薬事・食品衛生審議会

平成２４年度　第１回　血液事業部会運営委員会

議 事 次 第

日時：平成２４年５月２８日（月）
１０：００～１２：００
場所：弘済会館４階（菊の間）
東京都千代田区麹町５－１

議題：

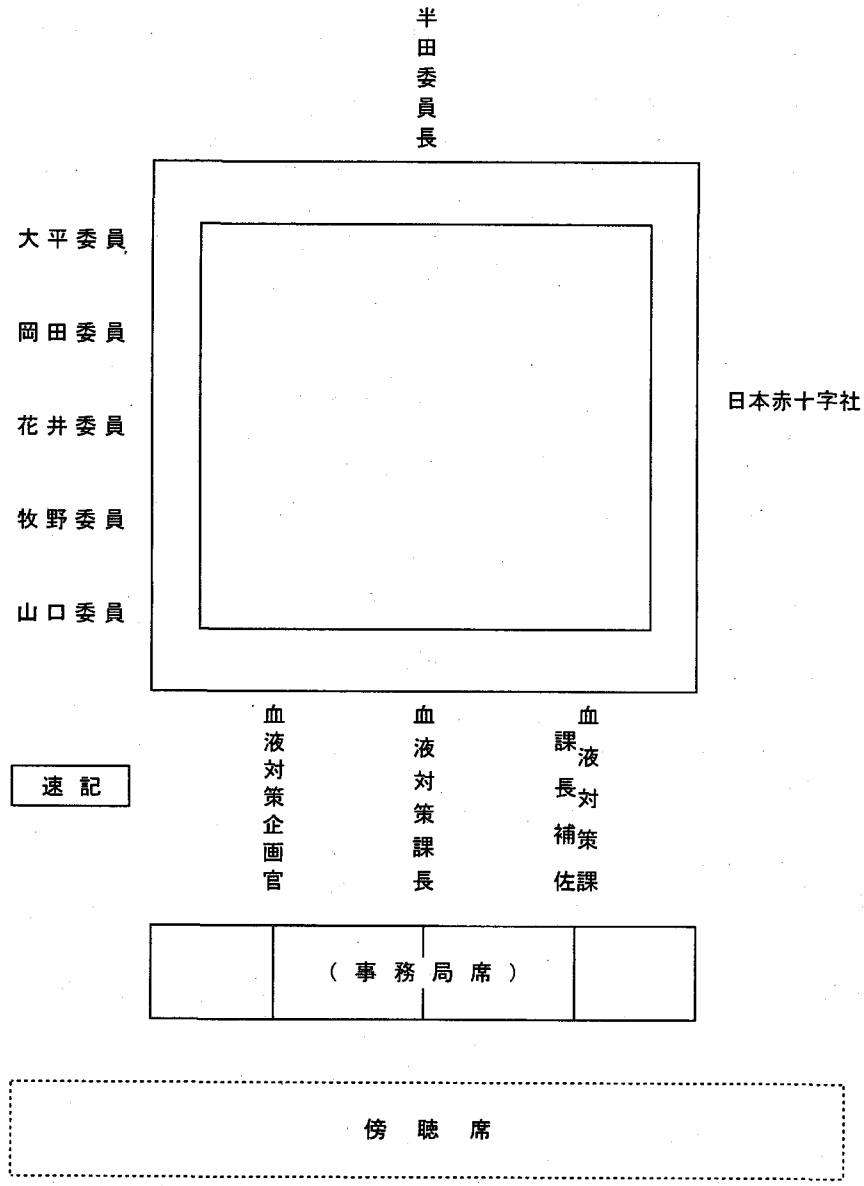
1.議事要旨の確認
2.感染症定期報告について
3.血液製剤に関する報告事項について
4.日本赤十字社からの報告事項について
5.日本赤十字社と田辺三菱製薬株式会社の血漿分画製剤の統合について（非公開）
6.その他

配付資料：

座席表
委員名簿
資　料　１　平成２３第４回血液事業部会運営委員会議事要旨（案）
資　料　２　感染症定期報告について
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　HBV 既往感染の血液に対する更なる安全対策の向上について（日本赤十字社提出資料）
資　料　５　欧州における病原体不活化技術の現状視察について
資　料　６　抗 HBs 人免疫グロブリン製剤の国内自給に係る原料血漿収集のためのＢ型肝炎ワクチン接種プログラムに関するガイドラインについて
資　料　７　「血液製剤の安全性の向上及び安定供給の確保に関する基本的な方針」の改訂スケジュールについて
資　料　８　日本赤十字社と田辺三菱製薬株式会社の血漿分画事業の統合について（平成 24 年 5 月 8 日公表資料）
資　料　９　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２４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24年5月28日(月)
弘済会館
4階 菊の間
10:00 ～ 12:00



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．花井 十伍 （はない　じゅうご）
　　ネットワーク医療と人権理事

4．半田 誠 （はんだ　まこと）
　　慶應義塾大学医学部輸血・細胞療法部教授

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23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23年3月14日（水）　14:00～16:00
場所：　厚生労働省12階　専用第15・16会議室
出席者：
（委員）
半田委員長、大平、岡田、花井、牧野、山口各委員
（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
（バクスター株式会社）
久保田シニアグループマネージャー
（事務局）
三宅血液対策課長、丈達血液対策企画官、伯野課長補佐

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. 日本赤十字社からの報告事項について
5. その他

（審議概要）

<u>議題1について</u>

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。

<u>議題2について</u>

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。

<u>議題3について</u>

事務局及び日赤から、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。

<u>議題4について</u>

（血小板製剤の不活化技術）

日赤より、血小板製剤に対する不活化技術（MIRASOL）導入の準備状況について報告がなされた。

委員より、MIRASOLのメーカーから得た情報ではなく、日赤が自ら調査・収集した情報や、国や第三者機関が収集した公平性のある情報を提示して欲しいとの意見や、血小板不活化技術の導入にあたっては、将来の献血者不足や大地震などの不測の事態に備え、成分由来血小板だけでなく、全血由来血小板にも対応できるよう準備して欲し

いとの意見が出された。

議題5について

（アルブミン関連）

バクスター社より、海外のアルブミン製造工場の培地充填試験で陽性反応が認められたことから、アルブミン製剤の出荷を停止するととともに、品質・安全性への影響について当局と検討中であることが報告された。また、今後の供給に支障をきたす見込みであるため、医療機関等に代替製品への切り替えを依頼したことが報告された。

バクスター社においては、引き続き、早期の供給再開に向けて努めることとされた。

（XMRV 関係）

岡田委員より、XMRV に関する最新文献の報告がなさ、いずれの文献も前立腺がんや慢性疲労症候群とXMRVとの因果関係を否定するものであった。因果関係に関して否定的なエビデンスが蓄積されつつあるが、事務局においては、引き続き、重要な文献報告がなされた場合には適宜報告することとされた。

（フィブリノゲン関係）

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。

以上

資料2

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。

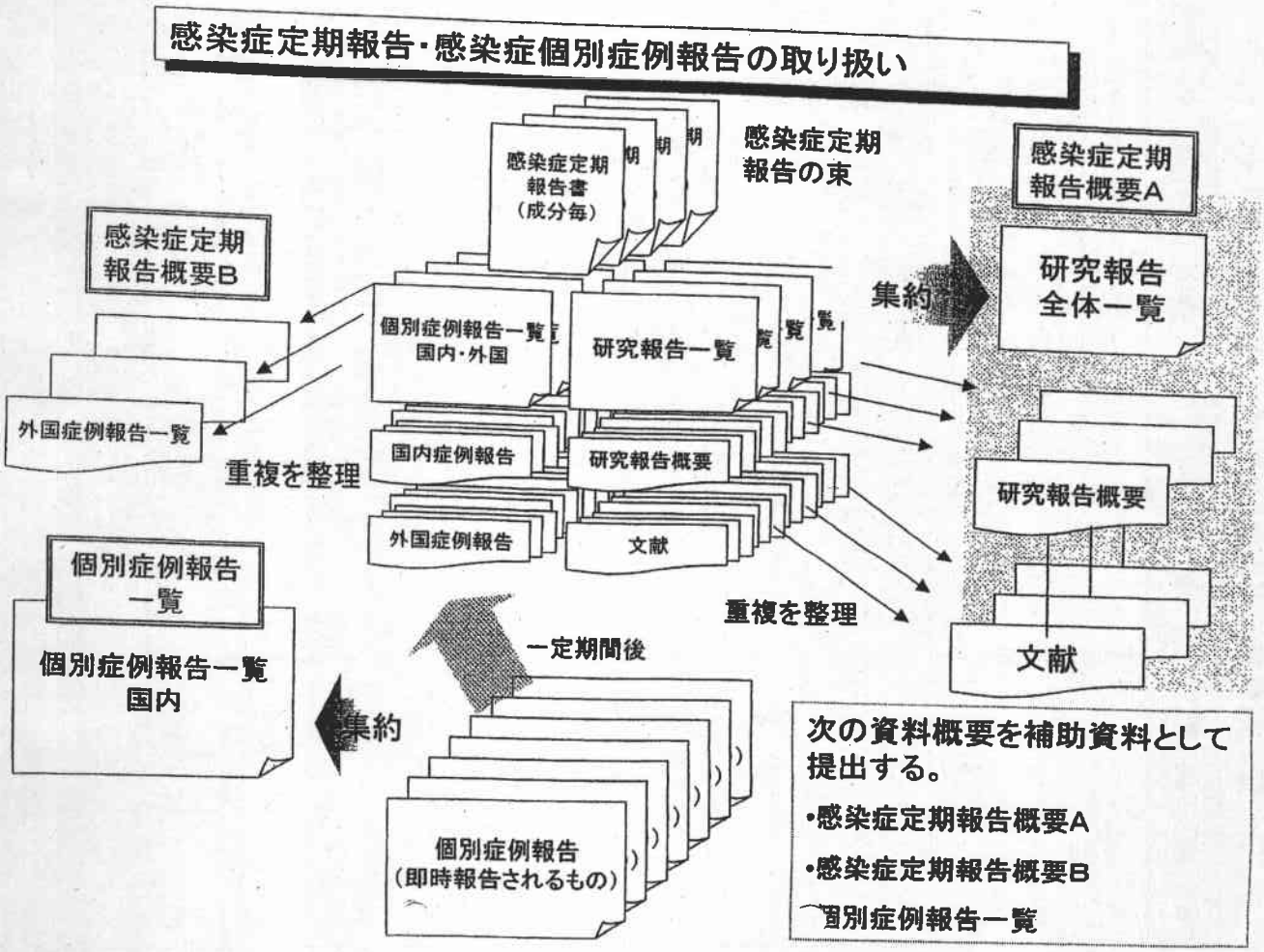
① 研究報告は、同一文献による重複を廃した別紙のような形式の一覧表を作成し、当該一覧表に代表的なものの報告様式（別紙様式第2）及び該当文献を添付した**「資料概要A」**を事務局が作成し、送付する。

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。



感染症定期報告概要

（平成２４年５月２８日）

平成２４年２月１日～３月３１日受理分

A　研究報告概要

B　個別症例報告概要

Ａ　研究報告概要

○　一覧表（感染症種類毎）
○　感染症毎の主要研究報告概要
○　研究報告写

研究報告のまとめ方について

１　平成２４年２月１日～３月３１日までに報告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　一覧表においては、前回の運営委員会において報告したもの以降の研究報告について、一覧表の後に当該感染症の主要研究報告の内容を添付した。

感染症定期報告の報告状況（2012/2/1～2012/3/31）

ID	受理日	番号	感染症（PT）	出典	概要	新出文献番号
100510	29-Mar-12	111075	C型肝炎	Ann Intern Med. 156(2012)263-270	C型肝炎ウイルス（HCV）スクリーニングの費用対効果に関する報告。米国において、最も感染者が多いとされる1945～65年生まれの人々に絞って積極的にHCVスクリーニングを行う場合の費用対効果について、コンピュータモデルにより解析を行った。その結果、スクリーニングを実施すればHCV持続感染者を新たに約80万人検出でき、検出した感染者に標準治療（ペグインターフェロンとリバビリン）を行うと質調整生存年（QALY）当たりの費用15,700ドルで死亡を約8万人減らせると推算された。また、遺伝子型2、3型の患者には標準治療を行い、1型の患者には標準治療と直接作用型抗ウイルス薬（DAA）を併用するとQALY当たりの費用35,000ドルで死亡が約12万人減少すると推算された。	1
100509	29-Mar-12	111073	HIV	IASR. 32(2011)290-292	日本の献血におけるHIVの検査状況の概略。献血血液のHIV抗体検査は1986年から開始され、2004年より20本プールに対して核酸増幅検査（NAT）を行っている。また、ウィンドウ期の献血を防止するために、問診票での質問を設けている。国内のHIV陽性献血者数は2009年102件、2010年86件であった。男女別ではこの5年間で男性が96%を占めている。都道府県別では東京、大阪が他と比べ高い。年齢群別では10～30代の若い世代が80%前後を占めている。2010年のHIV陽性血液は全てHIV-1であり、サブタイプ別内訳はBが77件（90%）、その他組換え流行株のCRF01_AE が6件、CRF01_AE/Bが 1件、CRF11_cpx が1件及びNAT陰性が1件であった。輸血によるHIV感染が確認されているのは1997年～2003年の合計4例であり、2004年の20本プールNAT実施以降、輸血感染事例は起きていない。	2
100509	30-Mar-12	111073	HIV	Transfusion. 52(2012)431-439	核酸増幅検査（NAT）によるHIV-1の検出に関する報告。欧州においてHIV-1 RNA陽性供血血液5例が3つの異なるNATで偽陰性となったことの原因調査のために、当該検体について異なる設計のHIV-1 NATシステム12種類を用いて比較調査した。各アッセイでHIV-1変異の相対的な増幅効率を調査し、偽陰性NATアッセイの対象領域の配列の変異によってプライマーとプローブの比較を行った。その結果、偽陰性結果のモノターゲットNATと同様の方法で設計された他のNATアッセイにも、ウイルス変異の検出において欠陥が見られた。それぞれの例において、増幅対象領域の配列が変異し、アッセイに用いるプライマー及びプローブとの不一致を起こした。デュアルターゲットアッセイでは増幅効果が減少したものがあったが、偽陰性結果は示さなかった。これらの結果より、モノターゲット領域のNATスクリーニングアッセイはデュアルターゲットアッセイよりも配列の変異に対してより脆弱であることが示唆された。	3
100514	10-Apr-12	111079	A型肝炎	Transfusion. 52(2012)181-187	A型肝炎ウイルス（HAV）の不活化に対する変異株の影響に関する報告。米国における人血清アルブミン（HSA）の低温殺菌について、各製造販売業者でHAVの不活化能力に最大3.9Logの相違があることが報告されていることを受けて、HAV変異株の低温殺菌への感受性に関して実験的に評価した。HAV HM175株の4種の細胞変性変異株が5%又は25%のHSAにスパイクされ、58±1℃、600±10分の条件でウイルス不活化が評価された。その結果、4つの変異体はウイルス減少率に有意な差がある2つのサブグループに分けられた。また、5%HSAよりも25%HSAにおいてウイルス減少率が高かった。この結果より、HAVの変異型とHSAのタンパク濃度が低温殺菌によるHAV不活化に影響を及ぼすことが示唆された。	4
100514	11-Apr-12	111079	E型肝炎	Vox Sanguinis Letter. 6 Jan, 2012	スウェーデン、ドイツ及び米国における献血血漿中のE型肝炎ウイルス（HEV）に関する報告。リアルタイムRT-PCR法を用いて、スウェーデン、ドイツ及び米国由来の献血血漿中のHEV-RNAについて調査された。その結果スウェーデン由来血漿においては95,835例中12例、ドイツ由来においては18,100例中4例が陽性であったが、米国由来においては51,075例中で陽性例が確認されなかった。HEV株の分析の結果、陽性となった全ての症例が遺伝子型3であることが示された。また、陽性検体の多くはウィンドウ期の献血であった。	5

ID	受理日	番号	感染症(PT)	出典	概要	新出文献番号
100517	28-Mar-12	111082	E型肝炎	Arch Virol. 156(2011)1989-1995	中国のブタとヒトにおけるE型肝炎ウイルス(HEV)の陽性率に関する報告。中国雲南省の養豚場と屠殺場から収集されたブタの血清、肝臓及び糞便検体について、血清学的検査とRT-PCRによりHEVの陽性率が調査された。また、加えて173例のヒト血清についても調査された。その結果、ブタ血清621例中490例(78.9%)が抗HEV抗体陽性であった。ヒト血清については173例中69例(39.9%)が陽性であった。HEV RNAは肝臓95例中6例、糞便60例中5例において検出され、ヒト血清では1例において検出された。12例のHEV株の配列分析により、9つの異なる塩基配列が確認され、これらは全てHEV遺伝子型4に分類されることが推測された。このことから、豊南省のブタとヒトにおいてHEV遺伝子型4が広まっていることが示唆された。	6
100514	12-Apr-12	111079	ウエストナイルウイルス感染	Transfusion. 52(2012)447-454	ウエストナイルウイルス(WNV)感染患者における血漿及び全血中ウイルス濃度に関する報告。米国において、WNVの個別NATを実施しているにも関わらず輸血感染症例が報告されたことを受けて、血漿及び全血検体に対するNAT検査の有用性を確認するため、WNV感染患者における血漿及び全血中のWNV-RNAをリアルタイムPCR法を用いて評価した。その結果、血清中抗WNV抗体陰性の患者29例においては全血より血漿中でWNV-RNAの濃度が4倍以上高かったが、抗WNV抗体陽性の13例においては血漿中より全血において10倍以上高かった。また、10例の抗WNV抗体陽性の患者について200日間の追跡調査を行ったところ、全血のウイルス量が血漿中より常に高かった。これらの結果より、血漿の代わりに全血を用いることによるWNV NATの感度向上は血清抗体陽性段階に限定されることが示唆された。	7
100509	08-Apr-12	111073	HTLV	J Med Viol. 84(2012)327-335	国内のHTLV-1感染率に関する報告。2006～2007年に、国内の初回献血者1,196,321例におけるHTLV-1感染率が調査され、そのうち3,787例が抗HTLV-1抗体陽性であると確認された。献血者以外の年齢層に適合曲線を適用したところ、国内の0歳～99歳における現在のHTLV-1キャリア数は108万人と推定された。これは1988年に報告された値よりも10%少なかった。調整後の全体的な感染率は男性、女性それぞれ0.66%及び1.02%と推定された。キャリア数のピークは70歳代で見られ、これは1988年のデータベースで50歳代に観察されたピークがシフトしたものである。キャリアは元々多いと言われている九州だけでなく日本全土、特に首都圏で増加していた。	8
100510	30-Mar-12	111075	レトロウイルス(XMRV)	HPS Weekly Report. 5 Jan, 2012	供血における異種指向性マウス白血病ウイルス関連ウイルス(XMRV)の影響に関する評価の報告。欧州委員会からの要請を受けて、2011年7月に欧州疾病対策センターは供血におけるXMRVの影響についてリスクアセスメントを発表した。現在得られている証拠の大半はXMRVと慢性疲労症候群(CFS)との間には因果関係がないという結論を支持している。現在、ヒトにおけるウイルスの潜在的な役割を示す十分な証拠は得られていないものの、輸血の安全性に影響を及ぼす別の新興感染因子である可能性はあり、他の因子と同様に最善の科学に基づいた迅速かつ効果的な行動が不可欠である。	9
100509	02-Apr-12	111073	インフルエンザ	http://abcnews.go.com/blogs/health/2011/11/25/new-swine-flu-strain/	新規ブタインフルエンザの発生報告。2011年7月以降10例の米国人がS-OtrH3N2ウイルスに感染した。このウイルスは北米で循環しているブタインフルエンザH3N2ウイルスと、2009年に流行したH1N1ウイルスが結合した新しいウイルスである。人々は免疫をまだ獲得していないため、健康へのリスクとなり得る。以前の7症例は全て、患者か患者の身近な人がブタに接触していたが、最新の3症例ではブタに接触した者はおらず、3人の子供が同じ保育園の集会に参加していたことから、ヒトとヒトの接触がウイルス感染に関連していると示唆された。	10

ID	受理日	番号	感染症(PT)	出典	概要	新出文献番号
100509	06-Apr-12	111073	デング熱	ProMED-mail 20111114.3364	世界各地における2011年11月時点のデングウイルス感染状況の報告。 パキスタン: パンジャーブ州のデング熱患者は計3,678例に上り、うち2,498例はラホール市の住民である。 インド: デリーにおけるデング熱患者数は945例に達するが、症例数は徐々に減少している。 台湾: 2011年6月以降、高雄市において10例のデング熱症例が報告され、うち8例が55歳以上である。 韓国: 韓国疾病管理予防センターの発表によると、2011年7月にデングウイルス感染と診断された32歳女性は、韓国国内で感染した可能性があることが判明した。 ブラジル: 2011年の9カ月間で70万症例以上のデング熱症例が報告されているが、1～2月の報告数は2010年に比べ24%減少し、死亡例も25%、重篤例も40%減少した。	11
100495	08-Feb-12	110892	ウイルス感染	Vector-Borne and Zoonotic Diseases. 12(2011)156-160	血小板減少を伴う発熱疾患(SFTS)の原因となる新規ブニヤウイルス(SFTSV)のヒト間感染に関する報告。中国において2006年に未知の感染症に罹患した2つのクラスターの患者13例の血清を用いて、RT-PCR法によるSFTSV-RNAの検出及び間接蛍光抗体法による抗SFTSV抗体検査を実施した。その結果、13例中12例においてSFTSV-RNAの検出又は抗SFTSV抗体力価の増加、セロコンバージョンのいずれかが認められた。残りの1例はサンプル量の不足により分析されなかった。また、対象患者全員に典型的なSFTSの臨床症状(発熱、血小板減少、白血球減少)があり、両クラスターの二次患者全員が一次患者の血液に接触後6～13日で発病していた。それぞれの一次患者と接触したが血液への曝露がなかった人においては発病した者がいなかった。このことから、SFTSVは血液との接触を通じてヒトからヒトへ感染することが示唆された。	12
100495	09-Feb-12	110892	ウイルス感染	Clin Infect Dis. 53(2011)1208-1214	血小板減少を伴う発熱疾患の原因となる新規ブニヤウイルス(SFTSV)のヒト間感染に関する報告。中国東部において2007年に血小板減少を伴う発熱疾患を発症した1家族の7例に対し疫学的調査を行い、そのうち二次患者6例において血清検体の解析を行った。その結果、一次患者が発病した6～9日後に家族であった6例が類似症状により入院しており、二次患者はいずれも一次患者と個人的に接触していた。また、6例共にRT-PCR法によりSFTSV-RNA陽性であり、抗SFTSV抗体も陽性であった。また、1例の検体よりSFTSVが分離された。これらの結果より、家族内で生じた疾患がSFTSVにより引き起こされたこと、接触により他者へ感染が広がったことが示唆された。	13
100509	04-Apr-12	111082	ウイルス感染	Vox Sang. 102(2012)82-90	核酸増幅技術(NAT)に関する国際調査の報告。国際輸血学会(ISBT)により、eメールのアンケートを用いて、全血とアフェレーシス供血におけるNATに関して国際調査が行われた。2009年8月に59カ国77人の専門家に送付され、37カ国50人が回答した。網羅される人口は計12億人であり、2008年にNATを実行したと報告した国の人口は計11億6千万人であった。2008年のNAT検査状況は以下の通り。 HIV-1: 3,350万人中2,189供血がHIV-1 NAT陽性であった。陽性結果の約2/3は南アフリカからであった。 HCV: 2,660万人中4,586供血がHCV NAT陽性であった。初回供血者陽性率が最も高い国はエストニアであった。 HBV: 902万人中3,081供血がHBV NAT陽性であった。ギリシャ及びマレーシアの陽性供血数は合計1,517件であり高かった。 この調査は、供血者の感染症スクリーニングに関してこれまで公表された中で最も広範な研究である。	14

ID	受理日	番号	感染症(PT)	出典	概要	新出文献番号
100509	05-Apr-12	111083	ウイルス感染	Transfusion. 51(2011)2820-2626	白血球除去血液製剤によるサイトメガロウイルス(CMV)感染に関する報告。1999～2009年に抗CMV抗体検査未実施の白血球除去血液製剤を使用した造血幹細胞移植(HSCT)患者を対象に、輸血関連CMV感染(TT-CMV)の有無についてプロスペクティブに調査した。その結果、CMV抗体陰性のHSCTを受けた23患者に対し、3,180供血者由来の1,847の血液製剤が輸血されたことが確認された。全ての患者はCMV DNAが陰性であったが、17患者に抗CMV IgG抗体の陽転化が起こった。陽転群は非陽転群に比べて1週間当たりの輸血量が顕著に多かった。このことから、HSCT患者における、抗CMV抗体検査未実施の白血球除去血液製剤によるTT-CMVのリスクは低く、抗CMV IgG抗体陽転化の原因は血液製剤中の移行抗体である可能性が最も高いことが示唆された。	15
					ウシ肝臓における腸管出血性大腸菌O-157に関する報告。厚生労働省による全国16自治体の食肉衛生検査所の調査により、ウシの肝臓内部からO-157が初めて確認されたことが分かった。約150頭のウシのうち2頭の肝臓内部からO-157が検出された。厚労省は生レバーの提供を禁止するかどうか検討している。	16
100508	22-Mar-12	111014	大腸菌性胃腸炎	共同通信(2011年12月15日配信)		
100508	23-Mar-12	111015	大腸菌性胃腸炎	薬事・食品衛生審議会食品衛生分科会乳肉水産食品部会資料(2011年12月20日開催)	ウシ肝臓における腸管出血性大腸菌O-157に関する報告。厚生労働省による全国16自治体の食肉衛生検査所の調査により、ウシの肝臓内部からO-157が初めて確認されたことが分かった。約150頭のウシのうち2頭の肝臓内部からO-157が検出された。厚労省は生レバーの提供を禁止するかどうか検討している。	17
100514	09-Apr-12	111091	梅毒	Medical News Today Nov 17, 2011	米国における梅毒発生状況の報告。疾病管理予防センター(CDC)の報告によると、2006年から2010年まで、米国における梅毒の症例数は36%増加した。特に、若いアフリカ系男性においては135%増加していた。CDCは、同性間性交渉のある男性は3カ月に一度STDのためのスクリーニング検査を受けるべきと勧告している。	18
100509	09-Apr-12	111087	異型クロイツフェルト・ヤコブ病	ProMED-mail 20120104.0027	各国における2012年1月時点でのプリオン疾患発生状況の報告。 英国:国立CJDサーベイランス研究所によると、2012年1月4日現在の異型クロイツフェルト・ヤコブ病(vCJD)確定または疑い症例の合計は176例である(生存0例/死亡176例)。全てのCJDについては、2011年の1年間で148例報告され、死亡者数は、弧発性CJDで74例、ゲルストマン・ストロイスラー・シャインカー病(GSS)で2例、家族性CJDで9例、vCJDで5例。医原性CJDで3例であった。 韓国:2011年11～12月、第1、2症例目の医原性CJDが報告された。韓国疾病管理予防センターによると、これらの患者はそれぞれ1987年と1988年に脳外科手術を受け、その際、ドイツで生産された人工硬膜であるLyodura®が使用されていた。	19
100510	31-Mar-12	111075	その他	Molecular Psychiatry advance online publication 4 Oct. 2011	アルツハイマー病(AD)のプリオン様伝播に関する報告。アミロイドベータ(Aβ)の蓄積がプリオン様に伝播するかを検討するために、マウスを用いてAD患者又は若年者由来の脳抽出液を脳内に接種して調査を行った。接種後脳内を観察したところ、AD脳抽出液を接種したマウスでは脳内にAβの凝集体が見られ、接種後の時間が経過するとともに増加していた。また、接種部位から離れた領域で病変が認められたが、対照マウスではAβ沈着がみられなかった。この結果より、ADに関連する脳異常の一部はプリオン様の疾病伝播メカニズムによって引き起こされている可能性が示唆された。	20

別紙様式第２－１

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	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	http://www.annals.org/content/early/2011/11/03/0003-4819-156-4-201202210-00378.full	公表国 米国	
販売名(企業名)	－					

研究報告の概要	使用上の注意記載状況・その他参考事項等
C型肝炎ウイルススクリーニングの対象を出生年により絞り込むことで、スクリーニングの費用対効果が高まる。米国では人口の約1.5%がHCVに感染しており、感染者が最も多いのは1945～65年生まれの人々で、感染者の50～75%は感染に気付いていない潜在的な感染者とされる。2005年のHCV感染による死亡は13,000人と推定され、現在の検出法や治療法を改善しない限り、2030年には35,000人に達すると予測される。 1945～65年生まれの人々に対し積極的にHCVスクリーニングを実施する場合の費用対効果について検討するため、 ①スクリーニングも治療も行わない ②現在行われているリスクに基づくスクリーニングと標準治療(ペグインターフェロン＋リバビリン)を行う ③45～65年生まれの人々を対象としたスクリーニングと標準治療を行う ④45～65年生まれの人々を対象としたスクリーニングを行い、HCV遺伝子2型と3型の患者には標準治療を行い、米国で最も罹患率の高い1型の患者には標準治療に加えて直接作用型抗ウイルス薬(DAA)を併用する との4つのシナリオを設定し、コンピュータモデルで解析した。その結果、45～65年生まれの人々を対象としたスクリーニングを実施すればHCV持続感染者を新たに約80万人検出でき、③のシナリオでは質調整生存年(QALY)1年あたりの費用15,700ドルで死亡を約8万人、④では質調整生存年(QALY)1年あたりの費用35,000ドルで約12万人死亡を減らせると推算された。 出生年で対象を絞った費用対効果は、乳ガン検診や大腸癌検診などルーチンで実施されている他の多くの予防法と同等であることが示された。	

報告企業の意見	今後の対応
HCVスクリーニングを感染者の最も多い1945～65年生まれの人々に絞り込んで行うことで、ルーチンで実施されている他の予防法と同等の費用対効果が得られるとの報告である。	今後ともHCVに関する情報に留意していく。

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Original Research

The Cost-Effectiveness of Birth-Cohort Screening for Hepatitis C Antibody in U.S. Primary Care Settings

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Abstract

Background: In the United States, hepatitis C virus (HCV) infection is most prevalent among adults born from 1945 through 1965, and approximately 50% to 75% of infected adults are unaware of their infections.

Objective: To estimate the cost-effectiveness of birth-cohort screening.

Design: Cost-effectiveness simulation.

Data Sources: National Health and Nutrition Examination Survey, U.S. Census, Medicare reimbursement schedule, published sources.

Target Population: Adults born from 1945 through 1965 with 1 or more visits to a primary care provider annually.

Time Horizon: Lifetime.

Perspective: Societal, health care.

Interventions: One-time antibody test of 1945–1965 birth cohort.

Outcome Measures: Numbers of cases that were identified and treated and that achieved a sustained viral response; liver disease and death from HCV; medical and productivity costs; quality-adjusted life-years (QALYs); incremental cost-effectiveness ratio (ICER).

Results of Base-Case Analysis: Compared with the status quo, birth-cohort screening identified 808 580 additional cases of chronic HCV infection at a

screening cost of $2874 per case identified. Assuming that birth-cohort screening was followed by pegylated interferon (pegIFN+R) and ribavirin for treated patients, screening increased QALYs by 348 800 and costs by $5.5 billion, for an ICER of $15 700 per QALY gained. Assuming that birth-cohort screening was followed by a direct-acting antiviral, pegIFN+R treatment for treated patients, screening increased QALYs by 532 200 and costs by $19.0 billion, for an ICER of $35 700 per QALY saved.

Results of Sensitivity Analysis: The ICER of birth-cohort screening was most sensitive to sustained viral response rate of antiviral therapy, the cost of therapy, the discount rate and the QALY losses assigned to disease states.

Limitation: Empirical data on screening and direct-acting antiviral treatment in real-world clinical settings are scarce.

Conclusion: Birth-cohort screening for HCV in primary care settings was cost-effective.

Primary Funding Source: Division of Viral Hepatitis, Centers for Disease Control and Prevention.

Editors' Notes

Context

- Most people in the United States infected with hepatitis C virus (HCV) were born from 1945 through 1965 and are currently undiagnosed. Because complications of hepatitis C increase with time, its burden is now rapidly increasing.

Contribution

- In simulated models, an approach of one-time screening for hepatitis C in this birth cohort followed by treatment with currently available therapies was cost-effective.

Caution

- Data on the real-world effectiveness of newer drugs for hepatitis C is extremely limited.

Implication

- A change from solely risk-based screening for hepatitis C to one-time screening of all "baby boomers" should be considered.

Approximately 4.1 million Americans are antibody-positive for hepatitis C virus (HCV), and approximately 75% of these are chronically infected; most of the latter were infected 20 to 40 years ago, before the discovery of HCV (1). In 2005, hepatitis C resulted in 7000 to 13 000 deaths (2–7). Because HCV progresses slowly, the risk for serious complications is increasing among infected Americans as time passes (8). Without changes in current case identification and treatment, deaths from HCV are forecasted to increase to 35 000 annually by 2030 (5).

In clinical trials, antiviral therapy with pegylated interferon and ribavirin (pegIFN+R) has resulted in a sustained viral response (SVR; that is, cure) of HCV infection in 46% of patients infected with genotype 1 (which infects 70% and 90% of chronically infected white and African-American persons in the United States, respectively) and as many as 81% of those infected with genotypes 2 or 3 (9, 10). PegIFN+R treatment is cost-effective at these rates of efficacy (11).

The Centers for Disease Control and Prevention currently recommends antibody screening of individuals with past behaviors, exposures, or health indicators associated with HCV infection, such as a history of injection-drug use, hemodialysis, or elevated alanine aminotransferase levels (12). Despite these recommendations, only 25% to 50% of patients with chronic hepatitis C are aware of their infections (12–16). Low case identification may result from difficulty in implementing risk-based screening given the limited time of primary care visits and the awkwardness of discussing behavioral risks.

Expanding screening recommendations to cover the birth cohort born from 1945 through 1965 (among whom HCV prevalence is highest) offers a potential complement to current risk-based screening recommendations. However, although birth-cohort screening would increase healthcare costs by increasing the number of individuals screened, the extent to which it would translate into health benefits is unknown. Currently, many diagnosed patients forgo treatment because of contraindications, inability to pay, lack of specialist access, or personal choice (17–20). Further, the effectiveness of antiviral therapy in community settings is lower than in clinical trials (17, 21–23).

In this paper, we used a previously validated simulation model to estimate the cost-effectiveness of birth-cohort screening for HCV in the United States (6). Our results can be used to inform ongoing discussions about the suitability of a birth-cohort screening strategy as policy recommendation.

