaths without prion filtration where two of the three would be prevented by filtration. Blank cells have zero probability. 2) For clarity the balloon plot only extends to 10 deaths—this excludes 0.8% (n = 194) of simulations with more than 10 deaths. Outcomes with a probability of less than 0.001 are not shown.

In 25.3% of simulations there were no deaths from vCJD infection through infected blood transfusions, irrespective of prion filtration. Indeed, this is the single most probable outcome. For simulations where there was at least one predicted death, the introduction of prion filtration was predicted to prevent all deaths in 96.8% of cases. The probability of different outcomes is shown in Fig. 1.

The benefits in terms of life-years gained follows a very skewed distribution (Fig. 2). The clustering of results with a gain of 0 is pronounced with 25.6% of simulations resulting in no life-years gained using prion filtration. Just over 83% of simulations had an incremental benefit of less than 50 life-years gained. Given the shorter life expectancy posttransfusion coupled with the older age profile of transfusion recipients, not all cases of prevented infections will result in life-years gained.

The median 5-year budget impact is €51.6 million. In the first few years, when only the P-Capt filter is available, the annual budget impact is approximately €11 million but this reduces to under €9 million by Year 5 when the PRF2BE filter is available. When the budget impact of prion filtration is distributed across the units transfused, it represents an additional cost of €74 (95% CI, €66-€82) per unit, in other words an additional 30% on the price of a unit of RBCs.

A univariate sensitivity analysis was carried out to assess the influence of different variables on the ICER (Fig. 3). The single most significant variable that impacts on the calculated ICER is the prevalence estimate of subclinical disease. The upper and lower bounds for prevalence are equivalent to 413 and 35 cases of subclinical disease nationally. If the prevalence of subclinical vCJD nationally is 35 cases, for example, then the ICER will be €13.4 million per life-year gained. If, on the other hand, the prevalence is 413 cases nationally, then the ICER will be €2.4 million per life-year gained. The susceptibility is the next most influential variable with an ICER of €7.8 million per life-year gained when susceptibility is at its lowest value and €2.8 million at its highest value. When the discount rate is at its lowest value the mean ICER is €3.1 million per life-year gained.

The cost-effectiveness acceptability curve for prion filtration is shown in Fig. 4. The cost-effectiveness acceptability curve shows the probability that prion filtration is cost-effective over a range of willingness-to-pay thresholds. The probability of cost-effectiveness is

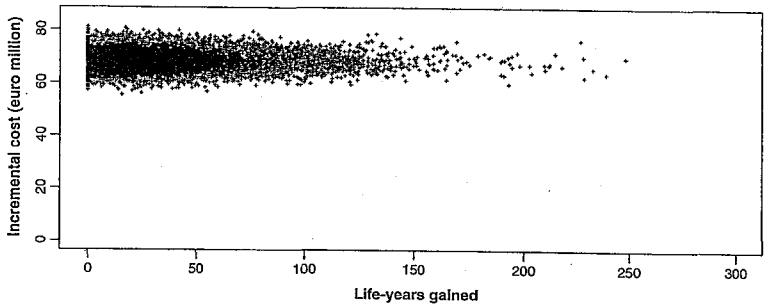

Fig. 2. Plot of cost against health benefit for 25,000 model simulations. Note: in 25.3% (n = 6334) of simulations there were no deaths from vCJD infection through infected blood transfusions, irrespective of prion filtration. Benefits are for the total transfused Irish population.

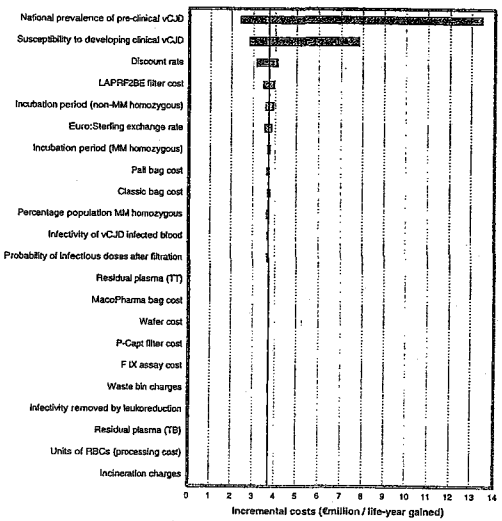

Fig. 3. Tornado plot of univariate sensitivity analysis. Note: base-case and upper and lower values for each variable are derived from Tables 1 and 2. The upper and lower bounds for the discount rate were 5.7 and 1.7%, respectively. Exchange rate variation is approximately ±5% per annum.