Methods

Decision Analytic Model

We programmed (Microsoft Visual Studio 2008, Redmond, Washington) a Markov chain Monte Carlo simulation model of the prevalence of hepatitis C antibody stratified by age, sex, race/ethnicity, and history of injection-drug use and of the natural history of chronic hepatitis C. A more thorough description of the disease components of the model is provided elsewhere (5). Briefly, we modeled chronic HCV infection based on Meta-Analysis of Histologic Data in Viral Hepatitis (METAVIR) scale units (24). We stratified annual disease progression by age at infection, sex, and alcohol consumption history and determined disease progression at model initiation by using historical HCV incidence data and published observations of annual progression in METAVIR units (Table 1) (2, 25). Patients who progressed to a METAVIR score of 4 were classified as having cirrhosis and experienced subsequent annual probability of 0.039 for progressing to decompensated cirrhosis (DCC) and a probability of 0.025 for developing hepatocellular carcinoma (HCC) (3, 26). Patients with DCC or HCC experienced an annual probability of transplantation or death (27–29). Data reported in Table 1 were obtained from multiple sources (30–48).

Model Cohorts

We modeled the U.S. population that was born from 1945 through 1965 and had at least 1 primary care visit in 2006. Using data from the National Health and Nutrition Examination Survey (NHANES, for 2001 through 2006), we divided this population into 40 mutually exclusive groups stratified by age group, race/ethnicity, history of injection-drug use, and prescription drug coverage (49). We further stratified these cohorts into those with and those without antibody to HCV and divided those with antibodies into those with chronic (75%) and those with cleared (25%) infections (8, 49). We estimated that 28% of chronically infected patients were already aware of their infection and would not benefit from additional screening (13–16).

Background Mortality

We used census life tables to calculate the annual probability of mortality from nonhepatic causes (50). We multiplied this background mortality probability by 1.42 for people age 40 years or older who reported ever injecting drugs. The relative risk for death was equal to a weighted average of the relative risk for death of inactive users (assumed to be 1.00) and the relative risk among active users; the relative risk was weighted by the proportion of people who admitted ever injecting drugs in the NHANES who did and did not report use within the past 12 months (49, 51).

Screening and Treatment Scenarios

We simulated 4 scenarios: 1) no screening or treatment; 2) risk-based screening, in which 18.5% (1% per year over the next 20 years) of individuals unaware of their chronic infection were screened and offered pegIFN+R treatment if identified; 3) birth-cohort screening in which all people born from 1945 through 1965 and unaware of their HCV antibody status were offered one-time HCV antibody screening during their 2006 primary care visit, then offered pegIFN+R treatment if identified; and 4) an identical birth-cohort screening scenario in which a) patients with genotype 1 disease who initiated treatment received direct-acting antiviral treatment (DAA) in addition to standard therapy and b) patients with genotypes 2 and 3 received pegIFN+R. Screening occurred once to identify prevalent cases. We did not consider repeated screenings because birth-cohort screening is not a useful strategy to prevent HCV incidence.

Screening, Contraindication, and Antiviral Initiation

We assumed that 91% of those offered screening would accept it, that 90% of those who tested positive would receive those results, and that all patients with prescription drug insurance coverage (87.6%) and no patients without prescription coverage would be considered for treatment (30, 49). We estimated that 23.1% of patients considered for treatment were contraindicated for modifiable reasons (such as substance abuse or major depression), 11.5% were contraindicated for nonmodifiable reasons (such as uncontrolled diabetes or autoimmune disorders), and 8.5% declined treatment (20, 31). After adjustment for these barriers, 40.8% of positive patients offered testing accepted, were identified, and initiated treatment.

Effectiveness of Antiviral Therapy

We set SVR rates for standard therapy to the average of that reported in 4 studies of antiviral therapy administered in primary care settings, yielding an SVR rate of 0.33 for genotypes 1/4 and 0.69 for genotypes 2/3 (17, 22, 43, 52). We set the SVR rate for DAA plus standard therapy to 0.54, a value equal to the ratio of the average SVR rate of standard therapy (0.33) divided by the SVR of standard therapy observed in clinical trials (0.46) multiplied by the SVR rate observed for 12-week DAA plus pegIFN+R in clinical trial data (0.75) (32).

Other Treatment

Diagnosed patients with insurance who did not undergo antiviral therapy or achieve an SVR received clinical management described in the *Cleveland Clinic Monograph* (39) or the American Association for the Study of Liver Diseases guidelines (40). Patients who achieved an SVR also received care in subsequent years. Clinical management other than antiviral therapy increased costs but did not result in any modeled benefit.

Medical Costs

We estimated screening costs from data provided by a federally qualified health center that conducted routine hepatitis B screening of at-risk patients, replacing the reimbursement costs for hepatitis B antigen testing with the costs of a hepatitis C antibody testing (38). We estimated the costs of standard antiviral therapy as the sum of the average monthly cost of pegIFN+R observed in the Kaiser Health System of Georgia in 2009 multiplied by the estimated months of therapeutic adherence observed in the control group of a published therapy-discontinuation study (43, 53). To these costs, we added the estimated monthly outpatient and laboratory costs of treatment as outlined in the *Cleveland Clinic Monograph* (39). We estimated the costs of adding DAA to standard treatment based on costs and response-based treatment algorithms obtained through personal communication (see the technical report available at www.norc.org/PDFs/Cost-effectiveness%20of%20BC%20Screening%20Technical%20Report_v7.pdf). We estimated the costs of clinical services used to treat patients in each disease stage by converting the procedures associated with each disease stage outlined in medical guidelines into their corresponding procedure codes. We assigned reimbursement costs to codes based on the Medicare fee schedule (39-42).

Productivity Losses

We estimated hours of productivity losses associated with the antiviral therapy by multiplying the number of hours per week lost during therapy estimated by 1 source by the discontinuation of therapy distribution (in weeks) observed in a second study (54, 55). We multiplied weeks of productivity losses by the median weekly wages obtained from the U.S. Bureau of Labor Statistics, adjusted by age and sex (56). We also estimated productivity losses from end-stage liver disease.

Utility Losses

Persons without hepatitis C experienced a background QALY that decreased as patients aged to account for the prevalence of other health conditions (57). For people with HCV, we collected utility losses from 5 empirical studies for 7 hepatitis

C disease states: SVR, METAVIR 0 to 1, METAVIR 2 to 3, compensated cirrhosis, DCC, HCC, and post-liver transplantation cirrhosis (58-62). We standardized results for each study by dividing the observed QALY value for each HCV state by the QALY value for the no HCV state. We multiplied the mean of the standardized values for each HCV state by the background QALY of the patient with disease. For patients undergoing antiviral therapy, we again multiplied the patient's QALY value by 0.0.88 for patients with genotype 1 and by 0.97 for patients with genotype 2 (26).

Simulation, Outcomes, and Sensitivity Analysis

We estimated medical outcomes, costs, and QALYs associated with each scenario, accounting for uncertainty in each of the model's key parameters. We simulated each scenario 1000 times, holding prevalence constant and using 1 of 1000 sets of parameters, wherein each parameter was selected randomly from its distribution. We report the mean of the simulated values for the overall population outcomes and the mean and the empirical 95% credible interval for per person costs and QALYs. We used these values to calculate the incremental cost-effectiveness ratios (ICER) and their credible intervals of the birth-cohort screening scenario compared with the baseline risk-based scenario. The ICER was calculated as the incremental difference in medical cost between 2 scenarios divided by the incremental difference in QALYs.

We tested the sensitivity of the ICER of birth-cohort screening with standard treatment compared to risk-based (status quo) screening to univariate differences in QALY losses, the discount rate, the probability of an SVR for genotype 1 and genotypes 2 and 3 of disease, the proportion of virus that is genotype 1, the cost of screening, and the costs of standard treatment. We tested the sensitivity of the ICER of birth-cohort screening with DAA plus standard treatment compared to standard treatment alone to univariate differences in the costs and effectiveness of treatment.

We calculated cost-effectiveness acceptability curves for willingness-to-pay (WTP) values per QALY gained ranging from $0 to $100 000 by calculating the probability that each scenario had the greatest net benefit (and thus was the most cost-effective) at each WTP value.

Role of the Funding Source

This research was funded by the Centers for Disease Control and Prevention's Division of Viral Hepatitis, which employed 4 authors (BDS, NP, JWW, and CMW) who participated in conceptualization, review, and revisions.

Results

We estimated that 66.9 million Americans born from 1945 through 1965 visited a primary care provider at least 1 time in 2006. Of these, 2.4 million were antibody-positive for HCV, 1.9 million were chronically infected, and 1.2 million were chronically infected and unaware of their status (Table 2). With no screening, we estimated that 618,000 birth-cohort members would develop DCC or HCC and die of hepatitis. Under risk-based screening, 14.8 million persons received antibody screening, 135 000 were treated, and 53 000 achieved an SVR. Under risk-based screening, 592 000 birth-cohort members developed DCC or HCC and died of

hepatitis C.

Under birth-cohort screening with standard treatment, 60.4 million persons received antibody testing, 1 070 840 new cases were identified, 552 000 patients were treated, 229 000 patients achieved an SVR, and the number of deaths from HCV was reduced to 509 000 (a decrease of 82 000 deaths compared with risk-based screening). Birth-cohort screening increased QALYs by 349 000, medical costs by $5.5 billion, and productivity losses by $6.9 billion.

Birth-cohort screening with DAA plus standard treatment increased screening, cases identified, and individuals treated by the same amount as did birth-cohort screening with standard treatment, but (compared with risk-based screening) increased the number of patients achieving an SVR by 311 000 and reduced the number of deaths from HCV to 470 000 (a reduction of 121 000 deaths compared with risk-based screening). Compared with risk-based screening, birth-cohort screening increased QALYs by 532 000, medical costs by $19.0 billion, and productivity losses due to therapy by $6.7 billion. Productivity losses were not used to calculate ICER values.

The ICER of birth-cohort screening with standard treatment was $15 700 per QALY saved compared with risk-based treatment (Table 3). The ICER of birth-cohort screening with DAA plus standard treatment was $32 000 per QALY saved compared with no screening, $35 700 per QALY saved compared with risk-based screening, and $73 700 per QALY saved compared with birth-cohort screening with standard treatment. When we considered only the incremental costs of screening, we estimated a cost of $2874 per new case of HCV identified.

The ICER of birth-cohort screening with standard treatment compared with risk-based screening was most sensitive to the inclusion of QALY losses from disease states before liver disease, the discount rate, and the probability of an SVR given genotype 1 disease. For this comparison, we estimated an ICER of $31 200 per QALY saved when we assumed no QALY losses from pre-liver disease states, $28 400 per QALY saved when we assumed a discount rate of 5%, and $20 100 per QALY saved when we assumed a 0.23 probability of an SVR for those with genotype 1 disease who initiated treatment (Figure 1).

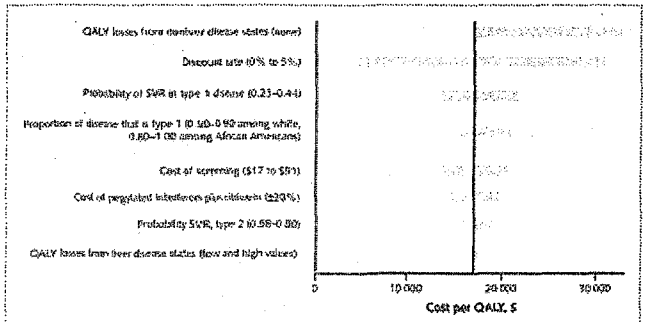

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Figure 1. Univariate sensitivity of the incremental cost-effectiveness ratio of birth-cohort screening with standard treatment compared with risk-based screening assuming pegylated interferon with ribavirin treatment.

QALY = quality-adjusted life-year; SVR = sustained viral response.

When we assumed birth-cohort screening in both scenarios, the ICER of additional DAA treatment compared with standard treatment alone was $39 600 per QALY saved when we assumed an SVR probability of 0.70; the ICER was $337 000 per QALY saved when we assumed an SVR probability of 0.38 (data not shown in Figure 1). The ICER of additional DAA treatment compared with standard treatment alone was $19 600 when we assumed the costs were half our baseline value and was $114 200 when we assumed costs were 50% higher.

We estimated that no screening was the most cost-effective up to a WTP of $16 000 per QALY gained, birth-cohort screening with standard treatment was the most cost-effective between a WTP of $16 000 and $75 000 per QALY, and birth-cohort screening with DAA and standard treatment was the most cost-effective at WTP values above that (Figure 2). When we dropped birth-cohort screening with standard treatment from our analysis and considered only the other 3 scenarios, no screening was the most cost-effective scenario up to a WTP of $16 000 per QALY, risk-based screening was most cost-effective between $16 000 and $36 000 per QALY, and birth-cohort screening with DAA plus standard treatment was most cost-effective at WTP values of $36 000 per QALY saved and higher.

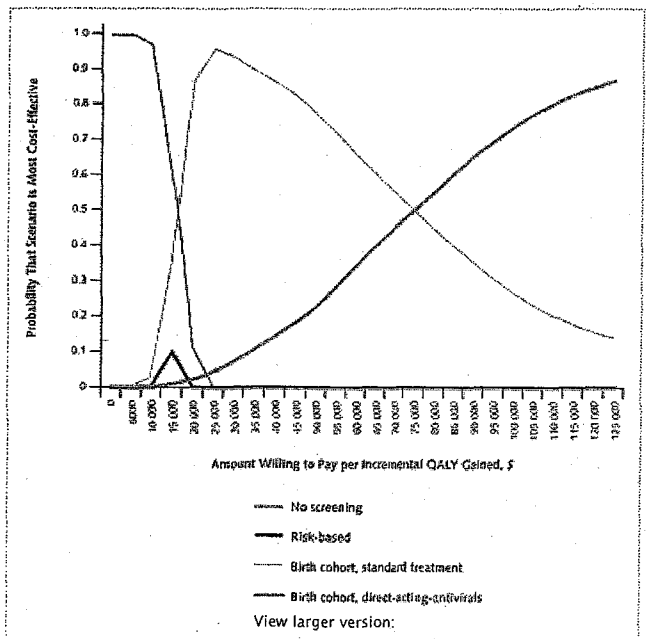

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Figure 2. Cost-effectiveness acceptability curve: probability that each screening scenario is the most cost-effective by willingness to pay per incremental quality-adjusted life-year gained.

QALY = quality-adjusted life-year.

Discussion

Without new case identification strategies, the adverse consequences of chronic HCV infection are forecasted to result in an increasing public health burden over the next 2 decades. Deaths from HCV are forecasted to double to over 18 000 per year by 2020 and to more than triple to 35 000 per year by 2030 (5). In this article, we investigated a new case identification strategy of screening those born from 1945 through 1965 (the birth cohort with the highest HCV prevalence) and estimated its cost-effectiveness using conservative assumptions about the uptake and effectiveness of treatment. We found that compared with the current strategy of risk-based screening, birth-cohort screening followed by standard treatment reduced deaths by 82 300 at a cost of $15 700 per QALY gained (95% credible interval, $11 500 to $30 100). Incorporating new DAA treatments would prevent approximately 121 000 deaths compared with risk-based screening at a cost of $35 700 per QALY saved (95% credible interval, $28 200 to $47 200).

No universally accepted standard exists to determine what level of cost-effectiveness is appropriate to justify the implementation of a new strategy. However, by using the standards outlined by the National Committee on Prevention Priorities, birth-cohort screening with standard treatment alone when compared with risk-based screening ranks equivalently to colorectal cancer screening, hypertension screening, influenza vaccination of adults age 50 years or older, pneumococcal vaccination of adults age 65 years or older, and vision screening of adults age 65 years or older (63). Birth-cohort screening with DAA plus standard treatment (when compared with risk-based screening) ranks below those interventions but equivalently to cervical cancer or cholesterol screening (63).

If fully implemented, birth-cohort screening in primary care would identify 808 580 new cases (85.9% of all undiagnosed cases in the birth cohort, compared with 21.0% under risk-based screening) at a screening cost of $2874 per new infection identified. This cost is similar to other estimated costs per new diagnosis of hepatitis B or C (30, 38). Birth-cohort screening is more costly than screening based on injection-drug use or elevated alanine aminotransferase levels, but those strategies probably miss many infected patients. The Centers for Disease Control and Prevention estimates that screening predicated on elevated alanine aminotransferase levels would identify less than half of the patients identified via birth-cohort screening (64). Furthermore, testing based on alanine aminotransferase elevations is already recommended in the Centers for Disease Control and Prevention's 1998 recommendations, but many persons chronically infected with HCV remain undiagnosed (12).

Our study has some limitations. First, to be conservative, we assumed that patients without insurance were not offered treatment, although many are currently offered treatment through compassionate use programs and clinical trial participation. Further, when the Affordable Care Act is fully implemented, insurance coverage will be extended to 95% of U.S. residents. Excluding uninsured persons from treatment limits our analysis by underestimating the aggregate benefits of the policy but has little effect on the cost-effectiveness. Of note, if birth-cohort screening received an A- or B-level recommendation from the U.S. Preventive Services Task Force, payment for screening would be mandated by the Affordable Care Act for all insurers (65).

Second, our estimates of the costs and the effectiveness of DAA plus standard treatment were necessarily speculative because clinical implementation data have yet to be reported. In our baseline analysis, in which we assumed a conservative probability of SVR (54%), our DAA plus standard results were favorable when compared directly with risk-based screening and acceptable when compared with birth-cohort screening with standard care. Future research should replicate this analysis using the real-world effectiveness and implementation costs of the DAAs telaprevir and boceprevir.

Third, fibrosis progression among undiagnosed persons is unknown. Our model capped the possible duration of disease before the start of the model at 20 years, an assumption that may underestimate disease progression in our population. The effect of this assumption is to make our screening intervention appear slightly less cost-effective than if we allowed for a longer possible duration at model initiation.

Fourth, as a simplification, we assumed that all screening (background and intervention) occurred in the first modeled year. This results in a slightly less favorable ICER than would a more realistic structure because it frontloads the costs of testing and treatment to the present time.

Fifth, our model does not incorporate elevated mortality risks from non-HCV causes among people with HCV but without past injection-drug use risks. Recent research indicates that excess mortality among these individuals for both hepatic and nonhepatic causes may be substantial, and this limitation probably led to a more favorable ICER (66).

Sixth, the NHANES data used for prevalence included only noninstitutionalized and nonhomeless populations. The institutionalized and homeless have an elevated HCV prevalence compared with NHANES respondents (67, 68), but they also have different competing risks for death and adherence to antiviral therapy. The effect of this limitation on cost-effectiveness is unknown, so these analyses do not apply to institutional or homeless settings. Finally, we excluded the benefits of lifestyle counseling to slow disease progression, as well any benefits from averting secondary transmission; this approach led to a less favorable ICER.

We predicted that, compared with the status quo, birth-cohort screening would identify an additional 808 580 cases of HCV infection and prevent 82 000 HCV-related deaths, at a cost of $2874 per new case identified and $15 700 per QALY saved assuming standard treatment and $35 700 per QALY saved assuming DAA with standard therapy. Birth-cohort screening appears to be a reasonable strategy

to identify asymptomatic cases of HCV.

Article and Author Information

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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
				2011. 10. 27	該当なし	
一般的名称	人血清アルブミン		研究報告の公表状況	IASR Vol.32 No.10(No.380) October 2011; Available from: http://idsc.nih.go.jp/iasr/32/380/dj3805.html	公表国 日本	
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン5%静注12.5g/250mL(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)					

研究報告の概要

○献血におけるHIV検査の現状と安全対策への取り組み
献血血液のHIV抗体検査は1986年から開始し、1999年から核酸増幅検査(NAT)を併用、2000年から50本プール、2004年から20本プールNATを行っている。現在ではHIV-1/2抗体検査(スクリーニング検査)として化学発光酵素免疫法(CLEIA)を、20本プールNATとしてTaqMan PCR法を用いている。ウィンドウ期の献血を防止するために、問診票でHIV検査目的の献血と感染症リスク行動についての質問を設けている。2011年4月から問診票を改訂し責任ある献血を強く促すとともに、感染症リスク行動の設問について期間を従来の1年以内から6カ月以内に短縮し、リスクのある性的接触の対象に「新たな異性との性的接触」を加えた。
国内のHIV感染者数の増加を背景に、HIV陽性献血者数は2007年に102件と100件を超え、2008年107件と増加したが、2009年102件、2010年86件と再び減少した。男女別ではこの5年間で男性が96%を占めている。都道府県別では東京、大阪が他と比べ群を抜いており、大阪は2004年以降東京を上回っている。その一方で陽性献血者の献血地は、全国的に拡散傾向となっている。年齢群別では10~30代の若い世代が80%前後を占めている。初回献血者の陽性者数は毎年30人前後であり、献血者10万人当たりの陽性者数は5~6人と、全献血者と比べ高い頻度であり、特に男性では2010年は1万人に1人の割合と、著しく高くなっている。
2010年のHIV陽性血液86件のHIV-1サブタイプは77件(90%)がBであり、その他、組換え流行株のCRF01_AE 6件、CRF01_AE/B 1件、CRF11_cpx 1件及びNAT陰性1件であった。輸血によるHIV感染が確認されているのは1997年~2003年の合計4例であり、2004年の20本プールNAT実施以降、輸血感染事例は起きていない。
今後も引き続きスクリーニング検査の向上により献血血液の安全性確保に努めるとともに、献血者には「責任ある献血」が強く望まれる。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン5%静注12.5g/250mL
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/20mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
日本の献血におけるHIVの検査状況や、安全対策への取り組みについての概説である。 これまで、本製剤によるHIV感染の報告はない。また本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれている。さらに最終製品についてHIV-NAT陰性であることを確認している事から本製剤の安全性は確保されていると考える。	日本これまでの使用実績やバリデーション成績に鑑み、特別の対応を必要としないが、国内外のHIV感染、AIDS発生の動向やHIV感染に関する新たな知見等について今後も情報の収集に努める。赤十字社では、化学発光酵素免疫測定法(CLEIA)により抗体検査を実施することに加えて、20プールNATについてもHIV-2及びHIVグループOの検出が可能な新NATシステムを導入し、陽性血液を排除している。

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献血におけるHIV検査の現状と安全対策への取り組み

(Vol. 32 p. 290-292: 2011年10月号)

献血血液のHIV検査と安全対策

献血血液のHIV抗体検査は1986年から開始し、1999年から核酸増幅検査(NAT)を併用し、2000年から50本プール、2004年から20本プールNATを行っている。全献血血液のHIV抗体検査(スクリーニング検査)を実施し、HIV抗体陰性であれば20本プールNATを実施する。確認検査としてウェスタンブロット(WB)法と個別NATを実施し確定している。現在では、HIV-1/2抗体検査(スクリーニング検査)として化学発光酵素免疫(CLEIA)法を、20本プールNATとしてTaqMan PCR法を用い、個別NATはTMA(transcription mediated amplification)法またはTaqMan PCR法により実施してHIV-1およびHIV-2の検出を行っている。

感染初期のウインドウ期の献血を防止するために、問診票でHIV検査目的の献血と感染症リスク行動についての質問を設けている。2011年4月から問診票を改訂し、責任ある献血を強く促すとともに、感染症リスク行動の設問について、期間を従来の1年以内から6カ月以内に短縮し、リスクのある性的接触の対象に「新たな異性との性的接触」を加え、以下のように変更した。

> 6カ月以内に次のいずれかに該当することがありましたか。
> (1)不特定の異性または新たな異性との性的接触があった。
> (2)男性どうしの性的接触があった。
> (3)麻薬、覚せい剤を使用した。
> (4)エイズ検査(HIV検査)の結果が陽性だった(6カ月以前も含む)。
> (5)上記(1)~(4)に該当する人と性的接触をもった。

HIV陽性献血者数の推移

国内のHIV感染者数の増加を背景に、HIV陽性献血者数は2007年に102件(献血者10万人当たり2.07)と100件を超え、2008年107件(同2.11)と増加したが、2009年102件(同1.93)、2010年86件(同1.62)と、再び減少した。男女別では、この5年間で男性が96%を占めている(図1)。

都道府県別では、東京、大阪が他と比べ群を抜いており、その頻度は、東京が10万人当たり3~4人台で推移しているのに対し、大阪は2004年以降東京を上回っており、特に2007年、2008年はそれぞれ6.92、6.70と著しく高かった(図2)。その一方で、陽性献血者の献血地は、2008年の24都道府県から2007年27、2008年30、2009年には33都道府県にわたっており、全国的に拡散傾向となっている(図3)。この5年間で陽性献血者が認められなかった県は福井、山梨、佐賀、大分の4県のみであった。年齢群別では、10~30代の若い世代が80. /後を占めている(図4)。初回献血者の陽性者数は毎年30人前後であり、10万人当たりの陽性者数は5~6人と、全献血者と比べ高い頻度であり、特に男性では2010年は1万人に1人の割合と、著しく高くなっている(図5)。

2010年のHIV陽性血液86件のHIV-1サブタイプは77件(90%)がBであり、その他、組換え流行株のCRF01_AE 6件、CRF01_AE/B 1件、CRF11_cpx 1件およびNAT陰性1件であった。

HIV輸血感染事例

輸血によるHIV感染が確認されているのは1997年1例、1999年2例、2003年1例の合計4例であり、NAT導入以降は2003年の50本プールNAT陰性(個別NAT陽性)の1例のみである。2004年の20本プールNAT実施以降、輸血感染事例は起きていない。

HIV陽性献血者への対応

HIV陽性者への対応について考慮すべき点は二つあり、一つは献血者の健康管理である。前者については、陽性者へ通知した場合、感染初期の検査目的の献血者を悲観させるマグネット効果で、ウインドウ期の献血によるリスクの増大につながる可能性があることから、「検査目的の献血」を防止する必要がある。後者については、陽性者に対し、受診勧奨、早期治療、二次感染防止などの留意点を伝え、心理的ケアを含めた告知するための配慮が必要である。

現在、日本赤十字社では、HIV陽性献血者に対しHBV、HCVのような陽性者への通知は行っていないが、感染拡大の防止、感染者の早期治療を促すために必要な措置を講じている。

最後に

2011年4月から問診票を改訂し、責任ある献血を強く促すとともに、HIVを想定した感染症リスク行動の設問をより具体的なものに変更した。しかし、2010年に「検査目的の献血」の設問に「はい」と回答し、献血をお断りした事例が337件あり、頻度でみると10～20代に多く見られた。

今後も引き続き、スクリーニング検査の向上により献血血液の安全性確保に努めるとともに、献血者には「責任ある献血」が強く望まれる。また、保健所等には高い利便性と迅速検査を主体とした検査体制のさらなる充実を強くお願いしたい。

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2)百瀬俊也、血液事業 34: 69-71,2011

3)厚生労働省医薬食品局血液対策課、平成22年版血液事業報告

日本赤十字社血液事業本部安全管理課　百瀬俊也

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別紙様式第2-1

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2012. 1. 23	該当なし	
一般的名称	人血清アルブミン		研究報告の公表状況	Chudy M, Weber-Schehl M, Pichl L, Jork C, Kress J, Heiden M, Funk MB, Nübling CM. Transfusion. 2012 Feb;52(2):431-9. doi: 10.1111/j.1537-2995.2011.03281.x. Epub 2011 Aug 2.	公表国	
販売名（企業名）	赤十字アルブミン20（日本赤十字社） 赤十字アルブミン5%静注12.5g/250mL（日本赤十字社） 赤十字アルブミン20%静注4g/20mL（日本赤十字社） 赤十字アルブミン20%静注10g/50mL（日本赤十字社） 赤十字アルブミン25%静注12.5g/50mL（日本赤十字社）				ドイツ	

研究報告の概要

○ヒト免疫不全ウイルス1型（HIV-1）に対する血液スクリーニング核酸増幅検査（NAT）における二つの異なる増幅対象の必要性

背景：HIV-1 RNA陽性供血血液5件が、3つの異なるCEマーク取得済みNATスクリーニングアッセイで検出されなかった。これらの事象は2件の輸血関連HIV-1感染に関係していた。関連するNATアッセイはモノターゲットアッセイで、異なるウイルスゲノム領域（グループ特異的抗原またはロングターミナルリピート）を増幅するものである。偽陰性結果の原因調査が始められた。

研究デザインと方法：NAT偽陰性の5つの血漿検体を、異なる設計のCEマーク取得済みHIV-1 NATシステム12種類において比較調査した。それぞれのアッセイでHIV-1変異の相対的な増幅効率を調査した。偽陰性NATアッセイの対象領域における配列の変異によってプライマーとプローブの比較を行った。

結果：偽陰性結果のモノターゲットNATと同様の方法で設計されたいくつかのNATアッセイにも、ウイルス変異の検出において欠陥が見られた。それぞれの例において、増幅対象領域の配列が変異し、アッセイに用いるプライマー及びプローブとの不一致を起こした。いくつかのデュアルターゲットアッセイは増幅効果が減少したが、偽陰性結果は示さなかった。

結論：HIVは新しいウイルス変異の急速な進化によって特徴付けられるが、新しい配列の進化は予測できない。モノターゲットNATアッセイによる偽陰性結果に基づき、ポール・エーリッヒ研究所はドイツのHIV-1供血スクリーニングにデュアルターゲットNATシステムを要請することを考慮している。

報告企業の意見

CEマーク取得済みのHIV-1 NATスクリーニングアッセイでHIV-1陽性供血血液が検出出来なかった件について調査した。その結果、デュアルターゲットNATの必要性が示唆されたとの報告である。

これまで、本製剤によるHIV感染の報告はない。また本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれている。さらに最終製品についてHIV-NAT陰性であることを確認している事から本製剤の安全性は確保されていると考える。

今後の対応

日本赤十字社では、化学発光酵素免疫測定法（CLEIA）による抗体検査を行い、陰性の検体について20プールNATを実施している。2008年よりさらに感度を上げHIV-1/2及びHIVグループOの検出が可能なNATを実施している。20プールNAT導入の2004年以降、輸血によるHIV感染例は発生していない。今後、次世代NATシステム導入の際には、本報告のようなウイルス変異にも対応できるよう検討を進める。HIVに関する新たな知見等について今後も情報の収集に努める。

なお、アルブミン製剤は70年も昔、HIV、HBV、HCVが発見されるはるか以前から、スクリーニングも施されていない血漿を原料に製造されても、いかなるウイルス感染を起こしたとの報告はない。極めて安全で特別な対応を必要としない。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
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赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/20mL

血液を原料とすることに由来する感染症伝播等

(3)

Blood screening nucleic acid amplification tests for human immunodeficiency virus Type 1 may require two different amplification targets

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BACKGROUND: Five cases of human immunodeficiency virus Type 1 (HIV-1) RNA–positive blood donations are described that escaped detection by three different CE-marked nucleic acid amplification technique (NAT) screening assays. These events were associated with two HIV-1 transmissions to recipients of blood components. The implicated NAT assays are monotarget assays and amplify in different viral genome regions (group-specific antigen or long terminal repeat). Investigations into the cause of the false-negative test results were initiated.

STUDY DESIGN AND METHODS: Plasma specimens of the five NAT false-negative cases were comparatively investigated in 12 CE-marked HIV-1 NAT systems of differing design. The relative amplification efficiency for the HIV-1 variant was determined for each assay. Sequencing of the variants in the region targeted by each false-negative NAT assay allowed comparison with the respective primers and probes.

RESULTS: Some of the NAT assays designed in a similar way to false-negative monotarget NATs also revealed deficiencies in detecting the viral variants. In each case sequencing of the assay target region in the variants demonstrated mismatches with primers and probes used by the assays. Some dual-target assays showed decreased amplification efficiency, but not false-negative results.

CONCLUSION: HIV is characterized by its rapid evolution of new viral variants. The evolution of new sequences is unpredictable; NAT screening assays with a single target region appear to be more vulnerable to sequence variations than dual-target assays. Based on this experience with false-negative tests results by monotarget NAT assays, the Paul-Ehrlich-Institut is considering requesting dual-target NAT assays for HIV-1 blood donation screening in Germany.

The most recent report of the Joint United Nations Program on HIV/AIDS (UNAIDS) and the World Health Organization (WHO) calculated that there were 33.4 million human immunodeficiency virus (HIV)-infected people worldwide by the end of 2008, with 2.7 million new infections alone in 2008.[1] These data reflect a continuing increase in the number of people living with HIV. Despite improved access to antiretroviral therapy in low- and middle-income countries, it is estimated that 2 million AIDS-related deaths occurred worldwide in 2008. Compared to the global HIV situation, Germany is among those countries with low prevalence and incidence rates in the general population as well as in blood donors. In Germany, between 2000 and 2007 the prevalence of HIV-1 infection in first-time donors was less than 10 HIV-1–infected donors per 100,000 applicant donors.[2] The HIV-1 infection rate (incidence) is also very low with less than 1 new infection per 100,000 repeat donors during the same period. Nevertheless, immense efforts have been made to prevent transfusion-associated HIV transmissions, including the use of highly developed technologies for blood screening. When nucleic acid amplification techniques (NAT) became available, Germany was one of the first countries in the world to implement this technology as a mandatory tool for blood screening in addition to serologic assays. NAT screening was introduced for hepatitis C virus (HCV) in 1999 followed by HIV-1 in 2004.[3,4] During the introduction of NAT, commercial assays designed for blood screening were scarce, and therefore flexible approaches were necessary. Compromises were made with regard to both test performance (e.g., sample pooling) and acceptance of different validated assay types for blood screening, including CE-marked diagnostic assays of high sensitivity or in-house–developed screening assays.[5] More recently, a variety of different HIV-1 NATs have become available on the common market in Europe following the CE marking process defined by the "in vitro diagnostic medical device (IVD) Directive."[6] In Germany, a minimal individual-donation (ID) sensitivity of 10,000 international units (IU) HIV-1 RNA/mL (based on the WHO international standard for HIV-1 RNA) was defined for HIV-1 NAT used for blood screening.[4,7] After several years of NAT in Germany the NAT yield (donations from the diagnostic window phase: NAT positive, anti-HIV negative) was determined.[8] From 2004 to 2010, a total of 23 HIV-1 NAT yield cases were found in more than 31 million NAT-screened donations, while two HIV-1 transmissions despite NAT were observed for this period. The first HIV-1 transmission was associated with a false-negative test result in the routine NAT assay. The assay targeted a region of the HIV-1 group-specific antigen (*gag*) gene and was shown to underestimate the viral load of the HIV-1 variant in this case.[9] Four additional cases (Cases 2-5) of nondetection of HIV-1 RNA by CE-marked assays were reported more recently to the Paul-Ehrlich-Institut (PEI), one of which resulted in another virus transmission to a recipient of a blood component. In this study we compare the detection efficiencies of different HIV-1 NAT assays for each of these cases and analyze the underlying HIV-1 sequences targeted by the assays. The suitability of monotarget NATs for the screening of strains of HIV is discussed. In our opinion, these cases demonstrate the need for at least two different amplification targets in "state-of-the-art" NAT blood screening systems for HIV-1 Group M.

ABBREVIATIONS: *gag* = group-specific antigen; ID(s) = individual donation(s); IVD = in vitro diagnostic medical device; LTR = long terminal repeat; NAT = nucleic acid amplification technique; PEI = Paul-Ehrlich-Institut; *pol* = polymerase.

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MATERIALS AND METHODS

Plasma specimens

Samples of the cases, testing falsely negative in CE-marked NAT assays, were provided to the PEI by the respective blood collection centers. The cases were numbered chronologically from 1 to 5, as reported to the PEI. The viral loads were determined using quantitative HIV-1 NATs. Based on the mean value obtained by different proficient quantitative assays, replicate plasma samples, either neat or serially diluted (in pooled negative human plasma) were subsequently used for comparative testing of a variety of different HIV-1 NATs. Furthermore, sequence analysis of NAT target regions in the viral genome was performed. An overview of the cases including the specimens analyzed is provided in Table 1.

HIV-1 NAT assays used in comparative study

Samples of the five false-negative cases were analyzed in 12 CE-marked NAT assays for HIV-1 RNA detection (six qualitative, six quantitative assays). The qualitative assays are CE-marked intended for use in blood screening, while the quantitative assays have been designed for diagnostic use, for example, patient monitoring. After validation, some of the highly sensitive quantitative assays are also used in blood screening in Germany. All assays were performed strictly following the instructions for use.

Comparative testing of the five plasma samples was performed by serial dilution of each of the HIV-1 RNA–positive specimens, and test results were assessed in comparison to the sensitivity claimed by the manufacturer. All investigations were performed in parallel with serial dilutions of the well-characterized PEI HIV-1 RNA reference preparation (3441/04, Subtype B, 80,000 IU/mL) which has been calibrated against the WHO international standard.[7] Assay performance characteristics described in the package inserts had to be reconfirmed using this reference material. Detection efficiency of individual assays for Cases 1 through 5 materials was determined by assessment of the obtained test results (expressed as IU/mL, Ct values, sample-to-cutoff values, or relative light units) in comparison to the results obtained with the PEI HIV-1 RNA reference preparation. Repeat experiments were performed to account for technology-intrinsic variation of results. Assay results for Cases 1 through 5 are expressed using symbols in Table 2 relative to the reported HIV-1 concentrations (quantitative assays) or to the detection efficiency of qualitative assays, when compared both to the 95% limit of detection claimed in the instructions for use, and to the performance with the PEI reference material. In Table 2 we used the following assessment scheme to report NAT assay efficiency: no detection, highly reduced detection (by a factor of >10), moderately reduced detection (by a factor of <10), and consistent detection.

Twelve CE-marked HIV-1 NAT assays were included as follows.

1. CAP CTM v1: COBAS AmpliPrep/COBAS TaqMan HIV-1 Test (quantitative; Roche Molecular Systems, Pleasanton, CA).
2. HPS CTM v1: HPS viral nucleic acid kit/COBAS TaqMan HIV-1 Test (quantitative; Roche Molecular Systems).
3. CAS v1.5: COBAS AmpliScreen HIV-1 Test, Version 1.5 (qualitative; Roche Molecular Systems).

TABLE 1. Summary of HIV-1 NAT nondetection cases

Case	Donor type, sex, age (years)	Donation dates	Screening NAT	HIV screening results	Viral load (IU/mL)	Donor status	HIV-1 transmission by
1	RD, male, 44	January 2007	CAP CTM v1	Ab neg RNA neg	10,000	WP	RBCs
		April 2007		Ab pos RNA neg	650	SC	
2	RD, male, 26	July 2007	CAP CTM v1	Ab neg RNA neg	0		
		October 2007		Ab pos RNA neg	80,000	SC	
3	RD, male, 26	May 2009	CTS MPX	Ab neg RNA neg	0		
		August 2009	CTS MPX	Ab neg RNA neg	20,000	WP	RBCs
		July 2010	VSPK v1.1	Ab pos RNA pos	280,000	SC	
4	RD, male, 42	March 2010	VSPK v1.1	Ab neg RNA neg	0		
		June 2010		Ab neg RNA neg	0		
		October 2010		Ab pos RNA neg	200,000	SC	
5	FTD, male, 18	October 2010	VSPK v1.1	Ab pos RNA neg	2,000	SC	

Ab = anti-HIV-1/2; FTD = first-time donor; neg = negative; pos = positive; RD = repeat donor; SC = seroconversion; WP = window period.

TABLE 2. Comparative testing of CE-marked HIV-1 NAT systems using Cases 1-5

Case	FN routine NAT	HIV-1 target regions:	CAP CTM v1 *gag*	HPS CTM v1 *gag*	CAS v1.5 *gag*	CAM v1.5 *gag*	CTS MPX LTR	VSPK v1.1 LTR	artus LTR	DRK LTR	Abbott RT *pol*	VSPK v1.2 LTR	CAP CTM v2 *gag* + *LTR*	Ultrio plus *pol* + *LTR*
1, 2	CAP CTM v1		((+))	–	+	+	+	+	+	+	+	+	(+)	+
3	CTS MPX		*(wd)*	+	+	+	–	+	+	+	+	+	(+)	+
4, 5	VSPK v1.1		*(wd)*	+	+	+	+	–	((+))	+	+	+	+	(+)

– = no detection; ((+)) = highly reduced (factor >10) detection efficiency; (+) = moderately reduced (factor <10) detection efficiency; + = consistent detection efficiency; FN = false-negative; *(wd)* = test version Version 1 has been withdrawn by the manufacturer.

4. CAM v1.5: COBAS Amplicor HIV-1 Monitor Test, Version 1.5 (quantitative; Roche Molecular Systems).
5. cobas TaqScreen MPX Test for use with cobas S201 system (qualitative; Roche Molecular Systems).
6. VSPK v1.1: Virus Screening PCR Kit, Version 1.1 (qualitative; GFE Blut mbH, Frankfurt, Germany).
7. artus: artus HIV-1 RG RT-PCR Kit (quantitative; Qiagen GmbH, Hilden, Germany).
8. DRK: DRK HIV-1 PCR kit (qualitative; DRK BSD Baden Württemberg-Hessen, Frankfurt, Germany).
9. Abbott RT: Abbott RealTime HIV-1 assay (quantitative; Abbott Molecular, Des Plaines, IL).
10. VSPK v1.2: Virus Screening PCR Kit, Version 1.2 (qualitative; GFE Blut mbH)
11. CAP CTM v2: COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, Version 2.0 (quantitative; Roche Molecular Systems).
12. Ultrio Plus: Procleix Ultrio Plus Assay (qualitative; Gen-Probe, San Diego, CA).

The CAM v1.5 assay is regarded as representative for the related Amplicor HIV-1 Monitor Test Version 1.5 and COBAS AmpliPrep/COBAS Amplicor HIV-1 Monitor Test, Version 1.5 because of the common amplification module; the Ultrio Plus assay is regarded as representative for the related assay Procleix Ultrio sharing the same HIV-1 amplification.

Sequence analysis

Viral RNA was extracted from 500 µL of plasma using the QIAamp DSP virus kit (Qiagen); amplicons of the 5′-long terminal repeat (LTR) were generated by cDNA synthesis and nested PCR using the outer antisense primer LTR-oa1 (5′-TAATACCGCTCTCGCACC-3′) and outer sense primer LTR-os1 (5′-CTTTTTGCCTGTACTGGGTCTC-3′). For the nested PCR procedure, the primers LTR-is1 (5′-CTGGGAGCTCTCTGGCTAACTA-3′) combined with LTR-ia1 (5′-TCCTTCTAGCCTCCGCTAGTC-3′) were used as sense or antisense primers, respectively. Direct sequencing of the amplification products was performed on both strands using a cycle sequencing kit (BigDye Terminator Version 3.1, Applied Biosystems, Foster City, CA) on a genetic analyzer (ABI 3730xl, Applied Biosystems). For all amplification and sequencing reactions the PEI HIV-1 RNA reference preparation (3441/04, Subtype B, 80,000 IU/mL) was analyzed in parallel as a positive control and to control for potential contamination.

Sequences representing 243 bp of the 5′-LTR region (Nucleotides 483-725) were aligned and compared with the corresponding sequence of the HIV-1 prototype HBX2 (Accession Number K03455). This part of the 5′-LTR region covers the target region of the VSPK, artus, and CTS MPX assays. Although we do not disclose the primer-probe sequences used in the different assays, data are reported concerning the frequency of mismatches and their relative position for several of the assays studied.

RESULTS

Different German blood establishments have recently reported five cases of HIV-1 RNA–positive blood donations missed by NAT screening. The five donors (all male) had not reported any HIV risk factors in the routine eligibility screening questionnaire. The NAT assays concerned were three different CE-marked screening tests (CAP CTM Version 1, CTS MPX, and VSPK Version 1.1) from two manufacturers (Roche Molecular Systems and GFE Blut mbH). Two of these cases resulted in HIV transmission to the recipients by transfusion of the corresponding red blood cells (RBCs).

The five cases were missed by the routine NAT assay despite viral RNA at a concentration level estimated to be sufficient for detection by the NAT screening system in place (Table 1). Samples from these cases were investigated using a variety of different HIV-1 NAT assays. The results are summarized in a semiquantitative manner describing the relative detection efficiency of the assays (Table 2).

Sequence analysis of the LTR target region was performed for the cases concerned (Fig. 1). There is high homology between sequences of primers and probes used in NAT assays and the prototype HXB2 sequence. When compared with the HIV-1 prototype HXB2, the sequence of the PEI HIV-1 RNA reference preparation exhibits five nucleotide changes in this in the part of the LTR analyzed (Nucleotides 483-725); these changes do not affect the efficiencies of the different NAT assays studied.

Case 1

Some details of the first case have already been published.[9] In April 2007, a male repeat donor was found to be anti-HIV-1 positive and HIV-1 NAT negative (CAP CTM v1, minipool and ID). The previous donation from January 2007 tested negative both by the serologic and the NAT assay (CAP CTM v1, pool of 96). The RBCs from the January donation infected the recipient with HIV-1, which was confirmed by molecular analysis. Retrospectively, it was shown that the CAP CTM v1 assay underquantified the HIV variant in both the donor and the recipient samples by a factor of up to 100.

Comparative tests

Performance of different NAT assays with the follow-up plasma from the April 2007 donation (650 IU HIV-1 RNA/mL; artus) confirmed the underquantitation performed by the CAP CTM v1 assay and the closely related HPS CTM v1 assay. Spurred on by reports of underquantitation for the CAP CTM v1, the manufacturer brought out a new

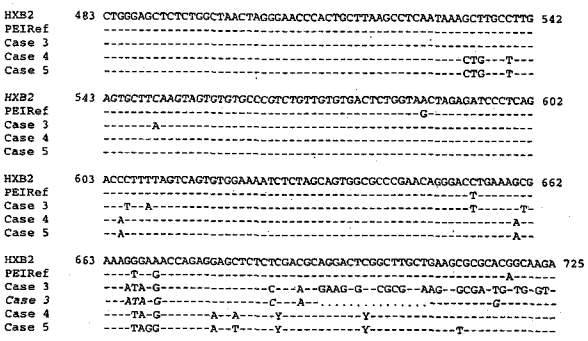

Fig. 1. Alignment of HIV-1 LTR sequences. Alignment of partial 5'LTR sequences (nucleotides 483-725) derived from amplified genomes of the "PEI HIV-1 RNA reference preparation" (#3441/04; subtype B), samples from non-detection Cases 3-5 and the HIV-1 prototype HXB2 (accession number K03455). Dashes represent homology. Differences are shown by the appropriate base letter or points (deletion). The alignment revealed a gap of 15 nucleotides (693-707) for the 3' part of the Case 3 sequence (see characters in italic).

version of the assay (CAP CTM v2) by incorporating a second target region, that is, the LTR, in addition to the *gag* region already amplified in the assay.[10,11] Although detection and quantification of Case 1 samples was clearly improved with the new version of the assay, its efficiency was still lower than that of other quantitative assays and is most probably due to the continued failure of the *gag* amplification by the improved assay.

Several qualitative or quantitative assays, when investigated with serial dilutions of the viremic plasma, showed no evidence of underestimation of this HIV variant, including the CAS v1.5 and CAM v1.5 assays as related monotarget *gag* amplification systems, when compared with the CAP CTM v1 assay.

Sequence analysis

Sequence analysis of the *gag* region of the HIV variant and alignment with primer and probe sequences used in the CAP CTM v1 assay has already been published.[9] It revealed three mismatches (one with the probe and two with the antisense primer). Subsequently, the mismatch at Position –3 at the 3' end of the antisense primer was shown to be responsible for the underquantitation of both this and a series of related HIV-1 variants (e.g., Case 2) by the implicated Roche CTM v1 assays (CAP CTM v1, HPS CTM v1).

Case 2

In October 2007 a repeat donor was tested positive for anti-HIV-1/2 but was negative by NAT screening (CAP CTM v1, pool of 96). Further investigations revealed positive test results with several other HIV-1 NATs. The screening results of the previous donation from July 2007 had been negative, and the RBCs were transfused. *The backup sample from July 2007 was* tested HIV-1 RNA negative by HIV-1 NATs (Abbott RT, Ultrio Plus), which picked up the HIV-1 variant of the October donation. The recipient of the RBCs had died in the meantime, without causal relationship to the transfusion or an infection. Both sequence analysis of the *gag* region and comparative testing in different NAT assays confirmed the HIV-1 variant of Case 2 as analogous to the Case 1 HIV-1 variant.

Case 3

In July 2010 an HIV-1 seroconversion was observed in a repeat blood donor whose HIV-1–positive status became obvious by a reactive test result in the HIV antigen and antibody combination assay confirmed in Western blot analysis (index donation). Furthermore, a reactive test result was obtained in the minipool (pool size of 96) with the multiplex PCR test system VSPK v1.1, which had been introduced as the routine NAT screening system at the blood collection site in May 2010. A donor-initiated lookback was initiated.

The previous donations by the donor were made in August and May 2009. These donations tested negative both in the serologic screening assay and in the previously used multiplex NAT system, the CTS MPX test. At the time this multiplex assay for HIV, HCV, and hepatitis B virus (HBV) had been used for minipools of 96.

However, reinvestigation of the backup samples from August 2009 resulted in a positive test result in the VSPK v1.1 assay both in ID-NAT and in a simulated minipool NAT of 96 donations. With the CTS MPX assay, however, the respective results were repeatedly negative for both kinds of sample. The HIV-1 RNA concentration was 20,000 IU/mL.

Backup samples of the previous donation from May 2009 tested negative in all HIV-1 screening assays. The data suggest HIV-1 infection of the donor during summer 2009, with the donor still being in the serologic window phase at the time of his donation in August 2009. The RBCs of the August 2009 donation were transfused in an 81-year-old male patient resulting in infection with HIV-1, which was confirmed by serology and NAT. The August 2009 donor plasma was still held in quarantine and available for further investigation; fortunately, the corresponding platelets had not been transfused.

Comparative tests

Comparative testing with the August 2009 plasma confirmed complete failure of the monotarget LTR assay CTS MPX, even with the neat plasma. Furthermore, there was an underquantitation of the viral load observed with the dual-target CAP CTM v2 (LTR, *gag*) when compared to the unmodified, original *gag* monotarget assay, HPS CTM v1, or other quantitative assays. This may be explained by reduced detection efficiency in the dual-target assay with the failure of one of the target regions (the LTR amplification systems may be related in both assays from the manufacturer, CAP CTM v2 and cobas TaqScreen MPX Test).

Sequence analysis

Sequence alignment of the LTR region of the Case 3 HIV-1 variant with primer and probes of the cobas TaqScreen MPX test showed good homology with one primer and the probe; however, it was highly divergent compared to the second Group M primer used in the test system. By contrast, comparison of the LTR from Case 3 with the oligonucleotides used in the VSPK v1.1 or artus assays resulted in good matches.

Case 4

An anti-HIV–positive test result (anti-HIV-1 enzyme-linked immunosorbent assay [ELISA] and Western blot positive) was obtained in a male repeat donor in October 2010. The screening NAT test VSPK v1.1 performed in pools of 96 was negative for this donation. By contrast, further analysis of the backup sample revealed a positive result with another NAT assay of different design indicating a false-negative test result by the NAT screening system used at this blood collection site. The two previous whole blood donations from June 2010 and March 2010 gave negative test results in the same routine serologic and NAT assays used in October 2010. Retesting with another sensitive NAT of different design (Abbott RT; shown to be positive with the index donation) did not detect HIV-1 RNA in the backup samples from June 2010 and March 2010. The most likely explanation for these findings is a seroconversion between June and October.

Comparative testing

Comparative testing with the backup sample of the index donation from October 2010 (200,000 IU/mL) was performed using serial dilutions of the material. The failure of the monotarget LTR assay VSPK v1.1 assay was confirmed while the monotarget LTR assay CTS MPX provided positive results. The sample was underquantified by a factor of more than 50 by the artus monotarget LTR assay. Although the dual-target (LTR, polymerase [*pol*]) qualitative Ultrio Plus assay detected the sample, a reduced efficiency (by a factor of 6) was observed with this assay compared to the relative detection efficiency of serially diluted PEI reference materials.

Sequence analysis

The sequence analysis of the LTR region revealed mismatches with one of the primers used in the VSPK v1.1 assay, apparently resulting in the complete failure of the assay to amplify this HIV-1 variant. Other assays based on LTR sequences are also affected by the sequence variations observed in this variant. The artus assay also shows mismatches with one of its primers, although these are situated toward the 5' end of the primer. This presumably explains the severe rate of underquantitation of the sample while still showing low residual amplification efficiency. The amplification of this variant by the artus assay might also be hampered by further mismatches in the binding region for the second primer.

Relative underestimation was observed for the Ultrio Plus assay (Table 2). The assay uses both the LTR and the *pol* as targets and might be affected by mismatches in the LTR. Although there are mismatches of this HIV-1 variant with one of the Ultrio LTR primers (*J. Linnen*, Gen-Probe, personal communication, 2011), additional studies (e.g., sequencing of the *pol* region of the HIV-1 variant) are needed to explain the lower efficiency of the assay. The CTS MPX assay amplifies this variant consistently, a finding substantiated by high homology between the viral variant and the primer-probe system of this assay with only a few mismatches at noncritical positions.

Case 5

The most recent case of a false-negative NAT result is a first-time blood donor (male, 18 years) who was tested anti-HIV-1/2 positive by ELISA and confirmatory Western blot in October 2010. NAT was negative, both in pools of 96 and in ID testing, using the VSPK v1.1 assay. To further investigate the possibility of an elite controller (i.e., an untreated HIV-infected individual without measurable viremia), the specimens were tested using another sensitive NAT assay of different design and shown to be reactive. The plasma from this whole blood donation was investigated using the panel of NAT assays. The overall reactivity pattern with the different assays for Case 5 is very similar to that obtained with Case 4. Sequence analysis revealed nucleotide changes very similar to those of Case 4, leading to the same explanation of differential reactivity in the different assays. However, there are few differences between these two related variants, with changes at three positions in the 243-bp region analyzed for the different primer-probe target sites of the different assays (Fig. 1).

DISCUSSION

The five HIV-1–positive blood donors described in this study, although positive by serologic screening (anti-HIV-1/2 ELISA), all showed false-negative NAT results in the respective screening assays (Table 1). The cases concern four recently seroconverted repeat donors (Cases 1-4) and one first-time donor with unknown infection history (Case 5). The cases occurred at different blood collection sites in Germany, and three different NAT systems from two manufacturers gave rise to false-negative test results. Two diagnostic window-phase donations, missed by the routine screening NAT assay, led to HIV-1 transmission to the RBC recipients.

For Case 1 the sequence analysis of the NAT target region (*gag*) has already been published.[9] Only a small number of entries in the public databases matched this specific sequence. Case 2 is of very similar *gag* sequence pattern and appeared at another blood donation center in Germany. Both sequence pattern and reactivity in different assays were highly similar between these cases confirming the urgent need for improvement of the CAP CTM v1 assay and withdrawal of the previous version from blood screening.

For Cases 3, 4, and 5, we analyzed the viral sequence of the 5′-LTR region targeted by the different NAT assays by comparison to the PEI HIV-1 RNA reference material and to the reference sequence HXB2 (Fig. 1). The three cases revealed unique sequence patterns not present in public HIV sequence databases.

The LTR region of the Case 3 HIV-1 variant differs at 13.6% of positions (33/243) compared to HXB2, with the lowest level of homology in the 3′ part of this LTR fragment. The low homology is mainly caused by a deletion of 15 nucleotides (Fig. 1, Case 3 sequence in italics). This deletion was not present in any public HIV sequence database. The deletion has no obvious inhibitory effect on the replication of HIV-1 as shown by the transmission event. Further investigations on this transmission event included HIV-1 sequence analysis in the index donation (July 2010) and in the RBC recipient (August 2010, 1 year after transfusion). Briefly, comparison of different regions of the HIV-1 genome (5′-LTR, *pol*) derived from donor (August 2009, July 2010) and recipient (August 2010) showed greater than 99.5% nucleotide identity, clustering with Subtype B sequences (data not shown). These results confirm the HIV transmission via RBCs from the HIV-infected blood donor and demonstrate sequence stability for this HIV variant in both donor and recipient for more than 1 year.

The HIV-1 variants of Cases 4 and 5 were missed by the same routine NAT system and furthermore exhibit similar reactivity in the different NAT assays studied for detection of this variant. These two variants exhibit a very similar (although not identical) pattern and rate of nucleotide exchanges in the 5′-LTR target region (243 bp) of the NAT assays analyzed, when compared to reference sequence HXB2 (differences at 13 [5.3%] or 15 [6.2%] positions) in Case 4 and Case 5, respectively. These variants show common patterns, with neighboring nucleotide exchanges. Several assays target one of the affected regions potentially explaining why different assays of related design show reduced efficiency for these two HIV-1 variants.[12]

Cases 4 and 5 appeared in different blood collection centers in different regions of Germany. The particular similarity in the LTR region is not reflected by nucleotide sequences in *env* (V3, V4). This region is of much lower homology between the two HIV-1 variants (data not shown). Therefore, there is currently no indication for a common infection source of the two donors.

Even if certain mismatches with primers or probes were prognosticated for viral variants, the extent of underquantitation or nondetection to be expected is often not predictable. While genetic change is inevitable, and may affect primer and probe regions, the extent to which viral loads are underestimated or viruses are simply not detected is often unpredictable. Although general rules have been established for the design of primers and probes to tolerate mismatches, experimental studies should be performed to measure the effect of individual mismatches and mismatch combinations on assay performance, exactly matching specified assay conditions. Besides the number and position of mismatches, several factors have been shown to contribute to the effect, including mismatch and primer type, neighboring sequences, and design of the amplification reaction.[13,14]

Monotarget versus dual-target screening NATs

There are now several actual examples of HIV-1 variants escaping detection by monotarget NATs targeting different regions of the HIV-1 genome. A recent study from Italy describes false-negative or underquantified results with the COBAS AmpliScreen HIV-1 Test, Version 1.5 and COBAS Amplicor HIV-1 Monitor Test, Version 1.5, which are monotarget assays.[15] These assays use *gag* primers and probes different from those of the CAP CTM v1 and are affected by different HIV-1 *gag* variants compared to Cases 1 and 2 described above.[9,15] Furthermore, the Italian case also does not seem to affect the *gag* portion in the dual-target CAP CTM v2 assay as the published quantitative data suggest. Therefore, the recent Italian case appears to be due to different *gag* sequence variation affecting different *gag* monotarget assays when compared to Cases 1 and 2 of our study. Another recent publication from Germany described new genetic polymorphisms in the LTR region affecting a real-time PCR blood-screening test developed in house.[16] Two unrelated HIV-1-positive blood donations

were missed by NAT, whereas parallel serologic testing was positive for both donations. The observed LTR sequence polymorphisms are different from those described in our study. The implicated in-house assay was subsequently improved to pick up the LTR polymorphisms. The CE-marked VSPK v1.1 assay was also modified to detect the viral variants from Cases 4 and 5 and was replaced by the new CE-marked assay VSPK v1.2. Modification of monotarget assays may provide an interim solution to deal with such deficiencies. However, from a conceptual point of view the inclusion of a second target region clearly appears to be the preferable approach long term.

The false-negative results were always obtained with screening NATs comprising a single amplification system designed for HIV-1 Group M sequences. The risk of a false-negative test results appears to be proportionally lower with more than one amplification target region in a screening NAT assay, especially for detection of a highly variable virus like HIV-1. We recognize that there are monotarget assays without reported mismatch-based failure in blood screening; however, evolution of HIV-1 sequence variants appears unpredictable and may therefore affect any NAT assay. The frequency of use in blood screening impacts the probability for the observation of adverse events for NAT assays. The risk of a false-negative screening test result may be directly translated into the risk of HIV-1 transmission by transfusion if the donor is in the diagnostic window phase at the time of donation. We show indirect evidence that reactivity of dual-target assays may be compromised in the case of failure of one of the targets; nevertheless, a significantly lower risk is expected for dual-target assays with the a second amplification system being able to widely compensate for the complete failure of the other component when affected by an emerging HIV-1 variant. There is currently no evidence of a dual-target assay missing one of the cases described in this study or in related reports. A recent publication describes a transfusion-associated transmission despite dual-target HIV-1 NAT.[17] However, the authors interpret this incident as being due to the low HIV-1 RNA concentration during the eclipse period of early HIV-1 infection. Nondetection of low-level viremia may still occur with any sensitive NAT system. This constitutes the nonpreventable residual risk in the era of NAT.

The detection failures, due to mismatches in monotarget HIV-1 NAT assays occurred during routine blood screening in two European countries with low HIV-1 rates in their blood donor populations. We therefore conclude from our study and from related cases published recently that dual (or more)-target HIV-1 Group M NAT assays should be considered for use in blood screening. We would define a dual-target assay for HIV-1 Group M screening NATs as a system where two different amplicons are generated with different primer sets, with the amplicons being detected by different probes. It might be of secondary importance if the different targets are located within one viral genomic region (e.g., the LTR) or in separate regions.

Critical assessment of blood screening laboratory data

In our view, both blood donation testing laboratories and NAT kit manufacturers should become aware of and regularly examine those test results that are not easily interpretable. Such results should include negative NAT tests for HIV-1, HCV, or HBV with simultaneous confirmed positive anti-HIV-1, anti-HCV, or hepatitis B surface antigen results, respectively. This constellation of diagnostic markers can occur, with higher probabilities for HBV (29%) and HCV (26%) compared to HIV-1 (11%), as recently published for US blood donors.[18] The review should include NAT assays of different design, to be in a position to identify false-negative results in the screening NAT. The PEI would be able to provide support by performing more extended analysis of discrepant test results, with the inclusion of a variety of assays (serologic, NAT). However, the availability of sufficient specimen volumes of important cases is often an issue. In our experience, specimens of great scientific value, identified by screening of blood donations, have often already been destroyed, after strictly following respective standard operating procedures, thus preventing follow-up investigations.

Viral sequences used for assay design

The treatment management of HIV patients is often based on sequence information of the *pol* (i.e., domains of protease and reverse transcriptase) and *env* regions. The use of these sequences for NAT assay design is limited. There is an urgent need to share newly identified virus sequences, particularly in the genomic regions targeted by blood screening NAT assays, for example, by making these sequences available in public databases. IVD manufacturers may gain knowledge about emerging viral sequences with respect to their own assays by investigating customer complaints. In our experience, individual IVD manufacturers are sometimes well aware of circulating variants potentially causing problems in their assays. Manufacturers may feel uncomfortable making this sequence information of viral variants publicly available. Despite commercial competition, it might be of mutual benefit to share new sequence data, especially for those manufacturers using similar viral genome regions as amplification targets. An IVD-specific sequence database at an independent institution may be helpful both for the design of new assays and for the surveillance of existing assays.

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CONFLICT OF INTEREST

The authors state no conflict of interest relevant to this manuscript submitted to **TRANSFUSION**.

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一般的名称	①②③④⑤⑥⑦ポリエチレングリコール処理人免疫グロブリン		研究報告の公表状況		TRANSFUSION 2012; 52 : 181-187	公表国 アメリカ，欧州	
販売名（企業名）	①献血ヴェノグロブリン IH5%静注 0.5g/10mL	（ベネシス）					
	②献血ヴェノグロブリン IH5%静注 1g/20mL	（ベネシス）					
	③献血ヴェノグロブリン IH5%静注 2.5g/50mL	（ベネシス）					
	④献血ヴェノグロブリン IH5%静注 5g/100mL	（ベネシス）					
	⑤献血ヴェノグロブリン-IH ヨシトミ	（ベネシス）					
	⑥ヴェノグロブリン筋注 450mg/3mL「ベネシス」	（ベネシス）					
	⑦ヴェノグロブリン筋注 1500mg/10mL「ベネシス」	（ベネシス）					

研究報告の概要	使用上の注意記載状況・その他参考事項等
ヒト血清アルブミン（HSA）の低温滅菌は米欧の薬局方に詳述されており、工程の生理化学的変数における少しの変動は許容している。にも拘わらず、低温殺菌によるA型肝炎ウイルス（HAV）不活化に関して、最大3.9Logの相違が報告されている。ここでは、低温滅菌で用いられるHAV変異型の選択が、この不活化変動性に寄与しているかもしれないという仮説を実験的に評価した。 HAV HM175株の4つの細胞変性変異は、部分的な塩基配列決定により測定された。これらの変異型は、5%、或いは25%のたん白質含有のHAVをスパイクしたHSAを58±1℃、600±10分に保ったGLPの厚制の下で実施される低温滅菌研究に使われ、ウイルス不活化が評価された。低温殺菌によるウイルス不活化で4つのHAV変異型は有意差のある（P<0.0001）2つのサブグループに分けられると考えられた。低温滅菌に使われたHSA溶液のたん白質濃度は、HAVの不活化に有意に影響を及ぼし、25%のHSAより5%のHSAでリダンクションファクターは有意に（P<0.002）低かった。 HAV変異型とHSA溶液のたん白質濃度は、低温滅菌のHAV不活化に影響を及ぼす。HAV不活化能力を過大評価してはならない。	代表として献血ヴェノグロブリン IH5% 静注 0.5g/10mL の記載を示す。 2. 重要な基本的注意 1）本剤の原材料となる献血者の血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体、抗HTLV-I抗体陰性で、かつALT（GPT）値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohnの低温エタノール分画で得た画分からポリエチレングリコール4000処理、DEAEセファデックス処理等により人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及びpH3.9～4.4の条件下での液状インキュベーション）及びpH3.9～4.4の条件下での液状インキュベーション処理を施しているが、投与に際しては、次の点に十分注意すること。
報告企業の意見	今後の対応
A型肝炎ウイルスはピコルナウイルス科ヘパトウイルス属に属するRNAウイルスで、形状は直径約27nmの裸の正20面体で、遺伝子型は7種類に分類されているが、血清型は1種類である。万一、原料血漿にHAVが混入したとしても、EMC及びCPVをモデルウイルスとしたウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

グロブリン

Inactivation of hepatitis A variants during heat treatment (pasteurization) of human serum albumin

Maria R. Farcet, Johanna Kindermann, Jens Modrof, and Thomas R. Kreil

BACKGROUND: Pasteurization of human serum albumin (HSA) is detailed in the US and European Pharmacopoeial monographs and therefore a process that allows for little variation in physiochemical variables. Nevertheless, differences of up to 3.9 log in hepatitis A virus (HAV) inactivation by pasteurization have been reported. Here, the hypothesis that the choice of HAV variant used in the pasteurization might contribute to this inactivation variability is evaluated experimentally.

STUDY DESIGN AND METHODS: The identity of four widely used cytopathic variants of the original HAV HM175 strain was determined by partial sequencing. These variants were used in pasteurization studies conducted under the principles of good laboratory practice, for which HAV-spiked HSA of 5 or 25% protein content was kept at 58 ± 1°C for 600 ± 10 minutes, and the virus inactivation was assessed. In addition, data from previous pasteurization studies were included in the analysis.

RESULTS: The four HAV variants could be divided into two subgroups, with significantly different ($p \leq 0.0001$) virus inactivation by pasteurization (4.7 and 4.8 log vs. 2.3 and 2.6 log, respectively). Also, the protein concentration of the HSA solution used for pasteurization had a significant effect on the achieved HAV inactivation, with reduction factors obtained in 5% HSA significantly lower than in 25% HSA ($p < 0.002$).

CONCLUSION: HAV variant and protein concentration of the HSA solution affect the overall HAV inactivation that is achieved during pasteurization. As the HAV inactivation capacity should not be overestimated, an HAV variant more resistant to heat inactivation should be used for studies investigating the viral safety profiles of plasma derivatives.

Pasteurization of human serum albumin (HSA), a heat treatment at 60°C for 10 hours, was the first virus inactivation step included into the manufacturing process of a plasma derivative,[1] and inactivation of lipid-enveloped viruses was consequently shown to be consistently effective. The process has been defined in both the US and European Pharmacopoeial monographs for HSA, is well standardized, and allows little variation in physiochemical variables and added stabilizers. Pasteurization would therefore be expected to result in similar levels of virus reduction, irrespective of where or by whom the pasteurization study is performed. However, regulators, that is, the only party with access to results from different manufacturers and contract research organizations (CROs), have indicated that significantly different reduction factors of between 3.0 and 6.9 log, that is, differences of approximately 10,000-fold, of hepatitis A virus (HAV) inactivation by pasteurization have been reported during the manufacture of HSA.[2] Similarly, publications of HAV pasteurization studies reported reduction values that differed by more than 4 log, that is, more than 10,000-fold, yet given that different products and different methods of HAV detection were used,[3-8] a direct comparison of these results was impossible. All these HAV inactivation studies used variants of the only cytopathic strain of HAV available, that is, HM175,[9] mainly HM175/18f[9,4,8] and HM175/24a,[5,7] or no further description of the used HM175 variant was given.[6] Genomic modifications have been described during the adaptation of the wild-type HM175 strain to persistence in cell culture (pHM175) and after the development of a cytopathic effect by the three variants HM175/18f, HM175/24a, and HM175/43c.[9,10] One factor that might affect the reduction factors achieved could thus be the HM175 variant used in the respective inactivation study, a theory that has previously been proposed[11] but has so far not been investigated. Pasteurized HSA has an excellent viral safety record[12] and HAV transmission by this product has never been reported. In addition to pasteurization, other contributing factors to the high safety margins of this product are nucleic acid testing of the plasma for HAV and also the ethanol fractionation process, which reduces HAV infectivity by several log 50% tissue culture infectious doses ($TCID_{50}$; unpublished data). Nevertheless, as HAV is known to be fairly robust and thus relatively resistant to inactivation,[13] the inactivation capacity of pasteurization for this virus is an important element of the overall viral safety margin and should thus not be overestimated in the context of plasma product safety. It was therefore the aim of this work to evaluate whether the differential HAV inactivation properties as observed when studying HSA pasteurization can be correlated to the respective HM175 variants used, which might explain some of the variability of HAV inactivation that has previously been observed.[2-8]

ABBREVIATIONS: CRO(s) = contract research organization(s); SNPs = single-nucleotide polymorphisms; $TCID_{50}$ = 50% tissue culture infectious dose; VSS(s) = virus stock solution(s).