zero below a willingness-to-pay threshold of €228,000 per life-year gained. The probability of cost-effectiveness is 0.09 at a willingness-to-pay threshold of €1 million per life-year gained. The probability of cost-effectiveness exceeds 0.5 at a willingness-to-pay threshold of €3.7 million per life-year gained.

DISCUSSION

In the absence of a policy to prion filter RBCs, it was estimated in this study that arising from transfusion of vCJD-infected RBCs over the next 10 years in Ireland there will be two (95% CI, 0-8) deaths from vCJD. This would correspond with 18.5 (95% CI, 0.0-102.5) life-years lost. A policy of universal prion filtration of RBCs is predicted to prevent these two deaths. However, the single most likely outcome is that there will be no deaths arising from vCJD transmission through infected RBCs. Compared to the base-case of no prion filtration, the estimated ICER is €3.7 million (95% CI, €0.7 m-€∞) per life-year gained. The 5-year budget impact of prion filtration would be €51.6 million (95% CI, €46.4 m-€57.5 m), which corresponds to an additional €74 per unit of RBCs transfused.

The ICER of €3.7 million per life-year gained is considered not cost-effective by traditional measures of cost-

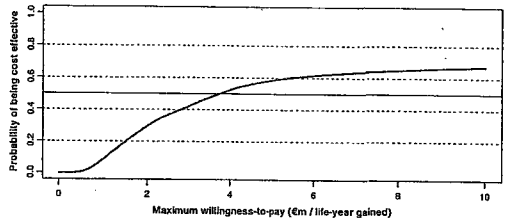

Fig. 4. Cost-effectiveness acceptability curve. Note: as prion filtration offers no benefit in 25.6% (n = 6397) of simulations, the probability of cost-effectiveness never exceeds 0.744.

effectiveness. Although there is no explicit threshold in Ireland, historically an upper limit of €45,000 per QALY has been used, although this has been lowered in recent years, notionally to €20,000 per QALY. Internationally, the cost-effectiveness of blood safety strategies does not compare favorably to that of other health technologies. For example, the addition of nucleic acid testing (NAT) to an existing strategy of viral serologic testing (including human immunodeficiency virus [HIV], hepatitis B, and hepatitis C) was found to be not cost-effective in published European and US studies, with ICERs ranging from €300,000 to €47 million per QALY.[28-32] Despite being not cost-effective, NAT has been adopted by blood transfusion services in most developed countries, including Ireland.[15] The introduction of NAT in Ireland must be viewed in context. From the 1970s to the early 1990s, some patients with hemophilia and von Willebrand disease received contaminated blood products leading to HIV and hepatitis C infections. Contaminated anti-D was also administered resulting in hepatitis C infections. This historical context, coupled with favorable economic conditions at the time, facilitated the introduction of NAT. With no reported Irish cases of vCJD transmission through infected RBCs and less favorable economic circumstances, the precedent of NAT is unlikely to influence a decision on prion filtration. A possible reason for the differing standards of cost-effectiveness of blood safety strategies relates to their purpose—they are aimed at risk reduction rather than improved effectiveness. It is possible that society has a greater preference for risk reduction than health gains.[33] Blood transfusion in Ireland is managed through a State agency so liability claims must also be considered. The negative publicity generated by failing to prevent cases of vCJD infection may also lead to a loss of donors.