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MATERIALS AND METHODS

HAV variants and propagation

HAV HM175 variants of four different origins and designations were used in this study (Table 1). Stock "HM175" was purchased from Advanced BioScience Laboratories (Kensington, MD) and two stocks of "pHM175," both derived from a common master stock, were purchased from BioReliance (Rockville, MD). Both CROs had sourced the original stock of the viruses from the US Food and Drug Administration (FDA; Silver Spring, MD). A stock of "HM175/24a"[9] (further referred to as "VSS 24a") was first obtained from Dr S. Emerson[7] (National Institutes of Health, Bethesda, MD) before 1994 and a stock of "HM175/18f"[9] (further referred to as VSS 18f) was purchased from the American Tissue Culture Collection (ATCC, Rockville, MD; Cat. No. VR-1402). HM175 and one stock of pHM175 were used as supplied by the CROs, the other HAV stocks were propagated on BSC-1 cells, obtained from the European Collection of Cell Cultures (Salisbury, UK; Cat. No. 85011422). For the preparation of virus stock solution (VSS), cells were cracked 14 days after virus infection by three freeze-thawing cycles and the virus-containing suspension was centrifuged to remove cellular debris.

Confirmation of HAV variant identity

Partial genome sequencing was done to confirm the identity of the HAV variants. Genome sequences available at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) of wild-type HM175 (Accession No. M14707),[14] variants HM175/18f (M59808), HM175/43c (M59809), and HM175/24a (M59810),[9] as well as the sequence of p16 HM175,[15,16] which can be deduced from data provided in literature,[9] were aligned using ClustalW2 software available at the European Bioinformatics Institute (http://www.ebi.ac.uk/Tools/clustalw2/index.html). From this alignment, four genomic regions (Region 1, Base Pairs 44-266; Region 2, Base Pairs 2570-3075; Region 3, Base Pairs 4881-5355; Region 4, Base Pairs 6729-7340; positions according to the wild-type sequence) with sufficient single-nucleotide polymorphisms (SNPs) to allow distinction between the HM175 variants were chosen. RNA was extracted from 100-µL aliquots of VSS 24a, VSS 18f, and one stock of pHM175 using an RNA purification kit (RNeasy Mini Kit, Qiagen, Hilden, Germany) and amplified using a one-step real-time reverse transcription–polymerase chain reaction (RT-PCR) kit (AgPath-ID, Applied Biosystems, Foster City, CA) and primers (5′→3′) R1F CCATGGTGAGGGGACTTGATAC, R1R GCAACGGCCAGAGCCTAG; R2F TGGTTTGCCATCAACACTGAG, R2R CATCCACTGATGACTCCAAGTCTC; R3F TGTGTTGATTTGATAATGGATGGAC, R3R TCTTC TCTCCAACTCCAAACTGAA; R4F GGTAGAATCATGAGT-

TABLE 1. Origin, designations, propagation, and identity of the HAV HM175 variants used in this study

HM175 variant (original designation)	Source	Designation used throughout the study	Propagation	Identity
HM175	ABL* (FDA)	HM175	No, as provided by CRO	HM175/24a†
pHM175	BRL‡ (FDA)	pHM175	One stock as provided by CRO One stock propagated	HM175/24a
HM175/24a	S. Emerson§	VSS 24a or HM175 "43c-18f"	Yes	Could not be confirmed
HM175/18f	ATCC¶	VSS 18f	Yes	HM175/18f

* Advanced BioScience Laboratories, stock was originally obtained from the FDA.
† Assigned based on the common origin (FDA) of HM175 and pHM175, as due to VSS restrains identity was not confirmed again by sequencing.
‡ BioReliance, stock was originally obtained from the FDA.
§ Barrett et al.[7]
¶ American Tissue Culture Collection.

GAACTATCTGGA and R4R CATCTCTTTCTCCAAACAG-GACTG. PCR products were sequenced using a cycle sequencing kit (BigDye Terminator, Applied Biosystems). Primer design, RT-PCR, and sequencing were done at Ingenetix GmbH (Vienna, Austria). Sequencing data was aligned to the whole genome sequences using computer software (ClustalW2, http://www.ebi.ac.uk/Tools/msa/clustalw2/) and illustrated with computer software (GeneDoc v.2.7.000, available at http://www.nrbsc.org/gfx/genedoc/index.html).

Pasteurization

Virus inactivation by pasteurization was investigated in studies conducted under the principles of good laboratory practices, using validated downscales of the manufacturing process as defined by regulatory guidance.[17] Five percent or 25% HSA was obtained from the Baxter manufacturing facilities in Vienna, Austria, or Los Angeles, California; spiked 1:10 or 1:20 with VSS (i.e., 9 or 19 parts of HSA plus one part of VSS, respectively); and mixed, and a sample was removed and titrated immediately to confirm the amount of virus added. Virus titers throughout the experiments were similar and did thus not bias the results obtained.The spiked material was then heated to 58 ± 1°C, that is, a temperature below the defined limit for manufacture (60 ± 1°C) to investigate worst-case inactivation conditions, within 90 minutes and kept at this temperature for 600 ± 10 minutes. Samples were removed and titrated immediately when the temperature of the spiked material reached 57°C and after 120 ± 2, 360 ± 5, and 600 ± 10 minutes at target temperature.

Infectivity assays

Infectious virus titers were determined by $TCID_{50}$ assays, using eightfold replicates of serial half-log sample dilutions of virus-containing samples that were titrated on an in-house FRhK4 cell line maintained in Dulbecco's modified Eagle's medium with 1 g/L D-glucose, 5% fetal calf serum, 0.5 mmol/L L-glutamine, 100 mg/L gentamycin sulfate, and 1 mmol/L sodium pyrovate. Unspiked HSA samples were tested for potential cytotoxicity and for interference with the detection of low virus titers on FRhK4 cells. Virus reduction factors for pasteurization were calculated in accordance to the EU Committee for Proprietary Medicinal Products guidance[17] and from at least two independent runs, for which different lots of HSA were used.

Data from previous pasteurization studies

Before this study, HSA pasteurization studies had already been done at Baxter or for Baxter at certain CROs. Studies done at Baxter in 1994 and 2000 used the original VSS 24a and "pHM175" (sourced from BioReliance, Table 1), respectively. A study done at BioReliance in 2001 used "pHM175" and a study done at Applied Bioscience Laboratories in 1995 used "HM175." The HSA used in all studies had originated from Baxter and contained 3.5, 5, or 25% protein. As the materials and methods used in the previous pasteurization studies were similar or the same as for this study, the data were included in the current analysis.

Statistical analysis

Statistical analysis of reduction factors as well as t tests was done using computer software (GraphPad Prism 5, GraphPad, San Diego, CA). Analysis of variance (ANOVA) and multiple comparison of the means by Tukey's test was done using computer software (MiniTab, MiniTab, Inc., State College, PA).

RESULTS

Confirmation of HAV variant identity

The published genomic sequences of the three HM175 variants are more than 99% identical to that of the HM175 wild type. The variants have in common a 14-nucleotide insertion in the 5'UTR and two deletions that resulted in the loss of five nucleotides.[9] Based on SNPs in the whole genome, the HM175/18f sequence is most similar to the wild-type sequence (41-SNP difference), followed by HM175/43c (44 SNPs) and HM175/24a (52 SNPs). With the exception of one base pair, an insertion at Position 115/116 (Region 1) in pHM175, the obtained sequences of the three partially sequenced HAV VSS 18f and VSS 24a and one stock of pHM175 were identical for Regions 1 and 3 and matched the published sequences of HM175/18f and HM175/24a.[9] An insertion at Position 117/118 (Region 1) that had been described for HM175/24a[9] was not seen in any of the sequenced VSS. Regions 2 and 4 contained SNPs that allowed differentiation of the sequenced HM175 variants. VSS 18f was confirmed as variant HM175/18f with 93% (one mismatch at Position 2864) base pair identity at the informative SNPs in Regions 2 and 4 (Table 2). VSS 24a matched the HM175/24a sequence with 43%, the HM175/43c sequence with 64%, and the HM175/18f sequence with 57% base pair identity at the informative SNPs in Regions 2 and 4 (Table 2). The sequence of VSS 24a lacked a SNP specific to HM175/43c in Region 3. Based on these results, the identity of VSS 24a could not be confirmed, as the regions sequenced shared more SNP identity with variants HM174/43c and HM175/18f than with HM175/24a (Table 2) and this variant was therefore termed HM175 "43c-18f." pHM175 was unambiguously identified as HM175/24a, with 100% base pair identity with the published sequence at the informative SNPs in Regions 2 and 4 (Table 2). The identity of HM175 could retrospectively not be confirmed by sequencing due to VSS restrains but, as both BioReliance and Advanced BioScience Laboratories had originally obtained their HAV stocks from the same source (FDA), there is some evidence that HM175 might also be variant HM175/24a. In summary, the identity of VSS 18f was confirmed as variant HM175/18f, pHM175—which originated from the same source as HM175—was shown to be variant HM175/24a and HM175 "43c-18f," which was propagated from the original VSS 24a was not variant HM175/24a but rather a VSS that shared most similarities with HM175/43c and HM175/18f.

TABLE 2. Results of Regions 2 and 4 sequencing: the sequences of VSS 18f, VSS 24a, and pHM175 were aligned to the sequences available in the databases, that is, wild-type HM175 (M14707), HM175/18f (M59808), HM175/43c (M59809), and HM175/24a (M59810)*

	Position													% identity			
	Region 2					Region 3			Region 4								
HAV variant	2646	2684	2780	2797	2864	2930	3018	3033	6813	6827	6920	7226	7247	7271	M59808 (18f)	M59809 (43c)	M59810 (24a)
VSS 18f	A	T	C	A	A	A	C	A	C	T	T	T	C	T	93	29	7
VSS 24a	A	C	C	A	A	A	T	G	C	T	C	T	C	T	57	64	43
pHM175	G	C	T	G	A	G	T	G	C	T	C	C	T	C	0	64	100
HM175 (wt, M14707)	A	C	C	A	T	A	T	A	C	T	T	C	T	T	71	64	29
HM175/18f (M59808)	A	T	C	A	T	A	C	A	C	T	T	C	T	T	100	36	0
HM175/43c (M59809)	A	C	C	G	T	A	T	G	C	C	C	C	T	C	36	100	64
HM175/24a (M59810)	G	C	T	G	A	G	T	G	T	C	C	C	T	C	0	64	100

* Only positions of informative SNPs are given; positions according to the wild-type sequence.

Inactivation of the HAV variants during pasteurization

For the current study, 21 independent pasteurization experiments were done, using four different batches of HSA with a protein concentration of 5 or 25%. HM175 variants of four different origins and designations were used, of which all but the HM175 variant obtained from Advanced BioScience Laboratories were used as two independently prepared VSSs. Complete HAV inactivation was not observed in any of these experiments. Similar reduction factors (p = 0.4) were obtained for HM175 (4.1 ± 0.1 log $TCID_{50}$, 3 [mean ± SD, n]) and pHM175 (4.6 ± 0.9 log $TCID_{50}$, 4). Significantly less ($p \leq 0.002$) overall inactivation through pasteurization was achieved for variant HM175/18f (2.6 ± 0.7 log $TCID_{50}$, 8) and for variant HM175 "43c-18f" (2.3 ± 0.4 log $TCID_{50}$, 6). For variants HM175/18f and HM175 "43c-18f" there was no significant difference ($p \geq 0.09$) in inactivation, irrespective of whether pasteurization was done in 5 or 25% HSA. For variants HM175 and pHM175 insufficient data sets were available to evaluate a differential inactivation in HSA of different protein concentrations.

During previous pasteurization studies done at or for Baxter, 13 independent pasteurization experiments were done, using different batches of HSA and of three different protein concentrations (3.5, 5, and 25%). HM175 variants of three different origins and designations were used as single VSS, with the exception of pHM175, for which two VSSs were used. Complete inactivation was observed only once, when variant pHM175 was subjected to heat treatment in 3.5% HSA (Table 3). When pHM175 was used at Baxter in 2000 or at BioReliance in 2001, similar (p = 0.7) reduction factors of 4.8 ± 0.7 log $TCID_{50}$, 4 [mean ± SD, n], or 5.1 ± 0.5 log $TCID_{50}$, 2, were obtained respectively, which did not differ (p = 0.5) from the reduction factor obtained for pHM175 in this study. The reduction factor obtained for HM175 at Advanced Bioscience Laboratories in 1995 (5.3 ± 0.4 log $TCID_{50}$, 3) differed significantly (p = 0.01) from the reduction factor obtained for HM175 in the current study. In addition, the study done at Baxter in 1994 that used VSS 24a resulted in a reduction factor (4.8 ± 0.7 log $TCID_{50}$, 4) that differed significantly

TABLE 3. Reduction factors according to HSA protein content and HM175 variant used in the current and previous inactivation through pasteurization studies

	Mean reduction factor (log $TCID_{50}$) ± SD, n			
HAV variant	3.5% HSA	5% HSA	25% HSA	All
HM175 (ABL)*	ND	4.1 ± 0.1, 3	5.3 ± 0.4, 3	4.7 ± 0.7, 6
pHM175 (BRL)†	>5.4, 1‡	4.2 ± 0.3, 5	5.3 ± 0.5, 4	4.8 ± 0.7, 10
VSS 24a (previous study)§	5.2 ± 0.9, 2	ND	4.4 ± 0.1, 2	4.8 ± 0.7, 4
HM175 "43c-18f" (current study)¶	ND	2.1 ± 0.1, 4	2.8 ± 0.6, 2	2.3 ± 0.4, 6
VSS 18f‖	ND	2.4 ± 0.2, 6	3.4 ± 1.3, 2	2.6 ± 0.7, 8
All	5.2 ± 0.7, 3	3.1 ± 1.0, 18	4.5 ± 1.2, 13	3.8 ± 1.3, 34

* Likely variant HM175/24a, based on the common origin with pHM175.
† Identified as variant HM175/24a through sequencing.
‡ > = no residual infectivity was observed.
§ Obtained as variant "HM175/24a."
¶ Stock made from VSS 24a but through sequencing shown to be more closely related to variant HM175/18f and HM175/43c.
‖ Confirmed in this study as variant HM175/18f through sequencing.

($p = 0.0001$) from the reduction factor obtained for HM175 "43c-18f," propagated from VSS 24a, in this study. The reduction factors obtained in the previous studies using variants HM175, pHM175, and VSS 24a were not significantly different ($p \geq 0.3$) from each other. These results however indicated that the variable "HAV variant" was not the sole contributor to reduction factor variability and the data was therefore further evaluated on the basis of HAV variant, the infectious dose of the different stock virus suspensions used for inactivation experiments, as well as the protein content of the HSA. The possibility that varying titers of virus stock suspensions would influence the assessment of HAV inactivation was evaluated by correlation analysis, which showed that, regardless of HAV variant, neither the stock titer infectivity ($r^2 = 0.004$) nor the infectivity of spiked starting material ($r^2 = 0.016$) correlated with the reduction factors obtained.

Effect of human albumin protein concentration on overall HAV variant inactivation

When data from this study and previous pasteurization studies were analyzed according to protein concentration of the HSA preparation and irrespective of the HM175 variant used, reduction factors of 5.2 ± 0.7 log $TCID_{50}$, 3 [mean ± SD, n]; 3.1 ± 1.0 log $TCID_{50}$, 18; and 4.5 ± 1.2 log $TCID_{50}$, 13, were determined for 3.5, 5, and 25% HSA, respectively (Table 3). The reduction factors differed significantly between 3.5 and 5% HSA ($p = 0.002$) and 5 and 25% HSA ($p = 0.002$) but not between 3.5 and 25% HSA ($p = 0.3$). When the data were analyzed according to protein concentration of the HSA preparation and taking into account the HM175 variant used for the inactivation, significant differences in reduction factors were seen between 5 and 25% HSA for pHM175 and HM175 ($p \leq 0.01$) but not for HM175/18f ($p = 0.09$) or the HM175 "43c-18f" variant (data from this study only, $p = 0.1$; Table 3). There was no significant difference in reduction factors obtained for the VSS 24a variant and 3.5 or 25% HSA (data from previous study only, $p = 0.3$). Further analysis by ANOVA showed that there were significant differences in mean reduction factors between HAV variants ($p = 0.000$) and HSA protein concentration ($p = 0.000$). Due to the limited number of data points available for subgroups "3.5% protein" and "VSS 24a" these were excluded from further statistical analysis. Multiple comparison of the means by Tukey's test showed that the simultaneous confidence intervals of HAV variants HM175 and pHM175 as well as VSS 18f and HM175 "43c-18f" grouped together, but were significantly different between the two groups ($p \leq 0.0001$). The achieved reduction factors in 5% HSA were significantly lower than the values achieved in 25% HSA ($p < 0.002$). Following this analysis, the results for HAV variants in the same group (i.e., HM175 and pHM175 as well as VSS 18f and HM175 "43c-18f") were grouped together according to protein concentration and presented as inactivation kinetics, which further illustrated the difference in overall achieved reduction between the HAV variant groups and the effect of protein concentration on inactivation (Fig. 1).

DISCUSSION

For more than a decade, the reason for the observed variability in HAV inactivation by pasteurization during the manufacture of HSA[2] was not explained. As this virus inactivation step and even the permitted stabilizers are closely defined in the respective US and European Pharmacopoeial monographs, the actual process of pasteurization cannot be the major source of variation. In this work, two factors that contribute to the variable HAV inactivation were identified: 1) in particular the different HAV variants as used in the pasteurization studies and 2) to some degree the different protein content of the HSA preparations. All pasteurization studies done at or for Baxter, as well as most pasteurization studies reported in literature, used variants of the HAV strain HM175.[3-8] As part of the initial HM175 variant characterization, the thermal stability of HM175/43c, HM175/18f, and the noncytopathic, cell culture–adapted p16 HM175 was determined but no difference in inactivation seen at the time.[9] However, variant HM175/24a, which was used in published inactivation studies,[5,7] was not included in this assessment.[9] In this work, a clear difference in overall achieved HAV reduction was seen when the HM175/24a[9] (represented in this study by HAV HM175 and pHM175) or the HM175/18f[9] (represented in this study by HAV VSS 18f and HM175 "43c-18f") variants were used, the latter being approximately 100-fold less sensitive to inactivation through pasteurization. These variants were generated through repeated cell culture passage on BSC1 (HM175/43c), FRhK4 (HM175/24a), or a combination of the two cell lines (HM175/18f)[9] and could therefore have collected further genomic modifications during cell culture for longer periods of time, a possible explanation for the observed difference in variant identity and inactivation properties between VSS 24a and the HM175 "43c-18f" variant. VSS 24a was originally obtained as HM175/24a before 1994 and at the time showed inactivation properties similar to the HM175/24a isolates used in the current work (HM175 and pHM175, Table 3). The HM175 "43c-18f" variant originated from VSS 24a, yet could not be confirmed as HM175/24a through sequencing in this work and also had inactivation properties more similar to HM175/18f (Table 3). These observations highlight the importance of stringent HAV culture conditions to minimize the possibility of further genomic modifications and a need to reverify isolate identity before use in inactivation studies.

Differences in inactivation were also seen for the different HM175 variants and HSA of different protein concentration, where pasteurization of 5% protein HSA consistently resulted in lower overall achieved reduction than pasteurization of 25% protein HSA, irrespective of the HAV variant used (Fig. 1). Therefore, another variable to consider in pasteurization studies using HSA is the protein content, which contributes to the variability of overall achieved HAV inactivation.

The results obtained in this study indicate that there is a substantial difference in overall achieved HAV reduction depending on which HAV variant is used for pasteurization. As HAV is known to be fairly robust and thus relatively resistant to inactivation,[13] the inactivation capacity of pasteurization should not be overestimated in the context of plasma product safety. For virus validation studies model viruses are used when for a specific virus of concern, that is, a target virus, no suitable system is available for quantitative investigation, for example, for hepatitis B virus and hepatitis C virus. This study shows that even when a specific target virus, here HAV, can be investigated in virus reduction studies, the choice of the particular virus variant is critical and requires verification with respect to compliance with regulatory guidance: ". . . where two similar viruses could be used for validation studies . . . , the virus considered to be the more resistant *should* be used."[17] From the data

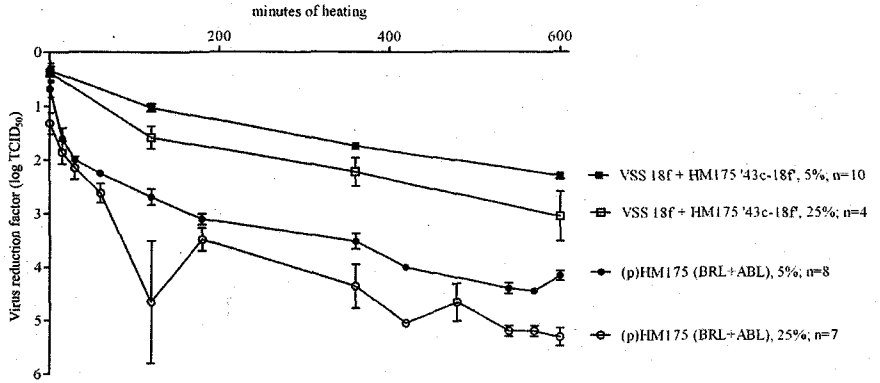

Fig. 1. Inactivation kinetics of the HAV HM175 variants during pasteurization in the manufacture of HSA. The results of HAV pasteurization studies done for/at Baxter using 5 or 25% HSA are illustrated; error bars indicate SEM. Data were grouped according to HAV variants that were shown to have similar reduction factors (variants HM175 and pHM175 as well as VSS 18f and HM175 "43c-18f"). n = number of experiments/data sets at time-points "0" and "600" minutes (not all other time points contain data from all experiments) BRL = BioReliance; ABL = Applied BioScience Laboratories.

presented here, the HM175/18f variant that was obtained from ATCC might be considered the variant of choice for pasteurization studies, with the HSA protein content taken into consideration as a further variable affecting overall HAV inactivation.

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CONFLICT OF INTEREST

MF, JK, JM, and TRK are employees of Baxter BioScience; JM and TRK have stock options.

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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年1月16日	新医薬品等の区分	厚生労働省処理欄
一般的名称	①②③④⑤ポリエチレングリコール処理人免疫グロブリン ⑥⑦人免疫グロブリン					
販売名（企業名）	①献血ヴェノグロブリンIH5%静注 0.5g/10mL（ベネシス） ②献血ヴェノグロブリンIH5%静注 1g/20mL（ベネシス） ③献血ヴェノグロブリンIH5%静注 2.5g/50mL（ベネシス） ④献血ヴェノグロブリンIH5%静注 5g/100mL（ベネシス） ⑤献血ヴェノグロブリン-IH ヨシトミ（ベネシス） ⑥ノグロブリン筋注 450mg/3mL「ベネシス」（ベネシス） ⑦ノグロブリン筋注 1500mg/10mL「ベネシス」（ベネシス）	研究報告の公表状況	Vox Sanguinis 2012; LETTER:	公表国 アメリカ，ドイツ，スウェーデン	

研究報告の概要

我々はリアルタイムRT-PCR法を用いて、最大96人分の献血血漿ミニプール中のE型肝炎ウイルス(HEV) RNAの存在について、ドイツ、スウェーデンと米国の165,010人分の血漿を調査した。
スウェーデン人:95,835人とドイツ人:18,100人の献血がスクリーニングされ、スウェーデン人12人とドイツ人4人がHEV RNA陽性であった。実際のミニプールサイズを考慮すると、HEV陽性献血の率は、それぞれスウェーデン人供血者で1/7,986、ドイツ人供血者で1/4,525であった。対照的に、米国の51,075人の献血はHEV陽性を確認できなかった。
ミニプールがHEV RNA陽性の場合、個別の陽性血漿は分解され、医薬品製造から排除された。
HEV株の分析において全ての症例が遺伝子型3であることが明らかになった。また、検体の多くはウインドウ期の献血であった。

報告企業の意見

HEVは直径27～38nmの球状粒子で、エンベロープはなく、長さ約7,300塩基対の一本鎖RNAを内包している。万一、原料血漿にHEVが混入したとしても、BMC及びCPVをモデルウイルスとしたウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考えている。

今後の対応

本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリンIH5%静注0.5g/10mLの記載を示す。

2. 重要な基本的注意
1) 本剤の原材料となる献血者の血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体、抗HTLV-I抗体陰性で、かつALT（GPT）値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohnの低温エタノール分画で得た画分からポリエチレングリコール4000処理、DEAEセファデックス処理等により人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及びpH3.9～4.4の条件下での液状インキュベーション処理及びpH3.9～4.4の条件下での液状インキュベーション処理を施しているが、投与に際しては、次の点に十分注意すること。

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Vox Sanguinis

LETTER

Occurrence of hepatitis E virus RNA in plasma donations from Sweden, Germany and the United States

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Dear Editor,

We have investigated 165 010 plasma donations from Germany, Sweden and the United States for the presence of hepatitis E virus (HEV) RNA in plasma mini-pools of up to 96 donations using a proprietary internally controlled real-time RT-PCR assay; the 95% cut-off of the assay is ~250 IU/ml as determined by dilution of the WHO International Standard for HEV RNA [1]. From Europe, 95 835 Swedish and 18 100 German donations were screened, of these, 12 Swedish and four German donations were positive for HEV RNA. Allowing for actual mini-pool size, the rate of HEV-positive donations was 1:7986 and 1:4525 for the Swedish and German donors, respectively. In contrast, no HEV-positive donations were identified in 51 075 donations from the United States. Whenever mini-pools were positive for HEV RNA, individual positive donations were resolved and excluded from pharmaceutical production; 12 of the samples were characterized by molecular and serological analysis (Table 1). Analysis of the HEV strains revealed genotype 3 in all cases. Genotyping was performed by amplification of the ORF2/3 region of the HEV genome using the OneStep RT-PCR kit (Qiagen GmbH, Hilden, Germany) and the forward primer 5′-GGGTGGAATGAATAA CATGT and reverse primer 5′-AGGGGTTGGTTGGATGAA or 5′-GGGGCGCTGGGMCTGGTCACGCCAAG. Amplification products were sequenced directly; all sequenced strains were distinct from each other (accession numbers JN995562-JN995573). The closest RNA sequence matches from BLAST searches were between an HEV strain from a wild boar (accession number FJ705359) and samples 6 and 7 obtained from German donors. Viral loads varied between ~3·2–5·7 $\log_{10}$ IU/ml HEV RNA and are in a similar range reported for Japanese blood donors [2]. Anti-HEV IgM and anti-HEV IgG in the individual donations were determined using EIAs from Wantai (Wantai, Beijing, China). The majority of samples were window-period donations. Only one viraemic sample was positive for IgM, another for IgG (confirmed by repeat testing using different kit lots). Three samples were initially reactive; however, repeat testing using different kit batches gave negative results. Such

Table 1 Summary of characterization of HEV RNA-positive plasma donations

Sample code	IgM[a]	IgG[a]	ALT[b]	Viral load ($\log_{10}$ IU/ml)[c]	Country of origin
1	+/−	−	Neg.	3·22	Sweden
2	+	+/−	Neg.	3·26	Germany
3	−	−	Neg.	5·35	Germany
4	−	−	Neg.	4·39	Sweden
5	−	+	Neg.	4·95	Sweden
6	+/−	−	Elevated	4·54	Germany
7	−	−	Neg.	4·19	Germany
8	−	−	Neg.	4·76	Sweden
9	−	−	Neg.	3·86	Sweden
10	−	−	Elevated	4·64	Sweden
11	−	−	Elevated	3·20	Sweden
12	−	−	Neg.	5·68	Sweden

ALT, alanine transaminase.
[a]Positive samples (+) defined as S/Co ≥1 (according to the kit specifications); equivocal samples (+/−) gave an S/Co ≥1 on initial round of testing and S/Co < 1 on repeat testing using alternative batches of kit. Negative samples (−). Positive control for IgG was performed using the WHO International Reference Reagent for anti-HEV IgG (95/584).
[b]> 80 IU/l.
[c]RNA titres determined by real-time RT-PCR in comparison to the WHO International Standard for HEV RNA – code number 6329/10 [1].

equivocal results highlight some of the problems with standardization of HEV serological assays. Alanine transaminase (ALT) levels were slightly elevated (80–110 IU/l) in only 3 of 12 viraemic donations tested. This indicates that screening of blood/plasma by ALT is not a reliable measure for exclusion of HEV viraemic donors.

Our results are in accordance with a recent investigation from the UK detecting 6 of 880 mini-pools (with mini-pool comprising 48 donors) positive for HEV RNA [3]. Considering a ratio of 1:4525 viraemic donations in Germany, the limited viraemic titre of some donations, and a size of ~3500 donations per plasma fractionation pool, this could explain why we found ~10% of large plasma pools for fractionation from Germany positive for HEV RNA [4]. We agree with Ijaz *et al.* [3] that such high numbers of HEV-positive blood donations in Europe suggest many subclinical infections [5], and the consequences of HEV contamination of blood and plasma warrants further investigation.

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一般的名称	①②③④人血清アルブミン ⑤⑥乾燥濃縮人アンチトロンビンⅢ ⑦人ハプトグロビン ⑧乾燥濃縮人血液凝固第Ⅷ因子	研究報告の公表状況	公表国 中国
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研究報告の概要

本研究は、雲南省のブタと一般人における E 型肝炎ウイルス(HEV)の陽性率と特徴を評価した。
960 の血清、95 の肝臓、60 の糞便検体が無作為に養豚場と屠殺場から集められ、加えて 173 人のヒト血清が血清学的調査と RT-nPCR 分析のために省都で採取された。スクリーニングの結果、養豚場から集められた 621 検体は、73.2%～83.5%の範囲で HEV-特異的 IgG 陽性率を示し、全体的な血清陽性率は 78.9%(490/621)であった。更に、血清陽性率は年齢と共に増加することが分かった。ヒト血清検体の陽性率は 39.9%(69/173)であった。HEV RNA はブタ糞便:5、ブタ肝臓:6 と抗 HEV-IgM 陽性ヒト血清検体:1 で検出された。12 の HEV 株の配列は、9 つの異なる核酸塩基配列を確認した。系統発生と分子進化解析は、9 つの配列が HEV 遺伝子型 4 に属している全ての分離株と共に、84.2%～100.0%の核酸塩基配列同一性を共有していることが分かった。
この研究の結果は、ブタ群とヒト集団の両方で HEV 遺伝子型 4 の流行は深刻で、当局は南西中国の HEV 流行により注意を払うべきことを示唆している。

使用上の注意記載状況・その他参考事項等

代表としてノイアート静注用 500 単位の記載を示す。
2. 重要な基本的注意
(1)本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-I 抗体陰性で、かつ ALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分から人アンチトロンビンⅢを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及びウイルス除去膜によるろ過処理を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見	今後の対応
HEV は直径 27～38nm の球状粒子で、エンベロープはなく、長さ約 7,300 塩基対の一本鎖 RNA を内包している。万一、ヘパリンの原料であるブタ小腸粘膜に HEV が混入したとしても、HPV1 及び PPV をモデルウイルスとしたウイルスクリアランス試験成績から、ヘパリンの製造工程において不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

ヘパリン

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Seroprevalence and molecular detection of hepatitis E virus in Yunnan Province, China

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Abstract In this study, the prevalence and characteristics of hepatitis E virus (HEV) in pigs and the general population in the Yunnan province, China, were evaluated. Nine hundred sixty sera, 95 liver and 60 feces samples were randomly collected from pig farms and abattoirs, in addition 173 human sera were sampled in the provincial capital city for a serological survey and an RT-nPCR assay. The screening results showed that among 621 samples collected from five pig farms, the HEV-specific IgG positive rate ranged from 73.2% to 83.5%, and the overall seroprevalence was 78.9% (490/621). A further analysis revealed that the seroprevalence increased with age. The positive rate of human serum samples was 39.9% (69/173). HEV RNA was detected in five swine feces, six swine liver and one anti-HEV-IgM-positive human serum sample by RT-nPCR. Sequence and alignment of the 348-nt PCR-amplified products of 12 HEV strains identified nine distinct nucleotide sequences. Phylogenetic and molecular evolutionary analysis revealed that these nine sequences shared 84.2% to 100.0% nucleotide sequence identity with each other, with all isolates belonging to genotype 4 HEV and clustering with other Chinese swine and human HEV sequences determined earlier. This study results suggest that the prevalence of genotype 4 HEV is serious, both in pig herds and in the human population, and authorities should pay more attention to the prevalence of HEV in southwest China.

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Introduction

Hepatitis E (HE), which is caused by hepatitis E virus (HEV), is the most frequent cause of acute hepatitis, acute liver failure, and acute-on-chronic liver failure. HE remains an important public health problem, mainly in developing countries, with sporadic cases increasingly diagnosed in some developed countries [1]. A World Health Organization (WHO) estimate, based on selected seroprevalence data and case series suggests that at least one third of the world population, residing mainly in Asia, Africa, the Middle East, and Central America has already been infected [18].

The HEV virion is non-enveloped, is approximately 27–34 nm in size, and contains a positive-sense, single-stranded RNA of approximately 7.2 kb, encoding three discontinuous partially overlapping open reading frames (ORF1, 2 and 3), with short 5′ and 3′ untranslated regions (UTRs). HEV was previously classified as a member of the family *Caliciviridae*, but it was classified as a member of the genus *Hepevirus* in the separate family *Hepeviridae* in 2004 [13]. Based on nucleotide sequence analysis, mammalian HEV has been divided into four genotypes, namely genotypes 1–4. Although a single serotype is recognized, extensive genomic diversity has been observed among HEV isolates, and it has been suggested that the four genotypes could be further divided into at least 24 subgenotypes (1a-1e, 2a-2b, 3a-3j and 4a-4g) [12].

It has been recognized that hepatitis E is a zoonotic disease and several species of animals have been reported as reservoirs. The virus has been isolated from humans and a variety of animal species, including swine, rabbit, deer, birds and horses, and still more domestic and wild animals have been found positive for anti-HEV IgG [14]. Within the family, avian HEV is now considered to belong to a

separate genus, while rabbit HEV is genetically related to but distinct from other known mammalian HEVs [5].

In China, genotype 4 was first reported in 1993 and has became the dominant cause of HE in China since 2000 [24]. Although molecular and seroepidemiological investigations of HEV have been performed in many provinces of China, there is a shortage of epidemiological data from Yunnan province (southwest China), and the prevalent genotypes have not been characterized previously. The aim of the present study was to investigate the prevalence of HEV infection in pigs and humans and to determine the extent of genetic variation of Yunnan HEV strains using phylogenetic and molecular evolutionary analysis.

Materials and methods

Sample collection

A total of 621 serum samples were randomly collected from five pig farms across the Yunnan province of China. Beside of this random sampling included 339 swine sera and 95 swine liver samples, all collected from four abattoirs and 60 swine feces samples originated from three pig farms in Kunming, the provincial capital of Yunnan. In addition, 173 sera (63 male and 110 female) were collected anonymously from the general human population in Kunming by the Center for Disease Control and Prevention of Yunnan province (Table 1). For collection of swine sera, 2-5 mL of blood was collected from swine in sterilized tubes. The samples were incubated in water for 1 h at 37°C. And after clotting and centrifugation at 3000 rpm for 10 min sera were separated and stored immediately in a −80°C freezer until use.

Detection of HEV antibodies in serum

Swine sera were tested for anti-HEV IgG, while human sera were tested for both HEV IgG and IgM using a commercial ELISA kit (Wantai Biological Pharmacy Co., Beijing, China) according to manufacturer's instructions. Human sera that were positive for anti-HEV IgM were kept for HEV RNA detection. The samples with an optical density less than the cutoff value (mean optical density for the three negative controls on each plate plus 0.12) were considered as negative. Samples with an optical density greater than or equal to the cutoff value were tentatively considered reactive and then retested to confirm the result. The absorbance was determined at 450 nm (BioTek ELx800).

Statistical analysis

Swine and human serum samples were subdivided into several age categories, and a possible correlation between HEV prevalence and age was identified. Statistical analysis was performed using the unpaired Student's t-test, and the results were considered significant when the p-value was less than 0.05. Descriptive statistical analysis was performed using SPSS software for Windows 13.0 (SPSS Inc., Chicago, USA), and the 95% confidence interval was calculated as described [23].

RNA extraction and reverse transcription-nested PCR

Total RNA was extracted from 100 μl of feces suspension, homogenized liver suspension and anti-HEV IgM serum using TRIzol (Invitrogen, USA) according to the manufacturer's instructions. RT-nPCR was performed as described elsewhere [10, 22].

Sequencing and phylogenetic analysis

The second-round PCR products were purified using a PCR product purification kit and ligated into the pMD18-T vector (Takara, Dalian, China). The plasmid was then used to transform *E. coli* DH5α. The plasmids were extracted, and the inserts were sequenced at Sangon Biological Engineering Company (Shanghai, China). The 348-nt consensus sequences were aligned using DNAman (version 6.0, Lynnon Corporation). Nucleotide sequence identity between isolated sequences and members of the four genotypes were calculated using Lasergene sequence analysis tools MegAlign (DNASTAR, Inc.). Phylogenetic and molecular evolutionary genetics analyses were conducted using the neighbor-joining method with MEGA 4 [19]. Thirty-five related HEV strains listed in Table 2 were used as references in the analysis, and an avian HEV strain (AY535004) was included as an outgroup.

Table 1 Samples used to assess the current epidemiology of HEV in humans and pigs in the Yunnan province

Sample	No.	Source (no.)	Location	Screening		
				HEV-IgG	HEV-IgM	HEV-RNA
Sera	621	Pig farm (5)	Across the province	Y	N	N
	339	Abattoir (4)	Kunimg	Y	N	N
	173	Normal population (-)	Kunimg	Y	Y	Y
Feces	60	Pig farm (3)	Kunimg	N	N	Y
Liver	95	Abattoir (4)	Kunimg	N	N	Y

Y-tested, N-not tested

Results

Prevalence of anti-HEV antibodies in pigs and humans

Swine

Among 621 samples collected from five pig farms, the positive rate for HEV-specific IgG ranged from 73.2 to 83.5%, and the overall seroprevalence was 78.9% (490/621). There was no significant difference in the serum

Table 2 HEV strains used in the phylogenetic and sequence analyses

Genotype	Strain	Host	Country	Accession no.
I	Burl	Human	Myanmar: Rangoon	M73218
	SAR-55	Human	Vietnam	M80581
	Uigh179	Human	China: Xinjiang	DI1093
	Hetian87	Human	China: Xinjiang, Hetian	D11092
	HEVNE8L	Human	Myanmar: Rangoon	D10330
	K52-87	Human	China: Xinjiang, Kashi	L25595
	hev037	Human	India	X98292
	Morocco	Human	Morocco	AY230202
II	M	Human	Mexico	M74506
III	HEV-US1	Human	USA	AF060668
	HEV-US2	Human	USA	AF060669
	Meng	Swine	USA	AF082843
	JRA1	Human	Japan: Tokyo	AP003430
	SwJ570	Swine	Japan: Tochigi	AB073912
	Arkell	Swine	Canada	AY115488
	Osh-205	Swine	Kyrgyzstan: Osh	AF455784
	swCNZJ304-06	Swine	China: Zhejiang	EF187823
	JMNG-Oki02C	Mongoose	Japan: Okinawa	AB236320
	WB1-Aichi	Wild boar	Japan: Aichi	DQ079628
	JDEER-Hyo03	Wild deer	Japan: Hyogo	AB114179
IV	swYN-01	Swine	China: Yunnan	FJ409465
	swGX40	Swine	China: Guangxi	EU676172
	ECh166	Human	China: Jiangsu	HM439278
	HC2-40	Swine	China: Hunan	EU620641
	HC10-44	Swine	China: Hunan	EU620651
	WHH18	Human	China: Hubei	FJ461770
	WH09	Swine	China: Wuhan	GU188851
	HE-JVN1	Human	Viet Nam	AB168096
	swIM12-9	Swine	China: InnerMongolia	AB550640
	CHN-XJ-SW13	Swine	China: xinjiang	GU119961
	Ch-shsw1	Swine	China: Shanghai	EU034707
	Ch-shsw6	Swine	China: Shanghai	EU034713
	Ch-shhu2	Human	China: Shanghai	EU034715
	SJ14	Swine	China: Zhejiang	AJ428856
	KNIH-hHEV4	Human	South Korea	FJ763142
		Avian	USA	AY535004

prevalence rate between different farms($P<0.05$. The seroprevalence rates of serum samples collected from four abattoirs were 78.0% (39/50), 80.6% (145/180), 78.9% (71/90) and 78.9% (15/19), and the average was 79.6% (270/339). Student's t-test showed no significant difference in the serum prevalence rates between the different abattoirs ($P<0.05$) (Table 3).

Swine serum samples collected from pig farms were divided into four age groups, under 2 months, 2-5 months, 6-10 months and over 11 months, and the anti-HEV IgG positive rate was analyzed with respect to age. The positive rate was 40.3%, 80.8%, 83.3% and 90.3%, respectively. The overall positive rate was 70.0%; increased accumulated with age (Table 4).

Humans

One hundred seventy-three samples were divided in to four age groups: 17-23 years, 24-30 years, 31-36 years and over 37 years. The anti-HEV-IgG-positive rates for the different age groups were 28.7% (17-23), 50.0% (24-30), 57.1% (31-36), and 50.0% (37-50). The 17-23-year-old group had a lower rate than other groups ($X^2=9.322$, $P<0.05$). The total positive rate was 39.9%. No significant difference was found between the males and females (41.3% vs 39.1%) ($P<0.05$). The positive rate for anti-HEV IgM was 6.4% (11/173) (Table 4).

RT-nPCR detection

HEV was detected by RT-nPCR at a rate of 8.3% (5/60) in swine feces, 6.3% (6/95) in swine liver samples, and 9.1% (1/11) in anti-HEV-IgM-positive human serum samples. The 348-nt PCR-amplified products of 12 isolates (five in feces, six in liver and one in anti-HEV-IgM-positive human sera) were sequenced and designated as swYN01 to swYN09, KMsw-1, KMsw-3 and HuYN01. Sequence alignment revealed that swYN01, swYN04, swYN05, swYN06 and HuYN01 shared the same sequence, and therefore nine sequences were finally submitted to GenBank: swYN01 (GU560156), swYN02 (HQ828102), swYN03 (HQ828103), swYN07 (HQ828104), swYN08 (HQ828105), swYN09 (HQ828106), KMsw-1 (HQ008863), KMsw-3 (HQ008864) and HuYN01 (HQ828107).

Table 3 HEV seroprevalence in sampled pig herds and abattoirs in the Yunnan province, China

Location	Farm	No.	HEV IgG positive	95% CI
Farms	1	41	30 (73.2%)	0.59-0.87
	2	54	42 (77.8%)	0.67-0.89
	3	98	72 (73.5%)	0.65-0.83
	4	258	204 (79.1%)	0.74-0.84
	5	170	142 (83.5%)	0.78-0.90
	Total	621	490 (78.9%)	0.76-0.82
Abattoirs	1	50	39 (78.0%)	0.67-0.89
	2	180	145 (80.6%)	0.75-0.87
	3	90	71 (78.9%)	0.71-0.87
	4	19	15 (78.9%)	0.61-0.97
	Total	339	270 (79.6%)	0.76-0.84
Total	-	960	79.2% (760/960)	0.76-0.82

Table 4 Age dependence of the prevalence of HEV antibodies in pigs and the general human population

Host	Age	No.	Anti-HEV IgG	95% CI	Anti-HEV IgM
Swine	<2 months	201	81 (40.3%)	0.34-0.47	-
	2-5 months	245	198 (80.8%)	0.76-0.86	-
	6-10 months	30	25 (83.3%)	0.70-0.97	-
	>11 months	145	131 (90.3%)	0.85-0.95	-
	Total	621	435 (70.0%)	0.66-0.74	-
Human	17-23	87	25 (28.7%)	0.19-0.39	5 (5.7%)
	24-30	54	27 (50.0%)	0.37-0.63	3 (5.5%)
	31-36	14	8 (57.1%)	0.31-0.83	1 (7.1%)
	>37	18	9 (50.0%)	0.27-0.73	2 (11.1%)
	Total	173	69 (39.9%)	0.33-0.47	11 (6.4%)

Phylogenetic and molecular evolutionary analyses

Phylogenetic and molecular evolutionary analysis revealed that nine isolates shared 84.2-100.0% nucleotide sequence identity with each other and 77.3-81.3%, 52.2-53.3%, 73.4-88.5% and 77.7-100.0% nucleotide sequence identity with strains representing genotypes 1, 2, 3 and 4. These isolates are all belonged to genotype 4 HEV and clustered with China swine and human HEV sequences generated earlier (Fig. 1). A analysis based on strains of known subtype revealed that the nine isolated strains could be further divided into four different subtypes (4b, 4c, 4e and 4?), including a newly identified subtype (swYN08) that, to our knowledge, has not been subtype in previous studies (Fig. 2).

Discussion

There is increasingly evidence that HEV is enzootic, and pigs are considered as one of the major reservoirs for human infection. Among the four genotypes of HEV, genotypes 1 and 2 are linked to large HEV epidemics in the human population and transmitted mostly by the fecal-oral route through contaminated water. These two genotypes are endemic in regions of Asia and Africa and have only been isolated from humans. In contrast, HEV strains of genotypes 3 and 4 infect both humans and a variety of mammalian species, in particular, domestic pigs and wild boars, and are responsible for sporadic cases of acute hepatitis E in both developing and industrialized countries [15]. The identification of HEV in essentially all swine-producing regions worldwide and the demonstrated ability of cross-species infection of this virus raise potential public-health concerns about zoonotic transmission [4, 20, 21]

In China, genotype 4 HEV is endemic in pigs, and sporadic human cases of hepatitis E throughout the country are causes by genotypes 1 and, perhaps more often, 4 [8]. Besides genotypes 1 and 4, genotype 3 also has been identified in eastern China since 2006 [16]. In this study, a serological and molecular survey revealed a 78.9% prevalence of anti-HEV IgG in selected pig farms and 79.6% in abattoirs respectively. An 8.3% HEV-RNA-positive rate in swine feces and 6.3% in swine liver samples indicated that HEV infection is highly prevalent among pigs in the Yunnan province. The prevalence was equal in the northern and southern parts of China (73.5–78.8%) [6, 10].

About 10-20% of people in China have signs of past HEV infection [25]. The 39.9% prevalence of anti-HEV IgG, 6.4% of HEV-IgM, and 9.1% of HEV-RNA in anti-HEV-IgM-positive human serum samples in the general human population revealed a more serious prevalence situation in Yunnan. Also, it is worth mentioning that human

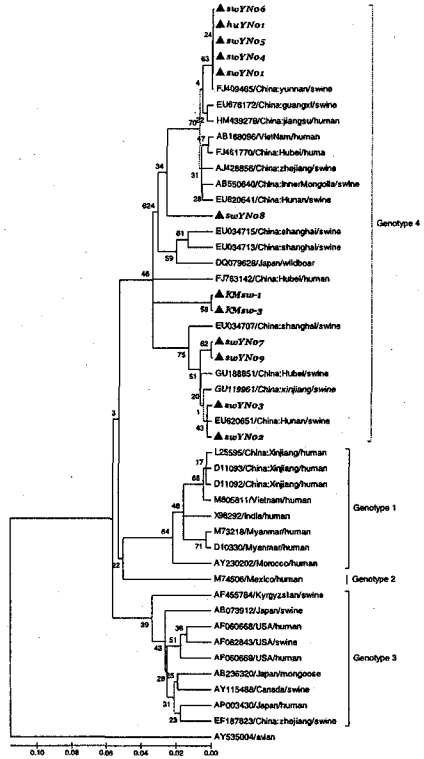

Fig. 1 Phylogenetic tree constructed by alignment of the 348-nt nucleotide sequence of ORF2 using the neighbour-joining method and evaluated using the interior branch test method with Mega 4 software. Percent bootstrap support is indicated at each node. The GenBank accession number, source, and country of origin are indicated. An avian HEV strain is included as an outgroup. The isolates identified in this study are indicated by a black triangle. The scale bar indicates nucleotide substitutions per position

strain HuYN01 shows a high degree of genetic relatedness to swine strains isolated in the same region, and this again confirms the capability of a zoonotic spread of genotype 4 HEV.

4a, 4b, 4c, 4d are the most prevalent in China of the seven subtypes (4a-4g) of genotype 4 HEV [6, 11, 23], and combined with our results, it can be suggested that there are at least four subgenotypes of genotype 4 (4c, 4d, 4b, and 4?) current prevalent in swine populations of Yunnan, and these

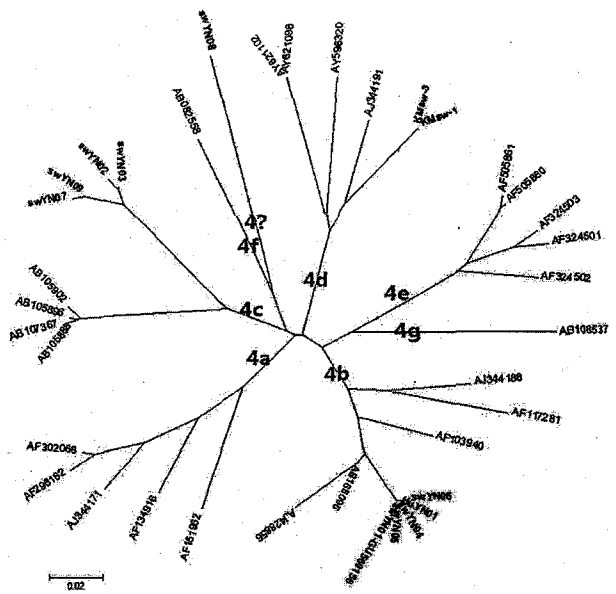

Fig. 2 Unrooted phylogenetic tree of HEV genotype 4 isolates based on partial ORF2 sequences The subgenotypes of these isolates were determined with seven confirmed subtypes of genotype 4 HEV as references. The results showed that 12 genotype 4 isolates found in this study were divided into four different subtypes. The tree was constructed using Mega 4, with 1000 replications

strains are genetically related to the strains that are prevalent in the general human populations in this region.

It has been demonstrated that viral sequences recovered from pig livers sold in local grocery stores are closely related, or identical in some cases, to the viruses recovered from human patients [2, 7, 10, 21], and the contaminating virus in commercial pig livers sold in local grocery stores remains fully infectious [3]. Sporadic cases of acute hepatitis E have been reported in patients who consumed wild boar meat [9]. This study found 6.3% of swine liver samples to be positive for HEV RNA, which once again raises additional public-health concerns over pork safety, the risk of HEV infection via the consumption of undercooked pork products. Moreover, results indicate the need for established quarantine methods towards HEV to prevent the flow of contaminated pig livers to the market chain, since there seems to be a lack of awareness from governments to deal with the situation.

As HEV has an animal origin it is suggested that some ancestral strains could have subsequently developed the capacity to be transmitted efficiently to and between humans. To prevent emergence of novel human diseases, it is important to understand the evolutionary and epidemiological processes facilitating this transition from enzootic to human-to-human transmission [17]. At present, the evolutionary relationship among the four HEV genotypes in humans and swine is not yet fully understood. The isolation of the nine HEV strains represented in southeastern China would facilitate study of the evolutionary history of this virus and the variation in selective pressures acting on different genotypes.

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医薬品
医薬部外品　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2012年1月26日	新医薬品等の区分	厚生労働省処理欄

一般的名称	①②③④⑤ポリエチレングリコール処理人免疫グロブリン ⑥⑦人免疫グロブリン	研究報告の公表状況	公表国 アメリカ
販売名（企業名）	①献血ヴェノグロブリン IH5%静注 0.5g/10mL (ベネシス) ②献血ヴェノグロブリン IH5%静注 1g/20mL (ベネシス) ③献血ヴェノグロブリン IH5%静注 2.5g/50mL (ベネシス) ④献血ヴェノグロブリン IH5%静注 5g/100mL (ベネシス) ⑤献血ヴェノグロブリン-IH ヨシトミ (ベネシス) ⑥グロブリン筋注 450mg/3mL「ベネシス」 (ベネシス) ⑦グロブリン筋注 1500mg/10mL「ベネシス」 (ベネシス)	TRANSFUSION 2012; 52 : 447-454	

研究報告の概要

ウエストナイルウイルス(WNV)の個別供血者核酸検査(NAT)を実施したにも拘わらず、3例のWNV輸血感染症例が報告され(2004-2008)、現在の血漿についての検査では全てのWNV感染献血を検出できないことを示唆した。WNV感染供血者からの赤血球成分の19/20は、血漿成分より1Log以上ウイルス量が高かった。
この研究の目的は、血液スクリーニング管由来のペア検体の差異のあるウイルス量により、血漿と比較して全血の検査に価値が高いことを確立することであった。
横断的研究では、血清陰性献血(n=29)は全血より血漿中でWNV RNAの濃度が4倍以上高かった、ところが血清陽性献血(n=13)ではWNV RNA濃度が血漿中より全血中の方が10倍高かった。全ての縦断的研究の参加者は、追跡調査を通して血清陽性であった。全血のウイルス量は血漿ウイルス量より常に高かった。
血漿の代わりに全血を用いたWNV NATの感度向上は確認したが、その向上は血清陽性段階に限定されていると思われた。しかし、血液スクリーニングにおけるこれらの知見の意味を明らかにするには、持続的な全血ウイルス血症の感染力を確立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

代表として献血ヴェノグロブリン IH5%静注 0.5g/10mLの記載を示す。
2. 重要な基本的注意
1)本剤の原材料となる献血者の血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体、抗HTLV-I抗体陰性で、かつALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査(NAT)を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohnの低温エタノール分画で得た画分からポリエチレングリコール4000処理、DEAEセファデックス処理等により人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及びろ過膜処理(ナノフィルトレーション)及びpH3.9～4.4の条件下での液状インキュベーション処理を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見	今後の対応
ウエストナイルウイルス（West Nile virus：WNV）は、フラビウイルス科フラビウイルス属に属し、大きさは40～60nmのエンベロープを有するRNAウイルスである。血清学的には日本脳炎ウイルス群に含まれ、蚊によって媒介される。 FDAは、2005年6月の業界向けガイダンス改訂版において、「FDAは全ての血漿分画製剤について現在行われているウイルス低減工程を再調査した。現在行われている方法は、WNVと分類上関連しているフラビウイルスを不活化することがバリデートされている。」と評価し、CPMPもまたポジションステートメントにおいて、血漿分画製剤の製造工程でWNVは不活化・除去されると評価している。万一、原料血漿にWNVが混入しても、BVDをモデルウイルスとしたウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

グロブリン

BENESIS 2012-003

Relative distribution of West Nile virus RNA in blood compartments: implications for blood donor nucleic acid amplification technology screening

Lori Lai, Tzong-Hae Lee, Lestie Tobler, Li Wen, Ping Shi, Jeff Alexander, Helen Ewing, and Michael Busch

BACKGROUND: Despite implementation of targeted individual-donor nucleic acid test (NAT) screening of blood donors for West Nile virus (WNV), three "breakthrough" WNV transfusion transmission cases were reported (2004-2008), suggesting that current plasma-based assays are unable to detect all WNV-infectious donations. *A 2007 report found that 19 of 20 red blood cell components from WNV-infected donors contained 1 log higher viral load than plasma components.* This study's aim was to further establish the value of screening whole blood relative to plasma for WNV RNA by generating differential viral loads on paired samples derived from blood screening tubes.

STUDY DESIGN AND METHODS: WNV RNA–positive donors identified by routine NAT screening were enrolled and quantitative viral data were generated using cross-sectional (index-donation) and longitudinal (follow-up) specimens. A real-time reverse transcription–polymerase chain reaction viral load assay was used on both study sample sets and replicate qualitative NAT screening assays were also used on the longitudinal study samples.

RESULTS: For the cross-sectional study, seronegative index donations (n = 29) had WNV RNA concentrations fourfold higher in plasma than in whole blood, whereas for seropositive donations (n = 13), the WNV RNA concentrations were 10-fold higher in whole blood than in plasma. All 10 longitudinal study participants were seropositive throughout the follow-up study; whole blood viral load was consistently greater than plasma viral load (mean difference, 343 copies; $p < 0.001$) up to 200 days after index.

CONCLUSION: The improved sensitivity of WNV NAT using whole blood instead of plasma was confirmed, but appears to be limited to better detection in seropositive stages. However, the implication of these findings for blood screening requires further study to establish the infectivity of persistent whole blood viremia.

In 1999, West Nile virus (WNV), a mosquito-borne flavivirus, was reported as the causative agent linked to a cluster of viral encephalitis cases in Queens, New York.[1] The first WNV transfusion-transmitted infection was observed in 2002.[2] Later that year, 22 additional transfusion-transmitted infections were confirmed.[3]

After these transfusion transmission reports, the Food and Drug Administration (FDA) asked blood screening *test manufacturers to develop WNV nucleic acid amplification technology (NAT) assays to detect infected donors at the earliest seronegative stages.*[4] By July 2003, two investigational qualitative WNV NAT assays were released, and by July 2005, more than 1000 viremic blood donors detected by these assays were reported.[3]

WNV NAT screening was initially implemented in minipool (MP) test formats employed for NAT screening for other viruses.[5] Cost-effective and logistically advantageous, MP-NAT screening involved pooling specimens from 6 to 24 donors and screening the combined specimen pool for viral RNA.[6] *After six transfusion transmission cases linked to MP-NAT screened donors*

ABBREVIATIONS: BSRI = Blood Systems Research Institute; Ct = cycle threshold; ID = individual donation; MP = minipool; S/CO = signal to cutoff; TMA = transcription-mediated amplification; WNV = West Nile virus.

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were reported in 2003, "triggering" strategies were developed and implemented that involved switching from MP-NAT to more sensitive individual-donation (ID) NAT when criteria indicating a higher risk of WNV infection in donors were met.[7] In addition, systems were developed to enable regional communication of WNV yield data among donor centers to support rapid triggering of ID-NAT when adjacent regions experience epidemic activity. Despite implementation of targeted ID-NAT, three more WNV transfusion-transmitted infections were reported between 2004 and 2008; all were linked to MP-NAT screened donations that were missed because ID-NAT triggering criteria were not reached at the screening sites.[7,8]

One approach to reducing WNV transfusion transmission risk could be to develop an alternative specimen preparation protocol that would increase the clinical sensitivity of current WNV NAT screening assays. In 2007, FDA investigators found an order of magnitude greater viral loads in the red blood cell (RBC) components collected from WNV-infected blood donors compared to that in the donors' corresponding plasma components.[9] This FDA study also reported that RBC-associated WNV was infectious in Vero cell cultures and another report from this group indicated that WNV antibody–positive specimens could also infect cells in vitro.[10]

Typically intended for transfusion, blood components are generally not initially screened for pathogens. Instead, plasma samples from vacutainer blood collection tubes collected at the time of donation are routinely screened. The 2007 FDA report suggested that further research exploring the use of whole blood as a screening sample is needed, potentially enhancing the extraction of cell-associated viral nucleic acids.[9] To this study group's knowledge, no research has been conducted to develop a blood donor screening method for WNV RNA in whole blood samples derived from blood collection tube specimens.

This study's objective was to further establish the sensitivity of screening whole blood relative to plasma for WNV RNA by generating differential viral loads using paired whole blood and plasma samples prepared from blood collection tubes instead of blood components. Our hypothesis was that, consistent with the 2007 FDA report, we would find 1 log higher viral loads in whole blood compared with plasma.[9] We also expected to see variations in the whole blood and plasma viral loads according to the stage of infection as defined by serology status, similar to our recent findings of compartmentalization in parvovirus B19 in infected blood donors.[11]

MATERIALS AND METHODS

Blood donor populations

This retrospective analysis was designed to gather quantitative viral data on cross-sectional and longitudinal specimens from WNV-infected donors that had been processed and stored in a repository at Blood Systems Research Institute (BSRI). The cross-sectional portion of the study involved using a previously described real-time reverse transcription–polymerase chain reaction (RT-PCR) assay to quantify viral loads in plasma and whole blood samples derived from specimens collected from donors on the index donation or index date.[12] The cross-sectional study samples were from donations that had been confirmed positive for WNV after reactive WNV RNA screening using a transcription-mediated amplification (TMA) NAT assay.[13] This TMA assay was used for blood screening at Creative Testing Solutions (Tempe, AZ). Viral loads were also determined in longitudinal follow-up whole blood and plasma specimens from enrolled participants whose WNV infections were confirmed by TMA reactivity at index and later seroconversion.

All samples were tested "blind" with no donor identification or outcome information and no data on bleed sequence or predicate WNV results provided during the testing process. Longitudinal study participants signed an informed consent form approved by the University of California at San Francisco Committee on Human Research to allow blood specimens to be collected during specific time intervals after their index date. This study's research protocol is compliant with the Declaration of Helsinki principles and was also approved by the A.T. Still University Mesa Institutional Review Board.

Serologic assay

Stage of infection for both the cross-sectional study participants and the longitudinal study participants was established at each specimen collection date using anti-WNV immunoglobulin (Ig)M and IgG immunoassays (Focus Diagnostics, Cypress, CA). The specimens were divided into seronegative (IgM negative and IgG negative) and seropositive (IgM positive and/or IgG positive) groups.

Sample preparation

Whole blood and plasma samples derived from specimens collected from both study groups were prepared by BSRI previous to this investigation. Anticoagulated blood specimens in collection tubes containing ethylenediaminetetraacetate (EDTA) were centrifuged at 872 × *g* for 10 minutes. Plasma was removed and divided into aliquots for storage in a biospecimen bank at −70°C. The buffy coat was resuspended with the remaining RBCs and plasma, and this volume, now called the whole blood sample, was also divided into aliquots and stored.

Sample aliquots from the longitudinal study group were further processed for replicate qualitative NAT testing using the TMA assay. These follow-up sample aliquots were thawed and diluted 1:5 by adding four parts of phosphate-buffered saline (PBS) to one part of whole blood or plasma. The samples were diluted to prevent the TMA inhibition that would likely occur if undiluted whole blood was tested.[14] To directly compare the proportion of replicate TMA assays that were positive using 1:5 diluted whole blood with corresponding plasma aliquots, the plasma was also diluted 1:5 in PBS before replicate TMA testing in parallel with diluted whole blood.

Real-time RT-PCR assay

The WNV RNA real-time PCR assay used in this study was based on methods reported in detail in a US patent.[12] A real-time RT-PCR assay using primer pairs described in the patent was previously validated by testing its ability to detect both US and Ugandan WNV strains.[12]

In this study, the assay protocol involved RNA extraction step using RNA kits (QIAamp, Qiagen, Valencia, CA) using procedures slightly modified from the package insert. (Of note, use of this RNA extraction method enabled testing of whole blood and plasma-derived RNA without predilution of samples before extraction, as was necessary before the target capture step TMA assay.) Real-time RT-PCR used primers and probes that targeted highly conserved sequences within the capsid region or the NS1/NS2 region of the WNV genome. After amplification, the mean cycle threshold (C_t) values from two replicate tests were determined for whole blood and plasma-derived samples processed in parallel.

WNV RNA–positive plasma with a known concentration, originally sourced from an FDA stock of WNV isolate (NY99) culture supernatant spiked into plasma, was obtained from CBER/FDA and used as the standard for viral load testing.[15] This standard was serially diluted into negative plasma or whole blood and replicate tests were performed on the serial dilutions to determine the limit of detection of the real-time RT-PCR assay on both sample types using Probit analysis.

TMA assay

A WNV assay (Procleix, NVD, Emeryville, CA) used in this study is FDA-licensed for donor screening.[13] The assay consists of three major steps including sample preparation with magnetic particle–based target capture, viral RNA target amplification based on TMA technology, and amplification product detection with chemiluminescent probes using a hybridization assay.[13]

To determine the specificity of the TMA assay on PBS-diluted specimens, 15 paired whole blood and plasma specimens were collected from donors who were not infected with WNV. These donors were anti-WNV IgM negative and shown to have no detectable WNV RNA in their plasma and whole blood using the real-time RT-PCR assay. Their specimens were diluted with PBS using the above protocol and tested in replicates along with the diluted follow-up specimens with the TMA assay using the automated NAT instrument platform (Procleix TIGRIS, NVD).

Statistical analysis

For the cross-sectional study specimens, the difference in C_t values (ΔC_t) between whole blood and plasma was calculated and used to estimate the differential viral load in the sample types. A ΔC_t of 3.3 is equivalent to a 10-fold viral load difference. The Mann-Whitney rank sum test (JMP, Version 7, SAS Institute, Inc., Cary, NY) was used to assess the difference in ΔC_t between the seronegative and seropositive groups—all tests were two-tailed ($t = 0.05$). In addition to calculating the proportion of replicate TMA-reactive results on the diluted whole blood and plasma samples at serial time periods after the index donation date, the data analysis for the longitudinal cohort also included plotting viral load data relative to time postindex donation date and serology results.

RESULTS

Probit analysis established that the 50 and 95% detection limits for the WNV RNA real-time RT-PCR assay were 9.5 and 73.7 copies/mL, respectively (Table 1). The detection limits for whole blood were 15.5 copies/mL (50%) and 89.0 copies/mL (95%). Since there were only one or two viral load levels that were within the range of 0% to 100% positive rate, our replicate dilution data did not contain

TABLE 1. WNV detection limits determined by Probit analysis using a CBER/FDA WNV standard dilution panel with a real-time RT-PCR assay

Dilution matrix	Copies/mL	Number reactive/ number tested	% Reactive	50% detection limit in copies/mL*	95% detection limit in copies/mL*
Plasma	10^5-10^2	16/16	100	9.5	73.7
	10^1	7/16	44		
	10^0	1/18	6		
Whole blood	10^5-10^2	16/16	100	15.5	89.0
	10^1	5/16	31		
	10^0	0/18	0		

* Determined by probit analysis.

TABLE 2. C_t values of paired whole blood (WB) and plasma (PL) specimens in the WNV cross-sectional study obtained using a real-time RT-PCR assay*

Antibody stage	IgM–/IgG– (n = 29)	IgM+/IgG– (n = 3)	IgM+/IgG+ (n = 9)	IgM+/IgG+ (n = 1)
WB WNV RNA (% detectable)	83	100	89	100
PL WNV RNA (% detectable)	86	0	11	0
WB C_t (mean)	37.2	36.3	36.9	34.9
PL C_t (mean)	35.2	40.0	39.9	40.0
ΔC_t = plasma C_t – whole blood C_t	–2.0	3.7	3.0	5.1
p value	0.0002	0.06	0.002	NA†
WB WNV fold difference vs. PL	–4	+12	+8	+34

* All specimens collected on the index date. All C_t values 40 or greater are indicated as 40 (undetectable).
† Not applicable; unable to calculate p value due to data from only one participant.

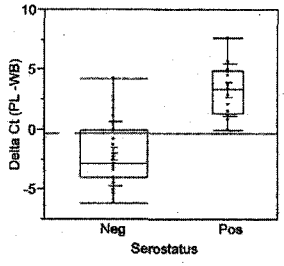

Fig. 1. Box-and-whisker plot of the ΔC_t values (plasma C_t – whole blood C_t [PL – WB]) based on serology status, negative (IgM– and IgG–, n = 29) and positive (IgM+ and/or IgG+, n = 13) for the cross-sectional study. The ΔC_t values are plotted on the y-axis and the serologic status (Neg and Pos) is plotted on the x-axis.

enough information to estimate confidence intervals (CIs) for these limit of detection values. All fivefold diluted whole blood samples from 15 non–WNV-infected donors tested nonreactive by replicate TMA assays, indicating that the TMA assay demonstrated 100% specificity when used with this sample set.

The cross-sectional study results revealed that the viral load was fourfold lower in whole blood compared to plasma in seronegative donor specimens (29/42), indicating that the virus was mainly suspended in the plasma and not cell associated during the viremic seroconversion stage of infection (Table 2). In contrast, we observed an approximately 10-fold higher viral load in whole blood versus plasma in seropositive index donation specimens (13/42). As Fig. 1 indicates, several seronegative specimens (3/29) showed slightly higher viral loads in whole blood compared to plasma, although this differential viral load distribution was less than that seen in whole blood relative to plasma samples from WNV-seropositive specimens.

All 10 longitudinal study participants were seropositive throughout follow-up, and their viral load results showed that more WNV RNA was typically detected in whole blood (mean *difference, 343 copies*; $p < 0.001$) than in plasma up to 200 days after index (Fig. 2). Plasma viral load levels were detectable only within 15 days after index for five of the participants, while the other five participants had no plasma viremia detectable by the real-time RT-PCR assay during their entire follow-up period. For six participants with follow-up specimens collected at least 90 days after index, five had detectable WNV RNA in whole blood to 3 months after index donation. All five study participants for whom sixth month specimens were available tested negative for WNV RNA in both the whole blood and the plasma compartments; by this point their IgG levels had reached a plateau while their IgM levels were decreasing or under the cutoff value of 1. As the IgM signal-to-cutoff (S/CO) values decreased, the whole blood viral load generally decreased (Fig. 2). Figure 3A presents a summary of the more sensitive qualitative RNA screening test results based on an mean percentage of reactive TMA tests out of five replicates performed on the diluted whole blood and plasma samples derived from specimens collected during six follow-up date ranges. These data confirm that there was a higher probability of detecting WNV in whole blood compared to plasma up to 3 months after index. The viral load mean for the same six follow-up date ranges show a pattern similar to that of the replicate TMA results (Fig. 3B).