The effect of discounting is not inconsiderable in this economic evaluation. Owing to the long incubation period after infection with vCJD, benefits from prion filtration (life-years saved) do not occur for many years. As per the Irish guidelines for the economic evaluation of health care technologies, the same discount rate is applied to both costs and benefits.[11] This practice is not without controversy, particularly when potential benefits do not accrue for a long time (such as in vaccination programs and other preventative public health strategies). Prion filtration is predicted to result in 18.4 (95% CI, 0-101.3) life-years gained with discounting compared to 36.3 (95% CI, 0-228.2) life-years gained without discounting. Differential rates of 3.5 and 1.5% for costs and benefits, respectively, have been used in the United Kingdom. By applying these differential rates, the ICER reduced to €2.3 million (95% CI, €0.5 m-€∞) per life-year gained. While this represents a substantial reduction from the estimate of €3.7 million per life-year gained, prion filtration would still be considered not cost-effective by traditional standards for cost-effectiveness.

The quality of the model was assessed by evaluating the plausibility of the results compared to similar studies. The only study to estimate future cases of vCJD in Ireland predicted that there would be one to two future clinical cases—this is in line with the findings of this study.[34] Until now, cases of vCJD have been driven by primary infection through consumption of BSE-infected beef. Based on the assumption of susceptibility, there is a large cohort who may never develop vCJD, but who are carriers that could infect others. It is therefore possible that a second wave of vCJD may occur in the future due to secondary transmission through infected blood products. In a study published in 2010, the possibility of a second wave of vCJD cases in the United Kingdom was investigated, distinguishing between primary and secondary transmission.[23] It was estimated that over the next 100 years the number of vCJD deaths due to secondary transmission would be approximately 1.7 times the number of vCJD deaths observed to date. If that ratio is applied to Ireland then there would be approximately seven cases in the next 100 years or less than one case per decade through blood transfusion. This would be within the confidence bounds estimated by this study.

The cost-effectiveness model is subject to a number of limitations that may impact on the results or their interpretation. There is substantial uncertainty around both the suitable point estimates and the associated ranges of probable values for many of the key model variables. By using a fully probabilistic model, the uncertainty in the variable values is reflected in the uncertainty in the estimate of cost-effectiveness. The prevalence in the model is assumed to be constant over the 10-year time horizon. This represents a pessimistic view: the risk of primary

transmission through the consumption of infected meat products is believed to have been eliminated; accordingly the number of subclinical donors in the population will decline over time. The cost-effectiveness results reflect the conservative approach adopted—that all genotypes can develop the disease. To date, however, all deaths due to confirmed clinical vCJD have been in MM homozygotes. In assuming, as we have done, that non-MM-homozygotes may develop clinical vCJD, we may be overestimating the number of cases of vCJD arising from blood transfusion. If only MM-homozygous individuals are susceptible to developing clinical disease, then prion filtration would have a true ICER that is substantially higher than €3.7 million per life-year gained. The costs considered in this study were limited to the direct costs to the publicly funded health care system. No costs attached to treatment of individuals with symptomatic vCJD were included. Costs to the individual (for example, out-of-pocket expenditure related to treatment or transport to appointments) or to society (for example, lost productivity in those diagnosed with vCJD) were not considered.

The knowledge of vCJD and its transmission is limited and constantly being updated with new information. While the evidence is sometimes seemingly contradictory or at odds with our understanding of the disease process, it provides an opportunity to refine variable values. The variable values used in this study may be viewed as conservative and reflective of a worst-case scenario. Emerging evidence suggests that some variables, such as infectivity, may be lower than previously thought and hence overstated in this model.[35] However, taking a public health perspective and viewing prion filtration as a means to prevent a civil risk, it is pragmatic to view the potential exposure in a pessimistic rather than optimistic light. Given Ireland's legacy regarding hepatitis C and HIV infection, a conservative approach is *more appropriate*. As knowledge improves, models can be refined to hopefully produce more accurate and precise estimates of the future course of vCJD.

In conclusion, in the absence of a reliable screening test for donors with subclinical vCJD, it has been proposed that prion filtration of RBCs would complement existing risk reduction strategies and further reduce the risk of transfusion-transmitted vCJD. The introduction of prion filtration for all transfusion recipients was found to be not cost-effective by traditional standards of cost-effectiveness. Although the results of this study may be closer to a worst-case scenario as a result of conservative modeling assumptions, they should not be viewed as improbable. Prion filtration could have a true ICER that is substantially higher than €3.7 million per life-year gained. Although other blood safety interventions regarded as not cost-effective have been implemented, the most effective use of finite resources in transfusion medicine must be taken into consideration.