DISCUSSION

Our retrospective study, using tube-derived specimens, revealed significantly higher viral loads in plasma than whole blood during the seronegative stage, in contrast to an earlier study that demonstrated higher levels of WNV RNA in RBC components compared to plasma irrespective of donation viral load or serologic status.[9] Although these different results may be due to a number of varying factors, the persistent whole blood viremia detected in this study may also present an opportunity for further studies exploring the diagnostic implications of this finding.

In the earlier study, confirmed WNV RNA–reactive samples from leukoreduced RBC units and their corresponding components were used—the RBC samples

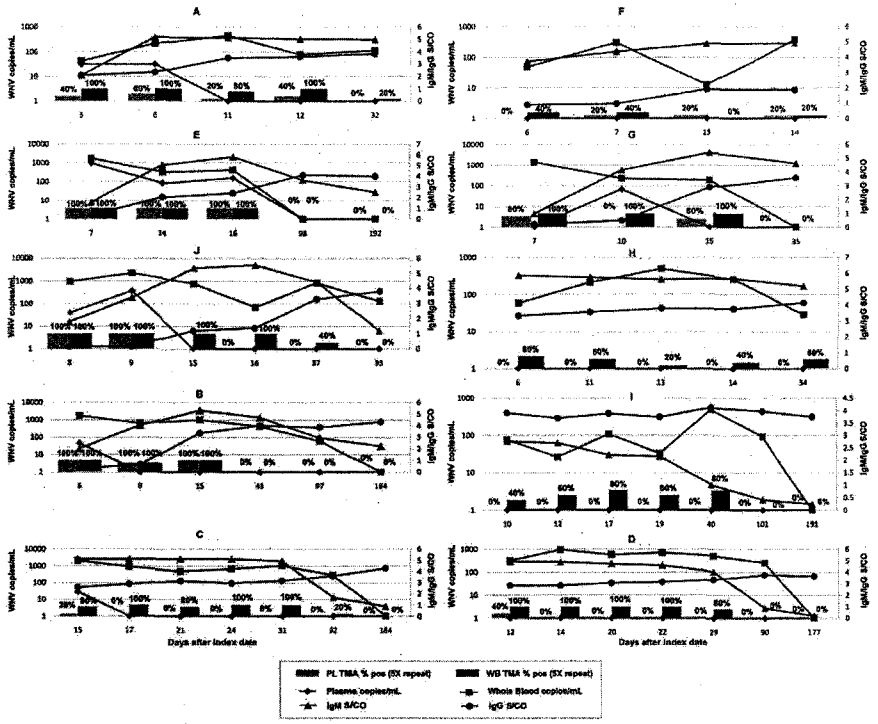

Fig. 2. WNV RNA concentration and serologic status over the follow-up period after index donation date for each of the 10 WNV-infected blood donors who participated in the longitudinal study. The corresponding plasma and whole blood WNV RNA concentrations in copies/mL is plotted on the left y-axis. IgM and IgG S/CO ratio is plotted on the right y-axis. The days after index donation date is plotted on the x-axis. The percentage of diluted plasma and whole blood samples that were TMA positive out of five replicate tests is indicated above the gray (plasma) and black (whole blood) bars above each follow-up collection day. The 10 participants are assigned a letter from A to J.

derived from fresh blood components were washed twice before extraction with Trizol (Life Technologies Corporation, Carlsbad, CA), whereas plasma was extracted with QiaAmp. In our study, the whole blood and plasma samples were prepared from blood donor specimen tubes routinely used for screening and then frozen and thawed before viral load quantification. The thawed whole blood in our study contained lysed RBCs, white blood cells (WBCs), and platelets (PLTs) while the leukoreduced RBC units most likely contained a greater amount of intact RBCs along with a minimal amount of WBCs and PLTs before sample processing for RT-PCR. The impact of starting with thawed, lysed RBCs, in addition to more WBCs and PLTs in our unwashed whole blood samples on real-time RT-PCR results relative to the RBC samples used in the earlier study, was not clearly understood. No specific stability data regarding WNV RNA in frozen whole blood samples could be found after a literature search; however, an earlier human immunodeficiency virus Type 1 RNA stability study found no significant change in mean viral loads in EDTA-anticoagulated plasma samples for at least 6 months when processed promptly and stored at –70°C.[16] The longitudinal sample pairs were thawed and tested at least 1 year after they were prepared and frozen so it may

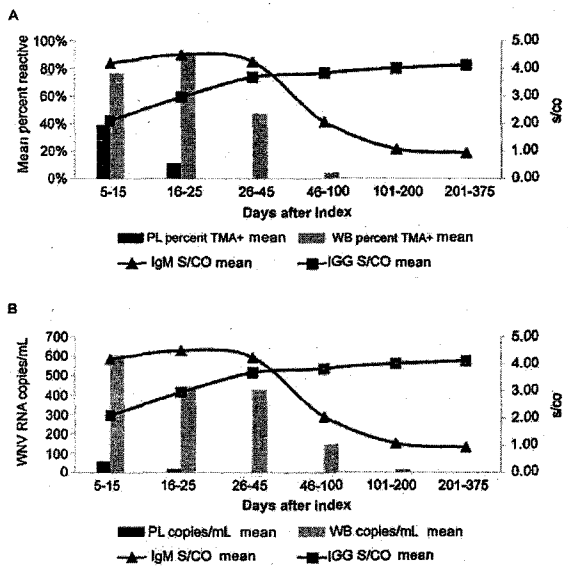

Fig. 3. (A) Mean percentage of TMA-reactive (Procleix WNV Assay, Novartis Diagnostics) for all participants' corresponding plasma (PL) and whole blood (WB) samples collected at designated time periods after index and the mean S/CO value for IgM and IgG for those samples over the same time periods. The mean percentages of TMA reactive (+) plasma and whole blood 1:5 diluted samples are plotted on the left y-axis as bar graphs and the mean S/CO values for IgM and IgG are plotted on the right y-axis. The time periods (5-15, 16-25, 26-45, 46-100, 101-200, and 201-375 days) of the follow-up collection days after index donation are plotted on the *x*-axis. (B) WNV RNA concentration (real-time RT-PCR assay used at BSRI) for all participants' corresponding plasma and whole blood samples collected at designated time periods after index and the mean S/CO value for IgM and IgG for those samples over the same time periods. The mean copies/mL for neat whole blood and neat plasma are plotted on the left y-axis as bar graphs and the mean S/CO values for IgM and IgG are plotted on the right y-axis. The time periods (5-15, 16-25, 26-45, 46-100, 101-200, and 201-375 days) of the follow-up collection days after index donation are plotted on the x-axis.

have been possible that some RNA degradation occurred in the both the plasma and the whole blood samples during storage; however, we could not demonstrate this because the whole blood and plasma samples were not tested for viral loads before being frozen. Per our protocol, our objective was to test samples without introducing further preservatives that could impact the findings of the study.

In our study, RNA extraction was done with RNA kits (QIAamp, Qiagen). We speculated that the different extraction methods might have resulted in different RNA extraction efficiencies, which may at least partially explain the different findings between the two studies. One study reported with foot-and-mouth disease virus spiked plasma samples, the Trizol extraction procedure detected 1 log less viral RNA 10^{-8} dilution compared with 10^{-7} dilution) than other extraction methods, including QIAamp RNA kit, using one RT-PCR assay. With another assay, the QIAamp RNA kit was approximately 2 log more sensitive than the Trizol method.[17]

Another possibility for the contrasting findings may be the different primers and probes used in each study. It is possible that primers and probes used in the earlier study could have been more efficient in detecting WNV RNA in RBC samples than those used in this study. If this could be demonstrated in a future study, the consistently higher RBC viral loads found in the earlier study, regardless of serostatus, could be explained.

Although there are no data to indicate that the lower percentage (83%) of seronegative samples with detectable whole blood viremia compared to that of plasma viremia (86%) was due to false-negative results, one of our current study's limitations was that there was no internal control in our real-time RT-PCR assay (the WNV TMA assay used in our study did include an internal control that had to be reactive for the results to be considered valid and included in our analysis; no such invalid results were observed with the 1:5 diluted whole blood and plasma samples tested from WNV-positive or control donor specimens). For real-time RT-PCR assays without a negative control, detecting false-negative results may be less likely compared to assays with an internal control. No internal control was used in the real-time RT-PCR assay in this study due to technical challenges associated with validating and optimizing the internal control input value. Future research using our real-time RT-PCR assay may include optimizing this assay to incorporate an internal control.

Our real-time RT-PCR assay's 95% limit of detection, established by probit analysis, was higher for whole blood (89.0 copies/mL) compared to plasma (73.7 copies/mL)

but it is not clear if this difference was significant since CIs could not be estimated. Future studies may must include more replicate testing of viral load levels between 10^2 and 10^0 copies/mL to obtain better limit of detection estimates and associated CIs for our real-time RT-PCR assay. Another published real-time RT-PCR assay was shown to reliably detect WNV RNA at a concentration of 10 to 30 copies/mL[18] so there may be opportunities to further improve the analytical sensitivity of our assay in future studies.

In our study, the viral loads in whole blood were approximately 1 log higher compared to plasma after seroconversion. Similar to the findings in our recent study of parvovirus B19 viremia in donors, WNV RNA levels varied with infection stage.[11] A potential explanation for the detection of higher viral loads in the plasma samples relative to the whole blood samples in seronegative donations is that without induced IgM specific for WNV, the dissemination of virus into the plasma compartment cannot be significantly slowed.[19] Again, similar to our recent study of Parvovirus B19,[11] whole blood WNV levels decreased considerably as soon as IgM became undetectable by the sixth month postindex follow-up collection date. Potential explanations of these findings could include WNV being preferentially bound to RBCs when the virus is present in IgM immune complexes, more WNV tending to bind at higher plasma concentration due to steric effects on receptor-mediated binding or WNV being present in high levels within a subset of peripheral blood RBCs in earlier compared to later stages of WNV infection. The last hypothesis is interesting because no sixth month follow-up whole blood sample was WNV RNA reactive and all available third month samples had detectable WNV RNA, which would be consistent with the 120-day survival period for RBCs.

From our study, we surmise that the clinical value of screening whole blood instead of plasma for WNV RNA may be limited to better detection during the seropositive stages that have shown little to no transfusion transmission risk to date. Only one seropositive (IgM-positive/IgG-negative) donation with a low plasma RNA load has been implicated in the 32 WNV transfusion transmission cases documented by the CDC.[10] Our group reported that in 2007, 9 of 34 ID-NAT only yield cases were IgM-positive/IgG-negative donations.[7] Another group had reported that in 2003 to 2004, a median viral load of 100 copies/mL was detected in 143 anti-WNV IgM-positive donations (including both donations without and with WNV-specific IgG).[6] Two inconclusive investigations of patients identified as having possible WNV transfusion-transmitted infection involved donors who were IgM positive but had plasma found WNV RNA negative by PCR, findings that are consistent with our observations in this study.[3] Based on the findings of this study, it may be possible to speculate that had the whole blood compartment of these two donors been tested, there is a greater likelihood that WNV RNA would have been detected in this compartment. Although not typically observed to date in vivo, another previous study showed that over 50% (15/28) of WNV antibody–positive samples, including some with high viral loads (8/15) and some with low viral loads (7/15), demonstrated infectivity for Vero cell culture and/or human monocyte-derived macrophage culture.[10]

Persistent whole blood viremia observed in some participants up to 90 days after index, combined with earlier findings of in vitro infectivity of RBC-associated WNV, suggest the need for future studies to evaluate related blood safety implications. Since plasma viremia can only be detected a few days after infection, diagnosis of WNV infection in humans is typically based on serologic test results.[20] Positive serologic results need to be confirmed by viral neutralization studies to rule out the presence of cross-reactive antigens such as Japanese encephalitis complex in the clinical sample; these neutralization studies require a Biosafety Level 3 facility.[20] If a whole blood NAT assay could be developed for diagnostic use, this could complement the currently used diagnostic tools and provide an opportunity for WNV RNA detection for several months instead of several days after infection.

More research is also needed to explore if whole blood screening could enhance the yield of MP-NAT during the early postseroconversion stages, extending the detection window of an active WNV infection for up to 90 days after infection as opposed to the current window period of approximately 6 to 7 days using current testing methods. Further longitudinal studies using a larger number of WNV-infected participants are ongoing to further explore these open research questions.

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CONFLICT OF INTEREST

LL and PS are NVD employees; MB is a Gen-Probe, Inc., and NVD grant recipient; LT, JA, HE, LW, and THL have nothing to disclose.

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研究報告の概要	使用上の注意記載状況・その他参考事項等
○献血者スクリーニングによって確定した日本国内の最新HTLV-1感染率 成人T細胞白血病（ATL）や関連疾患の主要な原因であるヒトT細胞白血病ウイルス1型（HTLV-1）は、九州に特有である。母乳による母子感染が主な経路であり、そのような感染を防止するためのプログラムを確立するには、正確なHTLV-1感染率及びキャリア数が必要である。 2006年から2007年に、日本の初回献血者1,196,321人におけるHTLV-1感染率が調査され、そのうち3,787人が抗HTLV-1抗体陽性であると確認された。献血者以外の年齢層に適合曲線を適用したところ、日本の0歳～99歳における現在のHTLV-1キャリア数は108万人と推定された。これは1988年に報告された値よりも10%少なかった。調整後の全体的な感染率は男性、女性それぞれ0.66%及び1.02%と推定された。キャリアの数のピークは70歳代で見られ、これは1988年のデータベースで50歳代に観察されたピークがシフトしたものである。キャリアは高浸淫地域である九州だけでなく日本全土、特に首都圏で増加していた。人口予測により、キャリア数は今後20年間で2分の1に減少すると計算された。	赤十字アルブミン20 赤十字アルブミン25 赤十字アルブミン5%静注12.5g/250mL 赤十字アルブミン20%静注4g/20mL 赤十字アルブミン20%静注10g/50mL 赤十字アルブミン25%静注12.5g/20mL 血液を原料とすることに由来する感染症伝播等
報告企業の意見	**今後の対応**
日本国内のHTLV-1感染率やキャリア数を正確に把握するため、初回献血者における抗体検査及び適合曲線を用いて計算したところ、現在のキャリア数は108万人であると推定され、また九州だけでなく首都圏にもキャリアが増加していることが分かったとの報告である。 HTLV-1は脂質膜を有するRNAウイルスである。垂直感染により日本では古代から広く侵淫しているが、本製剤による感染の報告はない。本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれていることから、本製剤の安全性は確保されていると考える。	日本赤十字社では献血時のスクリーニング法として、より感度の高い化学発光酵素免疫測定法（CLEIA）によるHTLV-1抗体のスクリーニング検査を行っている。特別な対応を必要としないが、今後も引き続き情報の収集に努める。

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Current Prevalence of HTLV-1 in Japan as Determined by Screening of Blood Donors

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Human T-cell leukemia virus type-1 (HTLV-1), a major source of adult T-cell leukemia and related diseases, is endemic to southwestern Japan. Mother-to-infant transmission via breast milk is an important route of infection, and establishing programs to prevent such transmission requires exact figures on the HTLV-1 prevalence rate and the number of carriers. Therefore, the seroprevalence of HTLV-1 among 1,196,321 Japanese first-time blood donors from 2006 to 2007 was investigated. A total of 3,787 of such donors were confirmed to be positive for anti-HTLV-1 antibody. By applying a fitness curve to the age ranges outside the blood donor age range, the present number of HTLV-1 carriers covering ages from 0 to 99 years was estimated to be at least 1.08 million in Japan; this value was 10% lower than that reported in 1988. The adjusted overall prevalence rates were estimated to be 0.66% and 1.02% in men and women, respectively. The peak in carrier numbers was found among individuals in their 70s, which is a shift from the previous peak observed in the 1988 database among individuals in their 50s. Carriers were distributed not only in the endemic southwestern region of Japan, but throughout the country, particularly in the greater Tokyo metropolitan area. By applying population projections, it was calculated that the carrier number will decrease by half in the next two decades; however, the carrier population will age over that interval, meaning that the age of patients with adult T-cell leukemia will also be higher. ***J. Med. Virol. 84:327–335, 2012.*** © 2011 Wiley Periodicals, Inc.

KEY WORDS: HTLV-1; seroprevalence; blood donors

INTRODUCTION

The human T-lymphotropic virus type 1 (HTLV-1; *Retroviridae, Deltaretrovirus, Simian T lymphotropic virus*) is a pathogenic retrovirus that causes adult T-cell leukemia, HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), and uveitis [Takatsuki et al., 1977; Hinuma et al., 1981; Gessain et al., 1985; Osame et al., 1986; Mochizuki et al., 1992]. HTLV-1 prevalence shows a peculiar geographical distribution pattern, with virtually exclusive concentration in southwestern Japan, the Caribbean islands, regions of South America, and tropical Africa [Manns and Blattner, 1991; Mueller, 1991]. The high endemicity of the virus and the seriousness of the resulting diseases have prompted epidemiological research on the mode of viral transmission. Three major routes of viral transmission have been established: (1) mother-to-infant transmission, mainly via breast-feeding [Yamanouchi et al., 1985; Kinoshita et al., 1987]; (2) sexual transmission, predominantly male to female [Tajima et al., 1982; Murphy et al., 1989]; and (3) transfusion by cellular blood components [Okochi et al., 1984]. Transmission of the virus via transfusion has virtually been eliminated since the implementation of viral screening of donated blood in 1986 [Ikeda et al., 1984; Inaba et al., 1989]; thus, transmission today is mostly confined to the mother-to-infant and sexual routes. Carriers of the virus are readily identified by screening for the anti-HTLV-1 antibodies, and the most effective measure for the prevention of mother-to-infant transmission is apparent (i.e., bottle feeding). Hence, the implementation of strategies to prevent transmission is considered a matter of both public health and public policy in endemic areas [Hino, 2003]. Although several local governments in southwestern Japan have implemented a screening and consultation system, national guidelines for HTLV-1 screening have not been created; the screening of pregnant women for the virus has been left to individual gynecologists. The establishment of a program for carrier management would be facilitated by data on HTLV-1 prevalence rates and carrier numbers. To determine the current status of HTLV-1 infection in Japan, the HTLV-1 carriage rate among first-time blood donors during 2006 and 2007 was investigated, and the results were used to deduce the number of carriers in the entire country. In combination with published data on population projections, the results permitted an estimate of future changes in the number of carriers.

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The authors declare no conflict of interest for this article.

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MATERIALS AND METHODS

Screening for Anti-HTLV Antibodies

In Japan, blood centers of the Japanese Red Cross (JRC) are the sole facilities authorized to handle blood collection, testing, processing, and distribution. In 1986, the JRC implemented an agglutination assay to screen for the presence of anti-HTLV-1 antibodies [Inaba et al., 1989]; use of this test continued until 2008. Prior to donation, all blood donors were informed that donated blood would be tested for anti-HTLV-1 antibodies. Blood donors examined in the current study were first-time donors (i.e., donors who have no record of blood donation for approximately the preceding 10 years) who donated blood during the 2 years from January 1, 2006 to December 31, 2007. Enrolling only first-time donors for the present study precluded the recounting of seronegative donors. Samples that were positive by the agglutination assay were subjected to a confirmatory test that employed immunofluorescence staining of the target MT-2 cell line [Miyoshi et al., 1981]. Only donors who were positive by the confirmatory test were included in the present study.

Estimation of Number of Carriers

Current and prospective population statistics for Japan were obtained from the vital statistics [National Statistics Center, 2009a] published by the Japanese Ministry of Health, Labour, and Welfare. The HTLV-1 prevalence rate among the first-time donors was obtained for each category defined by age, sex, and Prefecture. The number of HTLV-1 carriers in each category was estimated by projecting the detected prevalence rate to the population of that category. The ages of blood donors were categorized by intervals of 10 years. To deduce the carrier number for combined larger categories, the estimated carrier numbers were summed for the respective categories. Figures thus obtained are equivalent to those that are adjusted for age, sex, and Prefecture.

For most of the analyses, the 47 Prefectures of Japan were grouped into seven districts: Hokkaido, Tohoku, Kanto, Chubu, Kinki, Chugoku/Shikoku, and Kyushu. However, the number of carriers in Hokkaido and Tohoku were combined for the purpose of comparing changes in carrier numbers over the past two decades. To assess the distribution of the mean prevalence rates among Prefectures, the seven districts were grouped further into three larger regions, as follows (see also the map in Figure 4): eastern and central Japan (consisting of the Hokkaido, Tohoku, Kanto, and Chubu districts); western Japan (consisting of the Kinki and Chugoku/Shikoku districts); and southwestern Japan (consisting of the Kyushu district).

In Japan, the age of eligibility for blood donation was 16–64 years during the study period. For simplicity, the prevalence rate in the population in the 16–19 age category was assumed to correspond to that of the population in the 15–19 age category. The prevalence rates in the populations in the 0–4, 5–9, and 10–14 age categories were estimated by assuming that the prevalence rates in these cohorts form an exponential series with those of the 15–19, 20–29, 30–39, and 40–49 age categories. The corresponding curve was defined by the equation $R_a = R_0 \times e^{ba}$, where R_a and R_0 indicate the seropositivity rates at ages a and 0, respectively. The prevalence rate for the 10–19 age category was derived by combining the prevalence for the 15–19 age category with the estimated rate for the 10–14 age category. Similarly, the rate for the 0–9 age category was deduced by combining the rates for the 0–4 and 5–9 age categories.

The prevalence rate for the population in the 60–64 age category was assumed to correspond to that of the population in the 60–69 age category. The prevalence rates for the populations in the 70–79, 80–89, and 90–99 age categories were estimated based on the observation (1988 data) that prevalence rates increased in an essentially linear fashion between 30 and 64 years of age [Hashimoto et al., 1991]. Two decades later, prevalence rates in the population aged 50- to 84-year old are expected to be equal to or higher than that in the population aged 30–64 years observed in 1988.

Reevaluation of the Number of Carriers Surveyed in 1988

In 1988, a nationwide survey was carried out among blood donors to estimate the number of HTLV-1 carriers in the entire country [Hashimoto et al., 1991]. That study screened for seropositivity among all donors, not just first-time donors. However, data were collected only for donations made during a 1-month interval, which should have precluded data from repeated donations. In addition, at the time of that study, donors were not yet being notified of their HTLV-1 status, meaning that seropositive donors could continue donating blood. Prevalence rates obtained under these conditions (Table I) were regarded as equivalent to the rates expected for first-time blood

TABLE I. HTLV-1 Prevalence Rate Among Blood Donors in 1988 [Hashimoto et al., 1991]

Age categories	16–19	20–29	30–39	40–49	50–64
Hokkaido					
Male	0.17	0.47	0.63	0.83	0.83
Female	0.26	0.29	0.68	1.35	1.25
Tohoku					
Male	0.29	0.23	0.52	0.97	0.93
Female	0.23	0.33	0.59	0.84	1.17
Kanto					
Male	0.16	0.28	0.5	0.65	0.83
Female	0.15	0.29	0.59	0.84	1.21
Chubu					
Male	0.16	0.29	0.31	0.46	0.48
Female	0.19	0.36	0.57	0.86	0.84
Kinki					
Male	0.16	0.43	0.8	1.05	1.14
Female	0.35	0.52	1.13	1.47	1.84
Chugoku					
Male	0.08	0.39	0.53	0.87	0.88
Female	0.22	0.3	0.71	0.91	1.36
Shikoku					
Male	0.26	0.5	0.61	0.68	1.23
Female	0.29	0.46	1.29	1.03	1.82
Kyushu					
Male	0.75	1.44	2.69	3.71	5.21
Female	0.93	1.38	2.99	5.05	7.65
Whole country					
Male	0.23	0.44	0.8	1.03	1.37
Female	0.29	0.48	1.01	1.43	2.1

The 50–59 years category is combined with 60–64 years category in the 1991 study. Prevalence rates for the Chugoku and Shikoku districts are shown separately. Values are shown as percentages.

donors. Therefore, the 1988 survey estimated the total number of carriers at approximately 1.2 million for the entire country [Tajima et al., 1990]. There were, however, no published estimates on the number of carriers outside the eligible age range for blood donation. To address this issue, the numbers of carriers under 16 and over 64 years were re-examined and the analysis incorporated the assumptions described above, namely an exponential rate increase under 16 years, and a linear rate increase over 64 years.

Statistical Analysis

Differences in the mean prevalence rates in Prefectures among the three large regions were assessed using the Mann–Whitney *U*-test. Differences in prevalence rates between sexes and between 1988 and 2006–2007 were assessed using the chi-squared test. Unless otherwise indicated, a *P*-value of <0.05 was considered significant.

RESULTS

From 2006 to 2007, the JRC blood centers accepted 1,196,321 blood donations from first-time donors. Of the samples that tested-positive by agglutination assay, 3,787 (0.317% of all the first-time donors) were confirmed to be positive for anti-HTLV-1 antibody using the immunofluorescence test. The prevalence rates adjusted for age and sex were obtained for each Prefecture, and the maximum, minimum, and median were derived for each of the three large regions described above (eastern and central Japan, western Japan, and southwestern Japan; Table II). The prevalence rates for Prefectures were significantly different among the three large regions. As expected, prevalence rates were highest in southwestern Japan (where HTLV-1 is endemic); additionally, prevalence rates in western Japan were significantly higher than those in eastern and central Japan (Table II).

The nationwide prevalence rate, adjusted for district, is shown as a function of age in Figure 1. The observed rate increased sharply with the age of the donor. The difference in prevalence rate between sexes was significant for donors in their 20s, 30s, 50s, and 60s, and there was a trend toward increasing differences between sexes in the elderly. For example, the absolute prevalence rate differences between sexes were 0.29% and 0.37% for donors in their 50s and 60s, respectively; compare these values to differences of 0.05% and 0.06% for those in their 30s and 40s, respectively. Table III shows the age distribution of prevalence rate for each district. As expected, the highest rate was observed in the Kyushu districts (southwest Japan). The two Prefectures with the highest prevalence rates in the country were both located in the Kyushu district; the corresponding rates are shown in Figure 2. Notably, in both these Prefectures, the prevalence rates among under 40 donors were <2%, with large increases among older donors. For example, in one of the Prefectures (Fig. 2A), the mean rate for all donors was 1.95%, and the rates in the 60–64 age category were 14.0% and 8.7% for females and males, respectively. Thus, almost 1 out of 50 first-time donors were positive for the anti-HTLV-1 antibody in this Prefecture. For the entire nation, the total number of carriers aged 15–64 years in the Japanese population was calculated to be 510,000; this value was obtained by summing the total number of carriers from all districts.

The prevalence rates and the numbers of carriers in the populations under 15 years and over 64 years of age were estimated on the basis of the assumptions described above. The distribution of the estimated prevalence rate also is included in Table III; these estimates are indicated in italics. The overall prevalence rates covering all age categories from 0 to 99 years were calculated as 0.66% and 1.02% for males and females, respectively (data not shown). By summing up the projected carrier numbers within the blood donor age categories and the estimated carrier numbers outside the donor age categories, the total number of carriers aged 0–99 in Japan was calculated to be 1.08 million. This value was 10% lower than that derived in the 1988 study. The nationwide distribution of the number of carriers as a function of age is shown in Figure 3 (closed triangles). According to our analysis, carrier numbers are highest among individual in their 70s, followed by those in their 60s and 50s.

TABLE II. Mean Prevalence Rates Among Blood Donors (2006–2007) by Region

Region	Districts included (number of Prefectures included)		Min.	Max.	Med.
Eastern and Central Japan[a]	Hokkaido (1), Tohoku (6), Kanto (9), Chubu (8)	Male	0.04	0.26	0.11
		Female	0.04	0.32	0.15
		Total	0.06	0.26	0.13
Western Japan[a]	Kinki (6), Chugoku/Shikoku (9)	Male	0.05	0.41	0.25
		Female	0.09	0.53	0.30
		Total	0.10	0.45	0.27
Southwestern Japan[a]	Kyushu (8)	Male	0.48	2.01	0.93
		Female	0.83	2.43	1.29
		Total	0.62	1.95	1.07

Regional minimum (Min.), maximum (Max.), and median (Med.) of prevalence rates in Prefectures are shown (%), with the whole country divided into three regions as indicated. Note that the Niigata and Yamanashi Prefectures are included in the Kanto district.
[a]Statistically significant differences in prevalence rate among the three regions (Eastern/Central vs. Western vs. Southwestern; $P < 0.05$; Mann–Whitney *U*-test).

Comparison of country-wide prevalence rates between 2006 and 2007 and 1988 (bottom rows of Tables I and III) revealed that the 1988 rates were significantly higher than the 2006–2007 rates for both sexes in all donor age categories, that is, for donors aged 16–19, 20–29, 30–39, 40–49, and 50–64 years of age ($P < 0.01$; chi-squared test). The 1988 prevalence rates for those under 15 years and over 64 years of age were estimated using the methods described above, and the numbers of carriers in 1988 were deduced for all age categories. In Figure 3, the carrier numbers derived from the 1988 data (open circles) are overlaid with those derived from the 2006 to 2007 data. For the 1988 values, the carrier numbers form a roughly symmetrical curve, with values peaking for individuals in their 50s. Over the course of the subsequent two decades, the curve shifted to the right (i.e., skewed older). By 2006–2007, the number of carriers over 70 years of age had increased by 69% (178,000), whereas the number of carriers under 60 years of age had decreased by 50% (405,000).

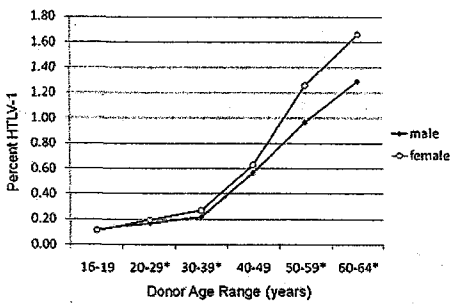

Fig. 1. Anti-HTLV-1 antibody prevalence rate among Japanese blood donors as a function of donor age. Prevalence rate was normalized by district population. Prevalence rates (%) of the anti-HTLV-1 antibody are plotted as a function of donor age range in males (closed diamonds) and females (open circles). Exact figures for the prevalence rates in each age and sex categories are derived from the bottom row of Table III. *Statistically significant difference between sexes ($P < 0.05$; chi-squared test).

Figure 4 depicts the changes in the total carrier numbers over the past two decades in relation to geography (district). Nationwide, the carrier number had decreased by 10% since 1988. The largest decreases were seen in the Hokkaido/Tohoku, Kyushu, and Kinki districts, which exhibited decreases of 44%, 19%, and 16%, respectively. Together, these three districts were calculated to have 208,000 fewer carriers by 2006–2007. On the other hand, the number of carriers increased by 46% (60,000 more carriers) in the Kanto district and by 41% (24,000 more carriers) in the Chubu district.

By using published data for population forecasts, and assuming that the Japanese population will age while maintaining its current HTLV-1 prevalence rate, both carrier number and the age distribution of carriers in the future was predicted. The analysis suggested that the number of carriers will decrease by approximately 2.5% each year, potentially reaching as low as 560,000 (a twofold decrease) by 2027. The projected age distribution of carriers is shown in Figure 5. It is predicted that the peak of the number of carriers will continue to be observed among individuals in their 70s, whereas the number of carriers in their 50s and 60s will decrease markedly.

DISCUSSION

Japan is one of the few developed countries with a high prevalence rate for HTLV-1 carriage. The country is thus in a unique position to establish the scientific basis for medical intervention and public policy for prevention and cure of HTLV-1-related diseases. There has, however, been no nationwide survey of HTLV-1 carriage in Japan since 1988, although several intensive screening tests have been performed for local residents and patients or pregnant women at medical facilities [Tajima et al., 1990; Morofuji-Hirata et al., 1993; Mueller et al., 1996; Taylor et al., 2005]. Our data not only clarify the current status of HTLV-1 in Japan but also reveal epidemiological changes in the carrier status over the past two decades.

HTLV-1 prevalence rate and the number of HTLV-1 carriers often have been deduced from the results of

TABLE III. HTLV-1 Prevalence Rate in 2006–2007 in All Age Categories

Age categories	0–9	10–19	16–19	20–29	30–39	40–49	50–59	60–69	70–79	80–89	90–99
Hokkaido											
M	*0.04*	*0.08*	0.09	0.17	0.15	0.49	0.54	0.70	*0.72*	*0.82*	*0.92*
F	*0.02*	*0.04*	0.04	0.18	0.10	0.46	0.79	1.13	*1.14*	*1.37*	*1.62*
Tohoku											
M	*0.03*	*0.05*	0.06	0.11	0.10	0.29	0.38	1.52	*1.13*	*1.33*	*1.53*
F	*0.02*	*0.04*	0.05	0.13	0.12	0.41	0.48	1.20	*1.22*	*1.47*	*1.72*
Kanto											
M	*0.03*	*0.05*	0.06	0.10	0.09	0.28	0.61	0.67	*0.90*	*1.06*	*1.22*
F	*0.02*	*0.05*	0.05	0.09	0.14	0.37	0.65	0.80	*1.22*	*1.53*	*1.84*
Chubu											
M	*0.03*	*0.06*	0.07	0.13	0.13	0.35	0.43	0.51	*0.55*	*0.64*	*0.72*
F	*0.03*	*0.05*	0.06	0.13	0.28	0.39	0.80	0.39	*0.97*	*1.10*	*1.24*
Kinki											
M	*0.05*	*0.10*	0.12	0.15	0.24	0.52	1.11	1.38	*1.46*	*1.63*	*1.80*
F	*0.05*	*0.08*	0.10	0.19	0.27	0.65	1.51	1.73	*2.16*	*2.51*	*2.86*
Chugoku/Shikoku											
M	*0.05*	*0.10*	0.11	0.21	0.17	0.40	0.66	1.00	*1.15*	*1.37*	*1.59*
F	*0.04*	*0.07*	0.09	0.19	0.22	0.32	0.87	0.98	*1.21*	*1.37*	*1.53*
Kyushu											
M	*0.19*	*0.35*	0.41	0.55	1.00	2.32	3.40	5.04	*6.23*	*7.49*	*8.75*
F	*0.20*	*0.37*	0.44	0.74	0.95	2.18	4.28	7.34	*9.44*	*11.77*	*14.10*
Whole country											
M	*0.06*	*0.10*	0.12	0.17	0.22	0.57	0.97	1.29	*1.59*	*1.92*	*2.19*
F	*0.05*	*0.09*	0.11	0.20	0.27	0.63	1.26	1.66	*2.36*	*2.96*	*3.48*

Figures for the 16–19 age category were derived from screening data and are indicated in non-italicized font; figures for the 10–19 age category were derived from the combination of screening data (for 16–19 years) and deduced data (10–15 years), and are indicated in italicized font. For the prevalence rate for the 60–69 age category, values for the 60–64 age category were used. Figures are shown as percentages; italics indicate estimated figures.

screening of local residents or patients. However, such surveys are of limited use for estimating figures for a larger population, either because of small population size, geographic focus, or skewing due to the underlying health status of the population examined. In this regard, the population of voluntary blood donors has been an appealing alternative for the estimation of the prevalence rate of asymptomatic diseases or carriage status; a large amount of data could be obtained from otherwise healthy individuals and from almost all areas of the country with a relatively even sampling rate. It is essential in a blood donor-based study to obtain data from first-time donors who have not been screened for the disease marker, thus

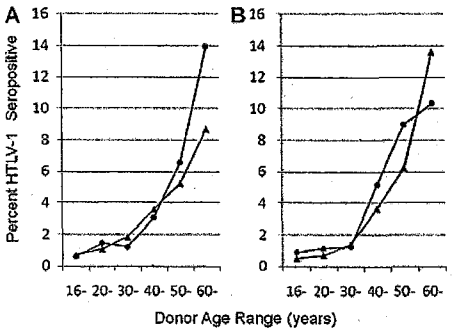

Fig. 2. Age distribution of HTLV-1 prevalence rate in Prefectures A and B in Kyushu district. Prevalence rates (%) of the anti-HTLV-1 antibody are plotted as a function of donor age range in males (closed triangles) and females (closed circles). Prefectures A and B were selected because these Prefectures have the highest mean prevalence rates in Japan.