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CONFLICT OF INTEREST

MF had previously been employed, until 2008, by Fannin Healthcare, the Irish distributors of the Macopharma range of products. CT, PH, MON, PSM, LM, and MR do not have any conflicts of interest.

REFERENCES

1. US Department of Health and Human Services, Centers for Disease Control and Prevention. Chronic wasting disease [Internet]. CDC; 2011. [cited 2011 Jan 21]. Available from: URL: http://www.cdc.gov/ncidod/dvrd/cwd/
2. Will RG, Ironside JW, Zeidler M, Cousens SN, Estibeiro K, Alperovitch A, Poser S, Pocchiari , M, Hofman A, Smith, PG. A new variant of Creutzfeldt-Jakob disease in the UK. Lancet 1996;347:921-5.
3. Hilton DA. Pathogenesis and prevalence of variant Creutzfeldt-Jakob disease. J Pathol 2006;208:134-41.
4. European Creutzfeldt Jakob Disease Surveillance Network. Current data (August 2011) [Internet]. EUROCJD; 2011. [cited 2011 Aug 31]. Available from: URL: http://www.eurocjd.ed.ac.uk
5. Knight R. The risk of transmitting prion disease by blood or plasma products. Transfus Apher Sci 2010;43:387-91.
6. Murphy CV, Eakins B, Fagan J, Croxon H, Murphy WG. In vitro assessment of red-cell concentrates in SAG-M filtered through the MacoPharma P-CAPT prion-reduction filter. Transfus Med 2009;19:109-16.
7. Orru CD, Wilham JM, Raymond LD, Kuhn F, Schroeder B, Raeber AJ, Caughey B. Prion disease blood test using immunoprecipitation and improved quaking-induced conversion. Mbio 2011;2:e00078-11.