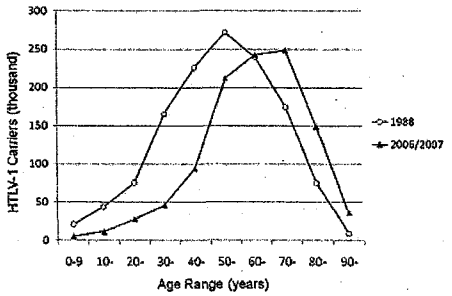

Fig. 3. Age distribution of HTLV-1 carriers estimated for 1988 and 2006–2007. Number of HTLV-1 carriers are shown for the respective age ranges in 1988 (open circles) and 2006–2007 (closed triangles). For the age categories between 20 and 59 years, carrier numbers were based on the results of blood donor screening; for the age categories 0–9 and 70–99 years, numbers were deduced from the assumed prevalence curve; for the age categories 10–19 and 60–69, numbers were derived from a combination of screening data and deduced data.

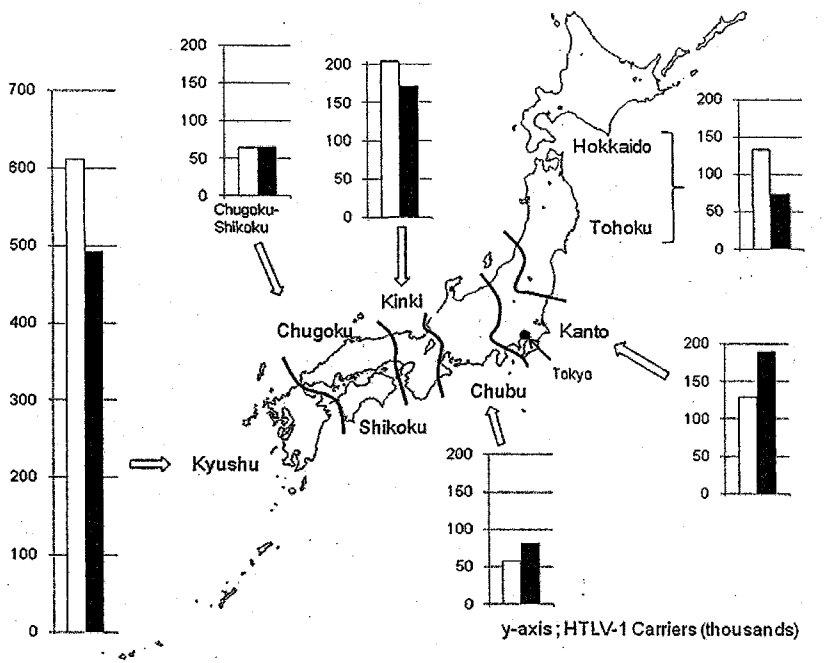

Fig. 4. Changes in the total number of carriers aged (0–99) in six districts of Japan over past two decades. Carrier numbers (thousands) are indicated for 1988 (white bars) and 2006–2007 (black bars). For this analysis, Japan was divided into six districts, such that the Hokkaido and Tohoku districts were combined, and the Niigata and Yamanashi Prefectures were included in the Kanto district.

reducing the chances of bias (e.g., repeated donation by marker-negative donors). This strategy makes it possible to project the obtained data to the general population.

Despite the exclusive use of first-time donors, our data set may still provide skewed conclusions, particularly in the study of HTLV-1. First, people diagnosed with HTLV-1-related diseases, and HTLV-1 carriers identified during the screening of pregnant women, are unlikely to be blood donors, but nonetheless should be counted as seropositives. Second, family members of patients with HTLV-1-related diseases and of HTLV-1 carrier women are less likely themselves to be blood donors. Third, blood donors are typically healthier than the general population, including a reduced likelihood of HTLV-1-associated illnesses [Atsma et al., 2011]. All of these factors would render the observed (donor) HTLV-1 prevalence rate lower than the actual value. Thus, the results of this study represent a lower estimate of HTLV-1 prevalence. Indeed, most studies on HTLV-1 prevalence rates obtained from the screening of local inhabitants, pregnant women, or hospital patients suggest higher prevalence rates than those seen in blood donor-based studies [Hlela et al., 2009; Koga et al., 2010]. In addition, although young first-time donors may be representative of their age group, the first-time donors in older cohorts are less likely to be representative, because older individuals donate blood after self-selection and medical exclusion as described above; the motives for blood donation also will differ between such groups. These distinctions make it difficult to evaluate prevalence rates evenly between younger and older donors. Nonetheless, our analysis provides key prevalence data in terms of sample size and geographic range, potentially permitting regional and even international comparisons.

Combining the prevalence rate deduced from donor measurement and the rate estimated from the theoretical curve, the overall prevalence rates covering all age categories were calculated to be 0.66% and 1.02% for men and women, respectively. The total number of HTLV-1 carriers in Japan was estimated to be 1.08 million, of which 0.51 million were derived from the

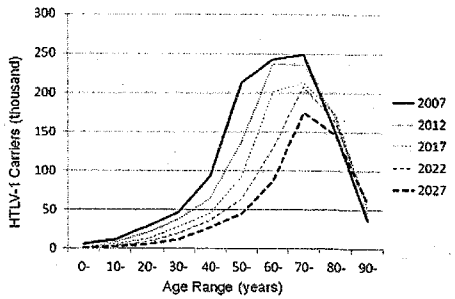

Fig. 5. Projected age distribution of HTLV-1 carriers over the *next two* decades. Age distribution for HTLV-1 carriage is projected at 5-year intervals for the next 20 years, from 2007 (bold solid line) through 2027 (bold broken line).

blood donor age categories. As noted above, these values are likely to represent lower limits: while the prevalence rate among people over 64 years of age was estimated based on the assumption that the rate increases linearly from 40 years of age, empirical results indicate that the increase in the rate becomes greater as the population ages.

Aside from transfusion-related transmission, horizontal transmission of HTLV-1 is believed to occur predominantly from men to women through sexual contact [Kajiyama et al., 1986; Murphy et al., 1989]. In fact, it may be possible to estimate horizontal transmission rates based on our data, by comparing the prevalence rates between the first-time donors in an age category in 1988 to those in the respective (20-year older) age category for 2006–2007. Such an analysis, however, risks the drawing of a skewed conclusion because of multiple assumptions needed to calculate the rate. Indeed, discordant results about sexual transmission have been reported even within cohort studies [Iga et al., 2002; Roucoux et al., 2005]. In the setting of blood donation, determination of the horizontal transmission rate would require following a large number of blood donors for several decades so as to track HTLV-1 seroconversion.

Most women under 30 years of age, and a considerable proportion of women in their 30s, are potential childbearers. The analysis suggests the existence of approximately 30,000 carriers among Japanese women in these age categories. Assuming that the total fertility rate in Japan is 1.32 [National Statistics Center, 2009b] and that the mother-to-infant HTLV-1 transmission rate is 20% [Nakano et al., 1986; Sugiyama et al., 1986; Wiktor et al., 1993], it is calculated that 7,900 children born to carrier mothers will be infected with HTLV-1 in the absence of future interventions to prevent mother-to-infant transmission.

Although the number of carriers 0–99 years of age decreased by 10% over the past two decades, the most remarkable change during that time was the aging of the carriers, as revealed by the rightward shift of the curve in Figure 3. Particularly noteworthy was the increase in the number of carriers over 70 years of age: this age category constituted 258,000 (22% of all carriers) in 1988, as opposed to 436,000 (40% of all carriers) in 2006–2007. Most likely, this aging of HTLV-1 carriers was a "birth cohort effect," whereby the high-prevalence cohort (those born 1930–1960) ages, while younger cohorts (those born after 1960) have lower prevalence rates. In addition, the extension of Japanese lifespan seems to have augmented the trend toward the carrier aging; the average lifespan of the Japanese has extended by 4 and 5 years for men and women, respectively, over the past two decades [Ministry of Health, Labour, and Welfare, 2009].

The geographical distribution of HTLV-1 seroprevalence among blood donors was highly variable, ranging from 0.06% in a Prefecture in eastern Japan up to 1.95% in a Prefecture in Kyushu. The carriers in the Kyushu district accounted for 45.7% of all carriers deduced for all age categories (0–99) in Japan. Changes in the distribution of carriers (0–99) over the past two decades revealed increasing numbers of carriers in the Kanto district, which includes the greater metropolitan Tokyo area, and in the Chubu district. In part, this pattern can be explained by the movement of population from northeastern and western Japan to the metropolitan and central areas. Over the past two decades, nationwide population rose 4.1%. During this interval, the population rose by 9.2% and 5.6% in the Kanto and Chubu districts, respectively, whereas population has decreased by 1.8% in the Hokkaido/Tohoku district or increased only marginally (1.1%) in the Kyushu district [National Statistics Center, 2009c]. Clearly, the carrier population has redistributed along with the movement of the general population. These results reveal that the prevention of HTLV-1 transmission is not an issue limited to the Kyushu district, but is now a challenge for the entire country.

On the basis of population projections, the changes in the number of HTLV-1 carriers were estimated for the next two decades. Although the number of carriers is expected to decrease by 50% in the next two decades, the age-based skew of the carriers is expected to become more prominent, with the peak number of carriers continuing to be those in their 70s. Accordingly, the population of adult T-cell leukemia patients is expected to become older. Since the conventional treatment strategy is inapplicable for these patients [Hermine et al., 1998; Tsukasaki et al., 2009; Tanosaki and Tobinai, 2010], this shift will represent a new medical challenge.

In conclusion, the study indicated that the number of HTLV-1 carriers in Japan is currently 1.08 million at the minimum, and that the carriers are distributed throughout the country. Intensive discussion and nationwide surveys will be needed to identify carriers, to educate seropositive individuals on the prevention

of mother-to-infant transmission, and to establish a consultation system for these people. Although the carrier number is expected to decrease by half in the next two decades, carrier population will age along with the general population. It is essential to establish a novel consensus regimen for the care and treatment of elderly patients with HTLV-1-associated diseases.

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別紙様式第２－１

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分		総合機構処理欄
一般的名称	－						
販売名（企業名）	－		研究報告の公表状況	http://www.documents.hps.scot.nhs.uk/ewr/pdf2012/1201.pdf	公表国 英国		

研究報告の概要	欧州委員会からの要請をうけて、2011年7月に欧州疾病対策センター（ECDC）は供血における異種指向性マウス白血病ウイルス関連ウイルス（XMRV）の影響についてリスクアセスメントを提示した。 公表文献のレビューからは、人間の病理学におけるウイルスの潜在的な役割を示す十分な証拠が得られず、人口あたりのXMRVの可能性有病率、慢性疲労症候群（CFS）におけるXMRV感染率、XMRVの遺伝的変異の程度について疑問が残っている。 XMRVは輸血を通して伝播することが仮定されるが、受血者において XMRV に関連する疾患や兆候は見られず、感染イベントも認められなかった。 XMRV は、輸血の安全性にリスクをもたらす別の新興感染因子の可能性があり、他の因子と同様に血液の安全性を確保するために利用可能な最善の科学に基づいて、迅速かつ効果的な行動が不可欠である。 現在、科学的データが不完全で矛盾しており、XMRV と CFS 患者におけるマウス白血病ウイルスに関する２つの研究での知見は、実験室での汚染によるものであった可能性が高く、証拠の大半は XMRV と CFS の間には因果関係が存在しないという結論を支持すると結論づけている。 近い将来、検証済みのアッセイの開発に伴って得られる追加データは、血液ドナーの適格性とスクリーニングに関する決定に役立つだろう。
報告企業の意見	今後の対応
慢性疲労症候群との関連が疑われるXMRVについては、検査の再現性が担保できず、XMRVとCFSの間には因果関係が存在しないという結論を支持するとの情報であった。	今後ともXMRVやその他のMLV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

9

in milk yield being observed in cattle and small livestock. Epidemiological, immunological and microbiological investigations are ongoing in Germany and the Netherlands. According to health authorities in Germany and the Netherlands, further cases in cattle and small livestock can be expected.

Previously, genetically similar orthobunyaviruses have not caused disease to humans. Therefore, it is unlikely that this virus would cause disease in humans, but it cannot be excluded at this stage. ECDC is urging close collaboration between animal and human health services to ensure rapid detection of any change of the epidemiology in animals and humans. It is also recommending, in particular, that the health of farmers and veterinarians in close contact with potentially infected animals is carefully monitored.

The full risk assessment *New Orthobunyavirus isolated from infected cattle and small livestock — potential implications for human health* can be accessed at http://ecdc.europa.eu/en/publications/Publications/Forms/ECDC_DispForm.aspx?ID=795. [Source: ECDC News, 23 December 2011. http://ecdc.europa.eu/EN/PRESS/Pages/index.aspx]

CMO's annual report 2010

46/0104 Action to improve the nation's health is proving effective but obesity, poor diet and excessive alcohol consumption continue to cause unacceptable levels of ill health, according to the sixth annual report from Scotland's Chief Medical Officer.

The report also notes that infections continue to be a major public health problem in Scotland. Its final chapter summarises significant trends in the incidence of the main communicable diseases of public health importance in 2010, as well as reviewing progress in Scotland in the management of two significant conditions namely tuberculosis and *Escherichia coli* O157 infections.

The CMO's report can be accessed at http://www.scotland.gov.uk/Publications/2011/12/14120931/0. [Source: Scottish Government News Release, 14 December 2011. http://www.scotland.gov.uk/News/Releases/2011/12/14091444]

Pet Travel Scheme

46/0105 Pet travel rules changed on 1 January 2012 when the UK brought its procedures into line with the European Union. From this date all pets can enter or re-enter the UK from any country in the world without quarantine provided they meet the rules of the scheme, which will be different depending on the country or territory the pet is coming from.

The Pet Travel Scheme has been successfully operating across Europe since 2004, and will allow the UK to maintain high levels of protection against animal disease whilst bringing it into line with scientific advances and updating a system first devised in the 1800s.

All pets will still need to be vaccinated against rabies and dogs must be treated for tapeworm, with important checks still being performed before animals are allowed to enter the UK. The scheme could however save UK pet owners around £7 million in fees - around £100 in vet fees per person travelling inside the EU and up to £2,500 in quarantine fees for those travelling outside the EU.

Further information on the new pet travel scheme can be found at http://www.defra.gov.uk/pets. [Source: Defra News Release, 30 December 2011. http://www.defra.gov.uk/news/2011/12/30/new-rules-will-make-it-easier-to-travel-with-pets/]

XMRV and implications for blood donation

46/0106 Following a request from the European Commission, the European Centre for Disease Prevention Control (ECDC) produced in July 2011 a risk assessment on the possible implications of xenotropic murine leukaemia virus-related virus (XMRV) on blood donation. A review of the published literature at the time showed that there was not enough evidence to reliably assess the potential role of the virus in human pathology.

Many questions remain regarding the possible prevalence of XMRV in the human population, the incidence of XMRV in cases of chronic fatigue syndrome (CFS) and the extent of genetic variation between XMRV isolates. Although it is theoretically presumed that XMRV could be transmitted through blood transfusion, no such transmission event has been identified, and there is no known evidence of XMRV infection, related illness or disease in transfusion recipients. XMRV may represent another emerging infectious agent that poses a risk to transfusion safety, and as with other agents, it is imperative that the action taken to ensure blood safety is swift and effective, yet based on the best available science. Currently, the scientific data are incomplete and conflicting. The assessment concludes that it seems likely that the findings in two studies on XMRV and murine leukaemia virus in CFS patients were due to laboratory contamination, and the majority of the evidence favours the conclusion that there is no causal relation between XMRV and CFS. With the development of validated assays, additional data will become available in the near future which will help inform decisions on blood donor eligibility and screening.

The full risk assessment can be accessed at http://ecdc.europa.eu/en/publications/Publications/111222_TER_Risk-assessment-XMRV-implications-for-blood-donation.pdf. [Source: ECDC News, 20 December 2011. http://ecdc.europa.eu/en/press/news/Pages/News.aspx]

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
				2011. 11. 25	該当なし	
一般的名称	人血清アルブミン		研究報告の公表状況	ABC News. Available from: http://abcnews.go.com/blogs/health/2011/11/25/new-swine-flu-strain/	公表国	
販売名（企業名）	赤十字アルブミン20（日本赤十字社） 赤十字アルブミン25（日本赤十字社） 赤十字アルブミン5%静注12.5g/250mL（日本赤十字社） 赤十字アルブミン20%静注4g/20mL（日本赤十字社） 赤十字アルブミン20%静注10g/50mL（日本赤十字社） 赤十字アルブミン25%静注12.5g/50mL（日本赤十字社）				米国	

研究報告の概要	使用上の注意記載状況・その他参考事項等
○新種のブタインフルエンザに対するCDCの警告 米国の政府保健担当官は今月（2011年11月）、アイオワ州の3人の子供に感染した新しいブタインフルエンザを警戒している。CDCによると、7月以降10人の米国人がS-OtrH3N2ウイルスに感染した。このウイルスは北米で循環している稀なブタインフルエンザウイルスH3N2と、2009年に大流行したH1N1ウイルスが結合した新しいインフルエンザウイルスである。人々はそのウイルスに対する免疫をまだ獲得していないため、健康へのリスクとなり得る。 以前の7症例は全て、患者が患者の身近な人がブタに暴露していた。 最新の3症例ではブタに暴露した者はおらず、3人の子供が同じ保育園の集会に参加していたことから、ヒトとヒトの接触がウイルス感染に関連すると示唆される。 CDCは新型インフルエンザの流行への予防対策として、S-OtrH3N2ウイルスに対するワクチンを開発するためのウイルス株を作り、ワクチン製造会社に送付したと発表した。 このウイルスは一般的な抗ウイルス薬（リマンチジン、アマンタジン）には耐性を持つが、オセルタミビル（タミフル）やザナミビル（リレンザ）には反応するとされる。	赤十字アルブミン20 赤十字アルブミン25 赤十字アルブミン5%静注12.5g/250mL 赤十字アルブミン20%静注4g/20mL 赤十字アルブミン20%静注10g/50mL 赤十字アルブミン25%静注12.5g/20mL 血液を原料とすることに由来する感染症伝播等
報告企業の意見	**今後の対応**
新種のブタインフルエンザウイルスに3人の子供が感染し、ヒト-ヒト間での感染が示唆されたとの報告である。 インフルエンザは毎年流行をみる最もポピュラーな疾患であるが、本剤によるいかなるインフルエンザウイルス感染の報告はない。本剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれているため、本剤の安全性は確保されていると考える。	特別な対応を必要としないが、新型インフルエンザが流行した場合、献血者減少につながることも予想されることから、今後も引き続き情報の収集に努める。

CDC Warns of New Swine Flu Strain

U.S. health officials remain on the alert for additional cases of a new swine flu (http://www.cdc.gov/h1n1flu/)strain that infected three Iowa children this month.

Since July, 10 Americans have been sickened by S-OtrH3N2 viruses that picked up a gene from the 2009 H1N1 swine flu pandemic, the federal Centers for Disease Control and Prevention reported. The new flu strain combines a rare influenza virus (H3N2) circulating in North American pigs and the H1N1 virus from the 2009 outbreak. New flu strains develop when flu viruses combine in new ways. They can pose health risks because people haven't yet developed immunity to them.

Of the other seven cases of the new swine flu, three occurred in Pennsylvania, two in Maine and two in Indiana, the CDC reported in a Wednesday dispatch in its Morbidity and Mortality Weekly Report (http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6049a1.htm?s_cid=mm6049a1_w). In all of those cases, either the patients or close contacts had been recently exposed to pigs (http://www.flu.gov/individualfamily/about/animal/index.html#protect_pec The lack of pig exposure in the three newest cases suggested that the new virus may involve limited person-to-person contact. As part of routine preparedness to counter pandemic threats from new flu viruses, CDC said it had developed a "candidate vaccine virus" that could be used to make a human vaccine against S-OtrH3N2 viruses and has sent it to vaccine manufacturers.

One of the three Iowa children, a previously healthy girl referred to as Patient A, became sick during the second week of November. Her doctor tested her as part of routine surveillance and sent a respiratory sample to the Iowa state laboratory for further analysis. Patient B, a boy, developed a flu-like illnesses two days after the Patient A became ill. A day after Patient B became sick, his brother, Patient C, also became ill. Both tested positive for swine flu. All three children had attended the same small gathering on the first day Patient A was fell ill.

After a detailed investigation, Iowa epidemiologists determined that the gathering was the only common link among the three children's illnesses. None of their families had recently traveled or attended community events, and none of the three or their families had been exposed to pigs, according to the CDC.

Eight days after Patient A became ill, Iowa state laboratory testing indicated the three might have S-OtrH3N2 influenza. The CDC subsequently confirmed the three youngsters had that strain, which included the so-called matrix (M) gene from the 2009 H1N1 pandemic. The new flu strain is resistant to two commonly used antiviral drugs, rimantidine and amantadine, but based upon their genetic structure, would likely respond to osteltamivir (Tamiflu) (http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0001054/) and zanamivir (Relenza) (http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0001049/).

CDC scientists said they expected this years' seasonal flu vaccine to provide adults with limited protection from the new flu virus, but that it wouldn't help children. They recommended that doctors who suspect swine flu infections in their patients treat them with Tamiflu where appropriate, obtain nose and throat specimens and send them to a state public health labs, which should report them to CDC. CDC also encourages anyone who has contact with pigs and develops a flulike illness to be tested.

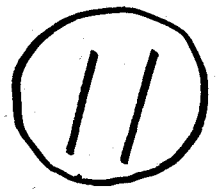
別紙様式第2-1

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2011. 11. 21	該当なし	
一般的名称	人血清アルブミン		研究報告の公表状況		公表国	
販売名（企業名）	赤十字アルブミン20（日本赤十字社） 赤十字アルブミン25（日本赤十字社） 赤十字アルブミン5%静注12.5g/250mL（日本赤十字社） 赤十字アルブミン20%静注4g/20mL（日本赤十字社） 赤十字アルブミン20%静注10g/50mL（日本赤十字社） 赤十字アルブミン25%静注12.5g/50mL（日本赤十字社）		ProMED 20111114.3364, 2011-11-14		パキスタンほか	

研究報告の概要	○デング熱／デング出血熱（DHF）更新情報2011年（抜粋） ・パキスタン：パンジャーブ州のデング熱患者数は合計3,678人に上り、うち2,498人はラホール市の住民である。11月10日に確性者が1人増え、ラホール市の死亡者数は合計で306人となった。 ・インド：11月11日、首都デリーの病院で24時間中に15人のデング熱患者が診断された。デリーにおける2011年のデング熱患者数の合計は945人に達するが、症例数は徐々に減少している。 ・台湾：11月10日に高雄市の69歳男性がDHFのため死亡したと報告された。今夏3件目の死亡例。6月以降、10例のDHF症例が報告され、うち8例が55歳以上である。 ・韓国：11月9日、韓国疾病管理予防センターの発表によると、7月にデングウイルス感染と診断された南キョンサン市の32歳女性は、韓国国内で感染した可能性があり、現在、他に患者がいるかどうか調査中である。女性はインドネシアに1週間滞在していたが、発症は帰国してから39日後であり、デング熱の平均潜伏期間である14日間よりはるかに時間が経過していた。 ・ブラジル：1982年、1986年、1988年、2002年、2008年及び2010年に続く7度目のデング熱流行を回避するための警戒体勢が敷かれている。2011年の9カ月間で70万症例以上が報告されているが、1～2月の報告数は2010年に比べ24%減少し、死亡例も25%、重篤例も40%減少した。
報告企業の意見	世界各地におけるデングウイルス感染状況についての最新情報である。 デングウイルスは脂質膜を持つ中型RNAウイルスである。これまで、本製剤によるデングウイルス感染の報告はない。本製剤の製造工程には平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれていることから、本製剤の安全性は確保されていると考える。
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渡航歴の有無を確認し、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。特別な対応を必要としないが、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。

使用上の注意記載状況・その他参考事項等
赤十字アルブミン20 赤十字アルブミン25 赤十字アルブミン5%静注12.5g/250mL 赤十字アルブミン20%静注4g/20mL 赤十字アルブミン20%静注10g/50mL 赤十字アルブミン25%静注12.5g/20mL 血液を原料とすることに由来する感染症伝播等



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Subject: PRO/EDR> Dengue/DHF update 2011 (45)
Archive Number: 20111114.3364

DENGUE/DHF UPDATE 2011 (45)
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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: Asia --- Pakistan (Lahore city and Punjab province) Pakistan (Karachi, Sindh province) Pakistan (Rawalpindi, Punjab province) India (Delhi and national) India (Churachandpur, Manipur state) India (Tamil Nadu state) Taiwan (Kaohsiung) South Korea (South Gyeongsang province) Philippines (national) Philippines (Northern Mindanao) Pacific --- Marshall Islands Latin America --- El Salvador Brazil (national) Brazil (Rio de Janeiro, Rio de Janeiro state) Brazil (Ilheus and Itabuna, Bahia state) Brazil (Caraguatatuba, Sao Paulo state) Brazil (Campinas, Para state) Bolivia (Amazon region)

****** Asia --- - Pakistan (Lahore city and Punjab province). 12 Nov 2011. Another patient fell victim to dengue virus on Thursday [10 Nov 2011], mounting the total death toll to 306 in Lahore city. According to Health Department only 23 persons with positive dengue symptoms in their blood reports were reported in Punjab during last 24 hours; out of them, 10 are from Lahore. The total number of dengue patients in the Punjab has risen to 3678, out of them, 2498 belong to Lahore.
http://www.thenews.com.pk/NewsDetail.aspx?ID=26354&title=One-more-falls-prey-to-dengue-fever

[A HealthMap/ProMED-mail interactive map of Lahore and Punjab province can be accessed at http://healthmap.org/r/1kdh. - Mod.TY]

- Pakistan (Karachi, Sindh province). 12 Nov 2011. A total of dengue fever patients reached 717 in Sindh, while 9 new patients were diagnosed dengue positive in Karachi on Friday [11 Nov 2011]. As per statistics of Sindh Dengue Surveillance Cell (SDSC), a total of 847 patients were admitted at different hospitals in Karachi and of them 717 were found dengue positive. SDSC reported that total 15 dengue patients had died in Sindh province of them 13 belonged to Karachi. Some 17 patients are still under treatment at different hospitals in the metropolis. http://www.dailytimes.com.pk/default.asp?page=2011%5C11%5C12%5Cstory_12-11-2011_pg12_11

[A HealthMap/ProMED-mail interactive map of Karachi, Sindh province can be accessed at http://healthmap.org/r/1kc-. - Mod.TY]

- Pakistan (Rawalpindi, Punjab province). 10 Nov 2011. Data revealed that the 3 teaching hospitals received less than 40 dengue fever suspects within past 3 days. Within 24 hours after Eid prayer on Monday [7 Nov 2011], the allied hospitals received 246 patients with fever at their dengue fever OPD [out patient departments] counters of which only 13 were admitted in wards after being suspected as patients of the infection. Focal person for dengue fever at the allied hospitals, Dr Javed Hayat, stated that the number of dengue fever suspects reaching allied hospitals is getting thin mainly because of the onset of chilly weather. http://www.thenews.com.pk/TodaysPrintDetail.aspx?ID=76690&Cat=6&dt=11/10/2011

[A HealthMap/ProMED-mail interactive map of Rawalpindi, Punjab province can be accessed at http://healthmap.org/r/1qVK. - Mod.TY]

- India (Delhi and national). 11 Nov 2011. Hospitals in Delhi have diagnosed 15 new dengue cases in the past one day, thus indicating that dengue has started ebbing in the city, MCD's [Municipal Corporation of Delhi] public health committee chairman VK Monga said. "The total number of people affected by dengue fever this year [2011] has reached the 945 mark. 15 cases were reported in the past one day. Dengue cases are gradually showing a downward trend. Unlike earlier when 20 and more dengue cases were being reported in just 24 hours, now it is less," Monga said. Over 10 000 dengue cases have been detected in the country this year [2011]. According to Union health ministry figures, states like Sikkim, Manipur, Nagaland, Himachal Pradesh, and Meghalaya have had no dengue cases this year. In contrast over 1000 dengue cases were detected in Punjab, Orissa and Tamil Nadu. "Dengue can be fatal," Monga said.
http://ibnlive.in.com/generalnewsfeed/news/chikungunya-cases-worrying-civic-authorities/896219.html

[The 9 Nov 2011 edition of IBN (http://ibnlive.in.com/generalnewsfeed/news/10000-dengue-cases-in-the-country-delhi-fifth-among-states/890746.html) reported that Punjab tops the list of affected states with 2016 dengue cases, followed by 1793 cases in Orissa, 1398 in Tamil Nadu, and 963 in Kerala this year [2011].