8. Edgeworth JA, Farmer M, Sicilia A, Tavares P, Beck J, Campbell T, Lowe J, Mead S, Rudge P, Collinge J, Jackson GS. Detection of prion infection in variant Creutzfeldt-Jakob disease: a blood-based assay. Lancet 2011;377:487-93.
9. Gregori L, Gurgel PV, Lathrop JT, Edwardson P, Lambert BC, Carbonell RG, Burton SJ, Hammond DJ, Rohwer RG. Reduction in infectivity of endogenous transmissible spongiform encephalopathies present in blood by adsorption to selective affinity resins. Lancet 2006;368:2226-30.
10. Cancelas JA, Rugg N, Pratt PG, Worsham DN, Pehta JC, Banks K, Davenport RD, Judd WJ. Infusion of P-Capt prion-filtered red blood cell products demonstrate acceptable in vivo viability and no evidence of neoantigen formation. Transfusion 2011;51:2228-36.
11. Health Information and Quality Authority. Guidelines for the economic evaluation of health technologies in Ireland. Dublin: HIQA; 2010.
12. Wells AW, Llewelyn CA, Casbard A, Johnson AJ, Amin M, Ballard S, Buck J, Malfroy M, Murphy MF, Williamson LM. The EASTR Study: indications for transfusion and estimates of transfusion recipient numbers in hospitals supplied by the National Blood Service. Transfus Med 2009;19:315-28.
13. Hilton DA, Ghani AC, Conyers L, Edwards P, McCardle L, Ritchie D, Penney M, Hegazy D, Ironside JW. Prevalence of lymphoreticular prion protein accumulation in UK tissue samples. J Pathol 2004;203:733-9.
14. Clarke P, Ghani AC. Projections of the future course of the primary vCJD epidemic in the UK: inclusion of subclinical infection and the possibility of wider genetic susceptibility. J R Soc Interface 2005;2:19-31.
15. Irish Blood Transfusion Service. Annual report 2009. Dublin: Irish Blood Transfusion Service; 2010.
16. Spongiform Encephalopathy Advisory Committee (SEAC) (UK). SEAC position statement on TSE infectivity in blood [Internet]. SEAC Website; 2008. [cited 2010 Apr 26]. Available from: URL: http://webarchive.nationalarchives.gov.uk/20110316162913/http://www.seac.gov.uk/statements/statement0806.htm
17. Gregori L, McComble N, Palmer D, Birch P, Sowemimo-Coker SO, Giulivi A, Rohwer RG. Effectiveness of leucoreduction for removal of infectivity of transmissible spongiform encephalopathies from blood. Lancet 2004;364:529-31.
18. Wiltshire M, Thomas S, Scott J, Hicks V, Haines M, Cookson P, Garwood M, Cardigan R. Prion reduction of red blood cells: impact on component quality. Transfusion 2009;50:970-9.
19. Zou S, Fang CT, Schonberger LB. Transfusion transmission of human prion diseases. Transfus Med Rev 2008;22:58-69.
20. Alperovitch A, Zerr I, Pocchiari M, Mitrova E, Cuesta JDP, Hegyi I, Collins S, Kretzschmar H, van Duijn C, Will RG. Codon 129 prion protein genotype and sporadic Creutzfeldt-Jakob disease. Lancet 1999;353:1673-4.
21. Ghani AC, Donnelly CA, Ferguson NM, Anderson RM. Updated projections of future vCJD deaths in the UK. BMC Infect Dis 2003;3:4.
22. Turner ML, Ludlam CA. An update on the assessment and management of the risk of transmission of variant Creutzfeldt-Jakob disease by blood and plasma products. Br J Haematol 2009;144:14-23.
23. Boelle PY, Cesbron JY, Valleron AJ. Epidemiological evidence of higher susceptibility to vCJD in the young. BMC Infect Dis 2004;4:26.
24. Garske T, Ghani AC. *Uncertainty in the tail of the variant* Creutzfeldt-Jakob disease epidemic in the UK. PLoS ONE 2010;5:e15626.
25. Gregori L, Lambert BC, Gurgel PV, Gheorghiu L, Edwardson P, Lathrop JT, Macauley C, Carbonell RG, Burton SJ, Hammond D, Rohwer RG. Reduction of transmissible spongiform encephalopathy infectivity from human red blood cells with prion protein affinity ligands. Transfusion 2006;46:1152-61.
26. Knight R. The diagnosis of prion diseases. Parasitology 1998;117(Suppl):S3-11.
27. R Development Core Team. R: a language and environment for statistical computing [Internet]. Vienna. 2010. [cited 2010 Oct 5]. Available from: URL: http://www.R-project.org
28. Borkent-Raven BA, Janssen MP, van der Poel CL, de Wit GA, Bonsel GJ, van Hout BA. Cost-effectiveness of additional hepatitis B virus nucleic acid testing of individual donations or minipools of six donations in the Netherlands. Transfusion 2009;49:311-9.
29. Jackson BR, Busch MP, Stramer SL, AuBuchon JP. The cost-effectiveness of NAT for HIV, HCV, and HBV in whole-blood donations. Transfusion 2003;43:721-9.
30. Korves CT, Goldie SJ, Murray MB. Cost-effectiveness of alternative blood-screening strategies for West Nile virus in the United States. PLoS Med 2006;3:e21.
31. Marshall DA, Kleinman SH, Wong JB, AuBuchon JP, Grima DT, Kulin NA, Weinstein MC. Cost-effectiveness of nucleic acid test screening of volunteer blood donations for hepatitis B, hepatitis C and human immunodeficiency virus in the United States. Vox Sang 2004;86:28-40.
32. Pereira A. Health and economic impact of posttransfusion hepatitis B and cost-effectiveness analysis of expanded HBV testing protocols of blood donors: a study focused on the European Union. Transfusion 2003;43:192-201.
33. van Hulst M, de Wolf JT, Staginnus U, Ruitenberg EJ, Postma MJ. Pharmaco-economics of blood transfusion safety: review of the available evidence. Vox Sang 2002;83:146-55.
34. Harney MS, Ghani AC, Donnelly CA, Walsh RM, Walsh M, Howley R, Brett F, Farrell M. vCJD risk in the Republic of Ireland. BMC Infect Dis 2003;3:28.
35. Gregori L, Yang H, Anderson S. Estimation of variant Creutzfeldt-Jakob disease infectivity titers in human blood. Transfusion 2011;51:2596-602.