A HealthMap/ProMED-mail interactive map of Delhi can be accessed at http://healthmap.org/r/1qVL. - Mod.TY]

- India (Churachandpur, Manipur state). 9 Nov 2011. 5 new cases of dengue were reported in Churachandpur [district] even as the district health officials struggled to contain the disease almost a month after the presence of its virus was confirmed [in the district]. Excel Diagnostic Centre reported that 2 blood samples they received on Sunday [6 Nov 2011] tested positive while all the 3 reports they received today [9 Nov 2011] from Babina Diagnostic Centre, Imphal were also found positive.
http://www.e-pao.net/GP.asp?src=4..101111.nov11

[A HealthMap/ProMED-mail interactive map of Churachandpur, Manipur state can be accessed at http://healthmap.org/r/1pSI. - Mod.TY]

- India (Tamil Nadu state). 13 Nov 2011. So far in the current year [2011], 1634 persons were affected with dengue fever. The samples of 8731 persons were tested for dengue fever. The number of people affected with dengue was high in border areas [with Kerala state] such as Kanyakumari, Tirunelveli, Coimbatore, Dindugal, Theni, and Nilgiris.
http://www.thehindu.com/news/cities/Madurai/article2622201.ece

[A HealthMap/ProMED-mail interactive map of Tamil Nadu state can be accessed at http://healthmap.org/r/1mm2. - Mod.TY]

- Taiwan (Kaohsiung) 10 Nov 2011. The Kaohsiung Department of Health (DOH) reported Thursday [10 Nov 2011] that a 69-year-old man died of DHF earlier in the week, the 3rd death from the mosquito-borne disease [virus] in the southern city this summer [2011]. There have been 10 DHF cases reported in the city since June 2011, 8 of which involved people aged 55 years old or above. http://www.taiwannews.com.tw/etn/news_content.php?id=1755090

[A HealthMap/ProMED-mail interactive map of Kaohsiung can be accessed at http://healthmap.org/r/1qVP. - Mod.TY]

- South Korea (South Gyeongsang province). 10 Nov 2011. The Korea Center for Disease Control and Prevention on Wednesday [9 Nov 2011] said it cannot rule out the possibility that a 32-year-old woman from South Gyeongsang Province [Gyeongsangnam-do], who was diagnosed with the virus in July 2011 was infected in Korea. It said it is testing mosquitoes and finding out whether there are other patients. The woman fell ill on 9 Jun [2011], suffering fever, cold shakes, headache, muscle ache, and a rash that spread to cover her whole body. She had spent a week in Indonesia, but her symptoms began 39 days after she came back, much longer than the average latency [incubation] period for dengue fever of 14 days.
http://english.chosun.com/site/data/html_dir/2011/11/10/2011111001233.html

[A HealthMap/ProMED-mail interactive map of South Gyeongsang [Gyeongsangnam-do] province can be accessed at http://healthmap.org/r/1qVQ. - Mod.TY]

- Philippines (national). 10 Nov 2011. The Department of Health [DOH] Dengue Surveillance Report showed that 97 158 dengue cases have been recorded from 1 Jan-22 Oct this year [2011]. The DOH-National Epidemiology Center (NEC) said the number is 36.87 percent lower than the 153 906 dengue cases during the same period last year [2010]. This year's 527 deaths were also lower than the 947 last year [2010]. Most of the cases came from Metro Manila (22 225), Central Luzon (19 995), the Calabarzon sub-region (13 746), and the Ilocos Region (12 754). http://www.malaya.com.ph/nov10/news6.html

[A HealthMap/ProMED-mail interactive map of the Philippines can be accessed at http://healthmap.org/r/1qJe. - Mod.TY]

- Philippines (Northern Mindanao). 9 Nov 2011. A total of 1605 dengue cases were admitted in different hospitals in Northern Mindanao during the 1st 10 months of 2011. This figure is 77.63 percent lower compared to the 7177 cases recorded in the same period last year [2010], said Dr Jaime Bernadas, regional director of the Department of Health (DOH). Bernadas sa' the ages of those afflicted by dengue ranged from less than one month to 90 years old, with the median age placed at 10.. years old. He said 56 percent of those afflicted with dengue were males. http://www.sunstar.com.ph/cagayan-de-oro/local-news/2011/11/09/dengue-cases-northern-mindanao-drops-first-10-months-189671

[A HealthMap/ProMED-mail interactive map of Northern Mindanao can be accessed at http://healthmap.org/r/1qVR. - Mod.TY]

Pacific --- - Marshall Islands. 10 Nov. 2011. There have been more than 250 confirmed cases of dengue fever in the Marshall Islands, according to Pacific Network, an information sharing network affiliated with the World Health Organization. According to the network, 68 people have been admitted to a Marshall Islands hospital, but there have been no deaths.
http://www.guampdn.com/article/20111110/NEWS01/111100309

A HealthMap/ProMED-mail interactive map showing the location of the Marshall Islands in the South Pacific can be accessed at http://healthmap.org/r/1qVS. - Mod.TY]

Latin America --- - El Salvador. 11 Nov 2011. The Ministry of Health reports 6163 cases of classical dengue fever and 138 of DHF to date, with 7 deaths. http://www.diariocolatino.com/es/20111111/nacionales/97320/Dengue-es-una-enfermedad-endémica-en-el-pa%C3%ADs.htm [in Spanish, trans. Mod.TY]

[A HealthMap/ProMED-mail interactive map of El Salvador can be accessed at http://healthmap.org/r/1kd0. - Mod.TY]

- Brazil (national). 13 Nov. 2011. Brazil is again on alert against dengue to avoid the 7th epidemic of the disease. The 1st case was registered in 1895 and the large epidemics up to now occurred in 1982, 1986, 1998, 2002, 2008, and 2010. In the 1st 9 months of 2011, more than 700 000 cases were registered, with a decline occurring between January and February of 24 percent compared with the previous year [2010]. [Dengue] deaths were also reduced 25 percent and serious cases

declined 40 percent. Most of the dengue cases were registered in the southeastern region, with 47.6 percent, followed by the northeastern region with 25.6 percent, the northern region with 15.7 percent, and the central-western region with 6.2 percent, and in last place the southern region with 4.8 percent of the national total. http://www.noticiasbr.com.br/brasil-esta-alerta-contra-a-dengue-29154.html [in Portuguese, trans. Mod.TY]

A HealthMap/ProMED-mail interactive map of Brazil can be accessed at http://healthmap.org/r/1n1t. - Mod.TY]

- Brazil (Rio de Janeiro, Rio de Janeiro state). 10 Nov 2011. Since the beginning of this year [2011], the Rio de Janeiro municipality has had 130 dengue deaths. http://radioitaperunafm.com/site/2011/11/10/mobilizacao-contra-a-dengue-em-itaperuna/ [in Portuguese, trans. Mod.TY]

[A HealthMap/ProMED-mail interactive map of Rio de Janeiro city, Rio de Janeiro state can be accessed at http://healthmap.org/r/1qJg. - Mod.TY]

- Brazil (Ilheus and Itabuna, Bahia state). 10 Nov 2011. Ilheus municipality registered nearly 1800 dengue cases this year [2011]. The other municipality that runs the risk of a dengue epidemic is Itabuna, with neighborhoods with indices of [vector mosquito] infestation over 30 percent and more then 500 registered dengue cases.
http://www2.uol.com.br/aregiao/2011/11/entry_5603.html

[A HealthMap/ProMED-mail interactive map of Bahia state can be accessed at http://healthmap.org/r/1qVT. - Mod.TY]

- Brazil (Caraguatatuba, Sao Paulo state). 9 Nov 2011. From January to 8 Nov 2011, the municipality registered confirmed 1157 cases of the disease and 2365 reported [suspected] cases. http://www.vnews.com.br/noticia.php?id=107634 [in Portuguese, trans. Mod.TY]

[A HealthMap/ProMED-mail interactive map of Caraguatatuba, Sao Paulo state can be accessed at http://healthmap.org/r/1qVU. - Mod.TY]

- Brazil (Campinas, Para state). 9 Nov 2011. This year [2011] there were 3010 dengue cases, versus 2647 last year [2010]. One person died of the disease in 2011. "We have dengue cases every week," said the coordinator of the Municipal Dengue Control Program, Andre Ribas Freitas. The most critical months are March and April.
http://diariodopara.diarioonline.com.br/N-145548-CAMPINAS+DEVE+TER+EPIDEMIA+DE+DENGUE+EM+2012.html [in Portuguese, trans. Mod.TY]

[A HealthMap/ProMED-mail interactive map of Campinas, Para state can be accessed at http://healthmap.org/r/1qJi. - Mod.TY]

- Bolivia (Amazon region). 9 Nov 2011. In Bolivia there are now 47 dead from a dengue outbreak and 7094 affected [by the infection]. The Amazon region is the most affected. According to official data, 16 people died of the disease in Beni [department -- state or province equivalent], 3 in the La Paz department, 1 in Cochabamba, and 27 in Santa Cruz. Also, 60 percent of the fatal victims are children and adolescents -- the most vulnerable [cohort].
http://www.infobae.com/notas/616093-Bolivia-47-muertos-por-nuevo-brote-de-dengue.html [in Spanish, trans. Mod.TY]

[A HealthMap/ProMED-mail interactive map of the Amazon region of Bolivia can be accessed at http://healthmap.org/r/1mkc. - Mod.TY]

See Also

Dengue/DHF update 2011 (44) 20111108.3320
Dengue/DHF update 2011 (43) 20111101.3242]
.....sb/dk/ty/mj/mpp

別紙様式第２－１

番号：1

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日 平成 23 年 11 月 16 日	新医薬品等の区分 該当なし	機構処理欄
一般的名称	ﾃｸﾈﾁｳﾑ大凝集人血清ｱﾙﾌﾞﾐﾝ（^{99m}Tc）		研究報告の公表状況	VECTOR-BORNE ZOONOTIC DISEASES Vol. 00, No. 00, 2011 /HPA(Emerging Infections Monthly Summaries 1-31 Oct. 2011) AND	公表国 中国	
販売名（企業名）	テクネ MAA キット（富士ファイルムＲＩファーマ株式会社）					

研究報告の概要	要約： 血小板減少症候群を伴う重度の発熱疾患（SFTS）は、新たに発見されたブニヤウイルス（SFTSV）に起因する新興感染症で、高い死亡率(平均 12%、最高で 30%)をもたらす。 本研究の目的は、SFTSV が人から人へ伝播できたかどうかを確認することである。中国安徽省で 2006 年 9 月～11 月の間に発生した未知の感染症に罹患した 2 つのクラスターの患者(13 人)の血清を、RT-PCR 法による SFTSV RNA 検出および間接蛍光抗体法による SFTSV 抗体検査にて分析した。全患者(n=14)に典型的な SFTS の臨床症状（発熱、血小板減少、白血球減少）があったこと、両クラスターの二次患者全員が、一次患者の血液に接触または曝露後 6～13 日で発病したことが判明した。我々は、クラスター1 の一次患者および二次患者 9 人全員と、クラスター2 の二次患者 3 人全員の急性期血清から、RT-PCR 増幅法により SFTSV RNA を検出したか（そして／または）、SFTSV に対する抗体力価が 4 倍に増加したか、セロコンバージョンが起きた、のいずれかを立証した。クラスター2 の一次患者は血清不足のため分析されなかった。同期間中に一次患者と接触したが血液への曝露がなかった人において、病気になったものはいなかった。我々は、SFTSV は患者血液との接触を通じて人から人へ伝播可能であったという結論に至った。
報告企業の意見	本研究報告は、RT-PCR 法と間接蛍光抗体法を用いて中国で発生した 2 つのクラスターの患者保存血清（13 人）を分析し、両クラスターにおいてそれぞれ人から人への感染があったことを示唆した新規感染経路に関する報告である。また死亡率も高いことから重大な感染症の可能性が高いと考えられ、感染症定期報告の対象と判断する。
今後の対応	本研究報告は、ヒト血液を原料とする血漿分画製剤とは直接関連しないことから、現時点で当該生物由来製品に関し、措置等を行う必要はないと判断する。

使用上の注意記載状況・その他参考事項等
特になし

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Person-to-Person Transmission of Severe Fever with Thrombocytopenia Syndrome Virus

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Abstract

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease caused by a newly discovered bunyavirus, SFTS virus (SFTSV), and causes high fatality (12% on average and as high as 30%). The objective of this study was to determine whether SFTSV could be transmitted from person to person. We analyzed sera of 13 patients from two clusters of unknown infectious diseases that occurred between September and November of 2006 in Anhui Province of China for SFTSV antibody by indirect immunofluorescence assay and for SFTSV RNA by RT-PCR. We found that all patients (n=14) had typical clinical symptoms of SFTS including fever, thrombocytopenia, and leukopenia and all secondary patients in both clusters got sick at 6–13 days after contacting or exposing to blood of index patients. We demonstrated that all patients in cluster 1 including the index patient and nine secondary patients and all three secondary patients in cluster 2 had seroconversion or fourfold increases in antibody titer to SFTSV and/or by RT-PCR amplification of SFTSV RNA from the acute serum. The index patient in cluster 2 was not analyzed because of lack of serum. No person who contacted the index patient during the same period, but were not exposed to the index patient blood, had got illness. We concluded that SFTSV can be transmitted from person to person through contacting patient's blood.

Key Words: Arbovirus(es)—China—Tick(s)—Transmission.

Introduction

SEVERE FEVER WITH THROMBOCYTOPENIA SYNDROME (SFTS) is an emerging infectious disease discovered in northeastern and central China recently and is caused by a novel bunyavirus, SFTS virus (SFTSV). The major clinical symptoms and laboratory abnormalities of SFTS are fever, thrombocytopenia, leukopenia, and elevated serum hepatic enzymes, and SFTS patients usually die due to multiple organ failure, with an average 12% case fatality rate and even 30% in some areas. SFTSV is classified in the genus *Phlebovirus* of the family Bunyaviridae (Yu et al. 2011). Except for *Hantavirus*, bunyaviruses are arthropod borne and are transmitted to humans through arthropod bites. Hantavirus is transmitted to humans through an aerosol generated from infected rodent's excreta (Nichol et al. 2005). SFTS was presumably transmitted by ticks, because SFTSV has been detected in *Haemaphysalis longicornis* ticks (Yu et al. 2011). Before discovery of SFTSV, several clusters of infectious diseases resembling the clinical manifestations and laboratory abnormalities of SFTS disease have been reported in China since 2005 (Zhou 2007, Zhang et al. 2008). In this study, we did a retrospective analysis of clusters of patients in China in 2006 to determine whether SFTSV was responsible for two clusters of unknown infectious diseases and whether SFTSV was transmitted from person to person.

Materials and Methods

Study design

All information and samples of the patients were collected from local hospitals in Anhui Province of China. Patients' sera were kept at room temperature during transportation and at −80°C in laboratories. Patients and patients' families provided informed written consent for the procedures of the investigation. When a cluster of unknown infectious diseases was reported, an investigation team of epidemiologists was

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dispatched to the hospitals and patients' houses to interview family members and healthcare workers who had been exposed to the patient and surveyed the environment surrounding the houses of the patients to determine the risk factors.

Indirect fluorescent antibody assay

Patient sera were tested for antibodies to SFTSV by indirect fluorescence assay (IFA). Sera that were reactive at a dilution of ≥1:64 were considered to be positive.

RT-PCR

RNA was extracted from patients' sera using RNeasy Mini Kit, and one-step RT-PCR Kit (Qiagen) was used to amplify viral RNA L-segment. The primers were designed at position 1324 (GGCAGCAAACCAGAAGAAAG) and at position 2326 (CATTTCTCCGAGGGCATTTA). PCR was performed for 1 cycle at 50°C for 30 min, 95°C for 15 min, and then 35 cycles of 94°C for 45 s, 58°C for 45 s, and 72°C for 1 min, with a final extension at 72°C for 10 min. PCR products were sequenced and the sequences were deposited in GenBank (JN398157 and JN398158).

Results

Cluster 1

Index patient. On October 31, 2006, a 50-year-old woman had sudden onset of fever (39.2°C), headache, myalgia, arthralgia, dizziness, and malaise, and she was treated with ribavirin, cephalothin, dexamethasone, and amidopyrine at the village clinic. On November 3, she was admitted to a local hospital because of high fever (39.7°C), gingival bleeding, nausea, vomiting, and a rash over her trunk. Laboratory testing revealed leukopenia, thrombocytopenia, elevated serum aspartate aminotransferase and alanine aminotransferase, elevated serum creatinine, and elevated blood urea nitrogen levels.

On November 4, she was bleeding from her nose and mouth, developed rapidly progressive dyspnea and worsening oxygen saturation, and required endotracheal intubation. She died on the next day.

Retrospective questioning of the patient's family revealed that she was bitten by a tick at 12 days before onset of fever.

Secondary patients. Within 12 days after the death of the index patient between November 9 and 17, 2006, nine patients were identified at the regional hospital with fever (≥38.0°C), myalgia, diarrhea, leukopenia, thrombocytoperia, and elevated serum aspartate aminotransferase and alanine aminotransferase (Table 1). All patients had contact with the index patient during the period that she was bleeding from her nose, mouth, and endotracheal tube between November 4 and 5, including five family members, two physicians, and two nurses (Fig. 1). None of 30 other family members and healthcare workers who were in contact with the index patient but not her blood had become ill and they were not investigated for SFTSV infection because of lack of sera. All secondary patients recovered after treatment with intravenous fluids and electrolytes and ribavirin.

Cluster 2

Index patient. The index patient was a 56-year-old female farmer who developed fever on September 28, 2006, and was admitted into a local hospital on the next day. On October 2 she had a black stool, and on October 4 she vomited blood five times. She was treated with antibiotics and ribavirin in the hospital. Her condition deteriorated, her family requested the hospital to discharge her, and she died at home on the same day.

The index patient lived in a village in Dingyuan County, Chuzhou City of Anhui Province, where 1560 residents lived, farming rice, wheat, peanuts, soybeans, and sesame, and raised cows, sheep, chickens, and ducks. Except for her family, no humans or animals were reported to be sick or dying in the village before or after she became ill. The index patient lived in an isolated farmhouse near a road. The house was described as unsanitary with numerous flies that were attracted by rotting persimmons that had fallen from a tree in the yard. The index patient had 20 chickens, one dog, and one cat. The animals ranged freely during the day and returned home at night. There were no chemical factories or other industries in the village. The patient had not traveled during the previous several months. No blood was collected from any of the animals; rodents and ticks were not investigated in the house or surrounding areas at the time because of lack of knowledge of the disease.

Secondary patients. From day 6 to 8 after the death of the index patient, her 58-year-old husband (case 2), 35-year-old son (case 3), and 33-year-old daughter (case 4) all had fever and were hospitalized (Fig. 1). Laboratory tests revealed that they all had leukopenia and thrombocytopenia (Table 1). All secondary patients were treated with intravenous fluids and electrolytes and ribavirin, and all patients recovered. Prior to onset of the disease, all of the secondary patients had participated in the care of the index patient in the hospital. Case 2 had been with the index patient from September 29 to October 4; case 3 was in contact with the index patient on October 2 and 4; case 4 was in contact with the index patient from October 2 to 4. On October 4, the index patient was vomiting blood, and the three secondary cases cleaned up the blood. Neither of six other persons in the family including the mother-in-law, three grandchildren, son-in-law, and the daughter-in-law of the index patient nor healthcare workers in the hospitals became infected despite contact with the index patient. However, they did not contact her blood. Son-in-law and daughter-in-law of the index patient were used as control and SFTSV antibody and viral RNA were not detected from their sera. Case 2 lived together with the index patient, and cases 3 and 4 did not live in the same area as the index patient. Case 3 worked in a factory in Nanjing City in Jiangshu Province, and case 4 worked in a food processing factory in a neighboring town. No coworker of case 3 or case 4 in the factories were ill during the period when cases 3 and 4 were sick.

The investigation had ruled out the possibilities of chemical poisoning, food poisoning, SARS, influenza, other respiratory infections, hemorrhagic fever with renal syndrome, dengue fever, malaria, gastrointestinal infections, *Streptococcus suis* infection, and human anaplasmosis based on laboratory and epidemiological data. However, the etiological agent of the disease was not known at the time.

Risk factors of person to person transmission of SFTSV

In cluster 1, all secondary patients were exposed to the index patient in the regional hospital. All secondary patients

TABLE 1. CLINICAL, LABORATORY, AND SEROLOGICAL FINDINGS OF PATIENTS IN TWO CLUSTERS OF SEVERE FEVER WITH THROMBOCYTOPENIA SYNDROME DISEASE

	Cluster 1										Cluster 2			
Patient information	*Index*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*Index*	*2*	*3*	*4*
Clinical symptoms														
Fever ≥38.5°C	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Chills	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	UN	UN	UN	UN
Diarrhea	Yes	Yes	No	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	Yes	No
Headache	Yes	Yes	No	No	No	No	Yes	No	No	No	Yes	No	No	No
Bleeding	Yes	No	No	No	No	No	Yes	No	No	No	Yes	Yes	No	No
Rash	Yes	No	No	No	No	No	No	No	No	No	No	No	No	Yes
Laboratory test														
White blood cell (normal 4000–10,000/μL)	3300	3700	2700	1100	1200	2600	3300	3700	2400	2000	2900	1400	3300	3600
Platelet(Normal: 150–300/μL)	18	54	61	54	41	95	54	47	114	40	71	45	52	40
Elevated AST and/or ALT	Yes	Yes	Yes	ND	Yes	ND	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
IFA														
Acute	128	128	256	512	–	128	128	64	64	128	ND	128	64	256
Convalescence	ND	1024	2048	4096	2048	1024	4096	2048	2048	1024	ND	ND	512	2048
RT-PCR	+	+	+	+	ND	ND	ND	ND	ND	ND	ND	+	ND	+

UN, unknown; ND, not done; AST, aspartate aminotransferase; ALT, alanine aminotransferase; IFA, indirect immunofluorescence assay.

were involved in endotracheal intubation and care during times of hemorrhage of the index patient. Of these nine individuals, three reported blood contamination of skin and possible mucocutaneous exposures.

In the second cluster, all secondary patients had direct contact with blood in vomit or bleeding from mouth of the index patient. These results suggest direct contact with blood or respiratory secretions as the mechanism of transmission.

IFA results. We collected sera from 13 patients and 12 of them had acute and convalescent sera. No serum was available from the index patient in cluster 2. Antibodies to SFTSV have been detected in all acute sera ($n=13$) and all convalescent sera ($n=12$); seroconversion or fourfold increase in antibody titer to SFTSV was detected in 4 of 10 patients in cluster 1 (Table 1).

RT-PCR results. L-segment of SFTSV RNA sequences was amplified from six patients including four patients in cluster 1 and two patients in cluster 2. RT-PCR was not performed for other patients because of limitation of sera (Table 1). DNA sequences of the viruses from patients were identical among each cluster, but were different between two clusters and known strains of SFTSV (Fig. 2).

Discussion

In this study we have retrospectively analyzed the etiology of the illness in two clusters of patients with mysterious infectious diseases. These two clusters of patients were not related and lived in different counties in Anhui Province. Epidemiological data indicated that the secondary patients were infected after taking care of the index patients in both

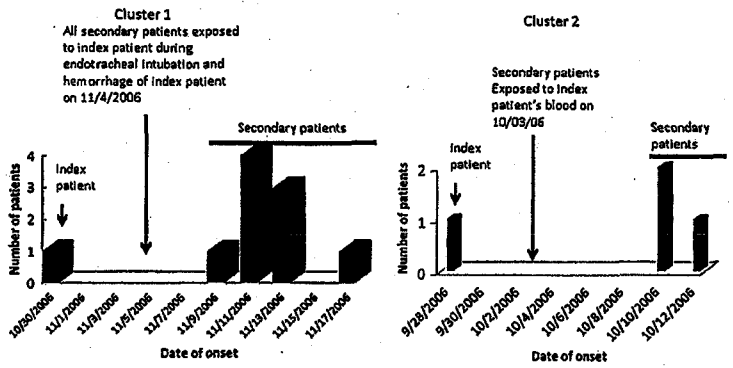

FIG. 1. Date of exposure and onset of secondary patients.

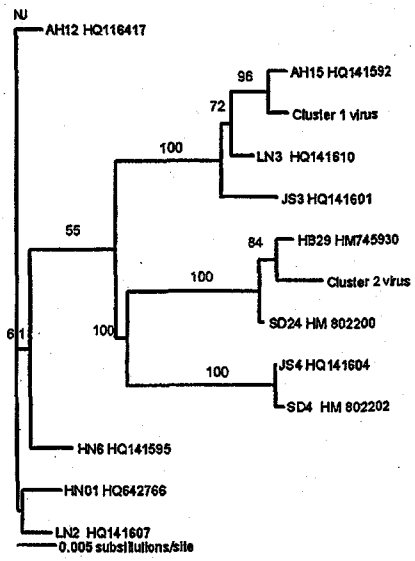

FIG. 2. Phylogenetic analysis of severe fever with thrombocytopenia syndrome virus (SFTSV) RNA L-segment amplified from patients in two clusters (cluster 1 and cluster 2). Phylogenetic trees were generated using Neighbor joining method with Paup 4.0 software. Strain name and GenBank accession number of each sequence of SFTSV are shown on each branch.

clusters; serological analysis showed that all patients were infected with SFTSV; molecular analysis further indicated that the genotype of SFTSV from patients in each cluster was identical, but the genotype of SFTSV from two clusters were different from each other and they were also different from known strains of SFTSV. No known strains of SFTSV are identical in the sequence of L-segment (Fig. 1). This study indicated that the secondary patients in both clusters nosocomially acquired SFTSV from the index patients. Given the fact that all secondary patients in cluster 2 had contacted with blood of the index patient and all secondary patients in cluster 1 had involved in endotracheal intubation and three of them had direct contact with blood of the index patient, it suggests that SFTSV in both clusters were most likely transmitted from person to person through contacting infected patient's blood. Aerosol transmission of SFTSV may be possible, but cannot be confirmed in both clusters. Person-to-person transmission may not be the major transmission route of SFTSV; however, our study demonstrated that patients with SFTSV should be isolated from family members and other patients. In China, currently, patients' family members are allowed to stay in the hospital ward to take care of patients with SFTS. Such a practice may be essential for noninfectious disease patients to reduce the medical costs, but clearly has increased the potential of nosocomial transmission of SFTSV to family members and other patients in the same hospital ward.

The correlations of contact of secondary patients with the index patients in both clusters are very strong. All secondary patients in the two clusters had onset of disease at 9–17 days after contact with the index patients. None of these patients had a history of tick exposure or rodent contact. All secondary patients had contacted the patient's blood during the patients' period of bleeding. We do not know whether any of the 30 persons who contacted the index 1 without contacting her blood had antibody to SFTSV because of lack of patients' sera, but none of them got illness after contacting the index patients. The fact that none of the persons who contacted index patient 2 without exposure to her blood had antibody to SFTSV further suggests that contacting SFTS patients' blood is a risk factor for person-to-person transmission of SFTSV.

The pathogen for the first cluster was ascribed to *A. phagocytophilum*, because *Anapiasma phagocytophilum* antibody and DNA were detected in the patients (Zhang et al. 2008). It has been always a doubt whether *A. phagocytophilum* can be responsible for nosocomial transmission of a severe hemorrhagic disease, because *A. phagocytophilum* had not been reported to cause hemorrhage or person-to-person transmission (Zhang et al. 2008). Because no virus was detected and only *A. phagocytophilum* was detected from the patients' blood samples in cluster 1, *A. phagocytophilum* was believed to be the sole culprit of the mysterious disease (Zhang et al. 2008). After the report of the cluster of human anaplasmosis in China, patients with fever, leukopenia, and thrombocytopenia resembling the clinical symptoms of SFTS were diagnosed and treated as human anaplasmosis (Li and Zhang 2008, Zhou et al. 2009, Tengxun News 2010) until Yu et al. (2011) isolated SFTSV, the actual etiologic agent of SFTS disease, from patients in 2009. The previous study was unable to detect virus in the patients (Zhang et al. 2008), because SFTSV is a novel virus that does not share antigenic cross-reactivity or genetic homology with previously known viruses and it does not cause cytopathic effect in Vero cells, which are commonly used for isolation of viruses. Fortunately, we used DH82 cells that are appropriate to isolate *Ehrlichia* in our previous study, and SFTSV causes severe cytopathic effect in DH82 cells, which can be recognized easily (Yu et al. 2011). In this study, we have detected antibody and/or viral RNA from secondary patients and index patient in cluster 1, which clearly demonstrated that all patients in the cluster were infected by SFTSV. In cluster 2 we demonstrated that all secondary patients were infected with SFTSV, but we could not directly demonstrate that index patient was infected by SFTSV because of lack of serum. We presume that the index patient was infected with SFTSV, because her clinical symptoms resembling SFTS disease and epidemiological data linked her to secondary patients who were all positive for SFTSV.

SFTS disease did not receive attention until the cluster of 10 patients with the unknown causative agent was observed in 2006, and unfortunately, the pathogen was ascribed as *A. phagocytophilum* (Zhang et al. 2008). Besides the two clusters of patients described in this study, a cluster of infectious disease resembling SFTS disease was actually reported as early as in 2005 (Zhou 2007). In that cluster of patients, the index patient was a 59-year-old male farmer who had onset of illness, on August 27, 2005, with fever, myalgia, leukopenia,

thrombocytopenia, and multiple organ failure. The patient died on day 13 after onset of illness, and within 5 days after the death of the index patient, five cases with similar clinical symptoms and laboratory abnormalities as the index patient were hospitalized. The secondary patients had been in contact with the index patient's blood and/or excreta. No known virus was detected from the patients' samples, and we were unable to obtain patient samples from this epidemic to confirm that it was caused by SFTSV.

Disclosure Statement

No competing financial interests exist for all authors.

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別紙様式第２－１

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日 平成 23 年 11 月 18 日	新医薬品等の区分 該当なし	機構処理欄
一般的名称	ﾃｸﾈﾁｳﾑ大凝集人血清ｱﾙﾌﾞﾐﾝ（^{99m}Tc）		研究報告の公表状況	Clin Infect Dis. 2011 ;53(12) :1208-14 /HPA(Emerging Infections Monthly Summaries 1-31 Oct. 2011)	公表国 中国	
販売名（企業名）	テクネ MAA キット（富士フイルムＲＩファーマ株式会社）					

研究報告の概要

要約：

（背景）中国東部の１家族７人は 2007 年５月に発熱と血小板減少症を発症した。しかし最初の調査では感染性の病因を同定するには至らなかった。2009 年 12 月、中国における同様の臨床症状を有する患者の病因として新規のブニヤウイルス（血小板減少症候群を伴う重度の発熱疾患を起こすブニヤウイルス[SFTSV]）が同定された。我々は SFTSV 感染したこの家族集団を再調査した。

（方法）一次患者と二次患者（６人）に対し、疫学的及び臨床データを分析した。６人の保存血液検体を RT-PCR 法、ウイルス培養、ゲノムシーケンス、マイクロ中和試験、間接蛍光抗体法により解析した。

（結果）80 歳女性は 2007 年４月 27 日に発熱、白血球減少症、血小板減少症を発症し死亡した。2007 年５月３～７日の間に 80 歳女性を除く家族６人が、発熱とその他の類似症状により地方病院に入院した。2007 年に採取された６人の血清検体は、RT-PCR 法で SFTSV-RNA が陽性、マイクロ中和試験と間接蛍光抗体法で SFTSV 抗体が陽性となった。保存血清検体の１つからは SFTSV が分離された。

二次患者間における唯一の共通点は、一次患者との個人的な接触であった：疑いのある動物やベクターに曝された報告はない。

（結論）臨床所見と臨床検査値は、2007 年に中国東部の家族内で生じた発熱や血小板減少症が、SFTSV によって引き起こされたことを立証した。疫学調査は二次患者の感染が、個人的接触により家族に伝播したことを強く示唆した。

報告企業の意見	今後の対応
本研究報告は、二次患者血清／血液（６人）を RT-PCR 法等にて分析した結果 SFTSV-RNA 及び SFTSV 抗体の陽性が確認され、臨床所見および二次患者間で唯一の共通点が一次患者との個人的接触であったことから、人から人へ感染したことが疫学的に示唆されたという新規感染経路に関する報告。また死亡率も高いことから重大な感染症の可能性が高いと考えられ、感染症定期報告の対象と判断する。	本研究報告は、ヒト血液を原料とする血漿分画製剤とは直接関連しないことから、現時点で当該生物由来製品に関し、措置等を行う必要はないと判断する。

使用上の注意記載状況・その他参考事項等

特になし

13

MedDRA/J Version(14.1)

MAJOR ARTICLE

A Family Cluster of Infections by a Newly Recognized Bunyavirus in Eastern China, 2007: Further Evidence of Person-to-Person Transmission

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Background. Seven persons in one family living in eastern China developed fever and thrombocytopenia during May 2007, but the initial investigation failed to identify an infectious etiology. In December 2009, a novel bunyavirus (designated severe fever with thrombocytopenia syndrome bunyavirus [SFTSV]) was identified as the cause of illness in patients with similar clinical manifestations in China. We reexamined this family cluster for SFTSV infection.

Methods. We analyzed epidemiological and clinical data for the index patient and 6 secondary patients. We tested stored blood specimens from the 6 secondary patients using real time reverse transcription polymerase chain reaction (RT-PCR), viral culture, genetic sequencing, micro-neutralization assay (MNA), and indirect immunofluorescence assay (IFA).

Results. An 80-year-old woman with fever, leucopenia, and thrombocytopenia died on 27 April 2007. Between 3 and 7 May 2007, another 6 patients from her family were admitted to a local county hospital with fever and other similar symptoms. Serum specimens collected in 2007 from these 6 patients were positive for SFTS viral RNA through RT-PCR and for antibody to SFTSV through MNA and IFA. SFTSV was isolated from 1 preserved serum specimen. The only shared characteristic between secondary patients was personal contact with the index patient; none reported exposure to suspected animals or vectors.

Conclusions. Clinical and laboratory evidence confirmed that the patients of fever and thrombocytopenia occurring in a family cluster in eastern China in 2007 were caused by a newly recognized bunyavirus, SFTSV. Epidemiological investigation strongly suggests that infection of secondary patients was transmitted to family members by personal contact.

In December 2009, a novel bunyavirus—named severe fever with thrombocytopenia syndrome bunyavirus (SFTSV)—was isolated from a patient in central China. Genomic sequencing indicated that the SFTSV comprises a third group within the genus phlebovirus, family *Bunyaviridae* [1]. Illness caused by this novel virus is characterized by sudden onset of fever and respiratory tract or gastrointestinal symptoms, followed by a progressive decline of whole white blood cell and platelet counts.

Three viruses in the family *Bunyaviridae* including Crimean-Congo hemorrhagic fever (CCHF) virus from the genus nairovirus, Rift Valley fever (RVF) virus from the genus phlebovirus, and Old World and New World hantaviruses, such as Andes virus, causing Hemorrhagic Fever Renal Syndrome (HFRS) and Hantavirus Pulmonary Syndrome (HPS), respectively, from the genus hantavirus are recognized as able to induce hemorrhagic fever disease in humans. Cases most likely encounter these viruses via direct contact with infected animal tissues, inhalation of contaminated material, or being bitten by arthropod vectors such as mosquitoes, sand flies, or ticks [2]. Some SFTS patients have reported that they had seen or been bitten by ticks before their illness onset; in their original study, Yu et al [1] reported that there was no epidemiological evidence for person-to-person transmission. SFTSV was therefore proposed to be transmitted by contact with animals and/or vectors such as the *Haemaphysalis longicornis* ticks.

In May 2007, Jiangsu Province Centre for Disease Control and Provention (JS-CDC) was notified about a family in which 6 persons developed fever and clinical symptoms compatible with human granulocytic anaplasmosis (HGA) [3]. In October 2006, an outbreak of HGA had been reported from an adjacent province [4]; therefore, initial investigation of this family cluster focused on identifying *Anaplasma phagocytophilium*. Neither extracted DNAs nor antibodies to the *A. phagocytophilium*, however, were detected from patient blood specimens. Additional laboratory studies ruled out infections from HFRS virus, dengue fever virus, typhoid/paratyphoid *Salmonella* bacteria, leptospirosis, *Rickettsia*-like spotted fever, Pullman's typhus, tsutsugamushi fever, or Q fever. In December 2009, Chinese scientists identified SFTSV in patients with a similar clinical syndrome as the 2007 family cluster. In this paper, we describe the laboratory evidence gathered through molecular and serological analysis and assess the possible routes of SFTSV in the 2007 family cluster.

METHODS

Patients

The 2007 family cluster occurred in a hilly area about 110 km south from Nanjing in eastern China. The first patient (index patient, patient A) was an 80-year-old female who lived in a small village with her husband. She visited the village clinic on 19 April 2007, complaining of fever and chills. She was treated with gentamicin and dexamethasone. On 20 April 2007, she was admitted to the local county hospital with a temperature of 39.0°C. Laboratory testing performed on admission revealed leukopenia (WBC count, 3.1×10^9/L) and thrombocytopenia (PLT count, 48×10^9/L). Chest X-ray examination showed bronchopneumonia. On 21 April, the patient was discharged home for her birthday party, then readmitted to the local hospital on 23 April. At this time, the patient was noted to be confused and unable to speak. Her neck was supple. There were no focal neurological abnormalities. Her temperature was 38.2°C. Blood tests showed that her white blood cell (WBC) count had declined to 1.6×10^9/L and platelet (PLT) count to 30×10^9/L. On 25 April, she was transferred to a municipal hospital. Her condition continued to decline rapidly; on admission, she was noted to have elevated liver-associated enzyme levels (serum aspartate *aminotransferase*, 3869 U/L; alanine aminotransferase, 573 U/L; lactate dehydrogenase, 3094 U/L) and acute renal insufficiency (creatinine, 1883 umol/L; urea nitrogen, 1306 umol/L). The next day, she developed bleeding gums, ecchymosis at an intravenous line puncture site, melena, and fecal incontinence. On 27 April, a massive amount of fresh blood effused from the needle puncture site. Family members elected to withdraw intensive medical support, taking her home, where she died a few hours later.

Within 10 days of patient A's death, 6 of her family members developed similar symptoms. The elder son-in-law (patient B) of patient A developed symptoms first, with onset on 3 May 2007, 6 days after his mother-in-law's death. Symptoms subsequently developed in patient C (patient A's elder daughter, patient B's wife) and patient D (patient A's younger nephew) on 4 May, patient E patient A's younger son-in-law) and patient G (patient's elder nephew) on 5 May, and patient F (patient A's second daughter, patient E's wife) on 7 May. All the secondary patients were previously healthy. None of them lived with the index patient. The secondary patients were between 53 and 72 years of age (mean, 58.3 years), and 4 of them were men. All had fever of at least 37.5°C for 3–6 days (median, 4 days). All complained of having loose stool, 1–3 episodes per day for 1–2 days. Three male patients (patients B, D, and F) developed pleural effusion, bronchitis, and pneumonia, respectively, during hospitalization. Patient E, who had a history of hepatitis B virus infection, experienced the lowest WBC and PLT counts among the 6 secondary patients. However, they all recovered through supportive treatment and were discharged in good condition. A timeline of key events is shown in Figure 1, and clinical features are shown in Table 1.

Epidemiological Investigation

On 8 May 2007, we received a report that 5 patients with fever of unknown cause were admitted to a county hospital. Epidemiological investigation and active surveillance were immediately initiated. On 9 May, another patient was identified. A standardized questionnaire was used to collect demographic information, clinical manifestations, history of exposure to the index patient (where, when, and how contact was made), history of exposure to wild animals, and extent of outdoor activity. Furthermore, public health officials interviewed family members, neighboring villagers, and all hospital staff who had provided medical service for any of the patients, and cross-checked several written timelines of the outbreak. Medical records were also reviewed for the time of discovery of the index patient and the secondary patients' onset and progression of illness.

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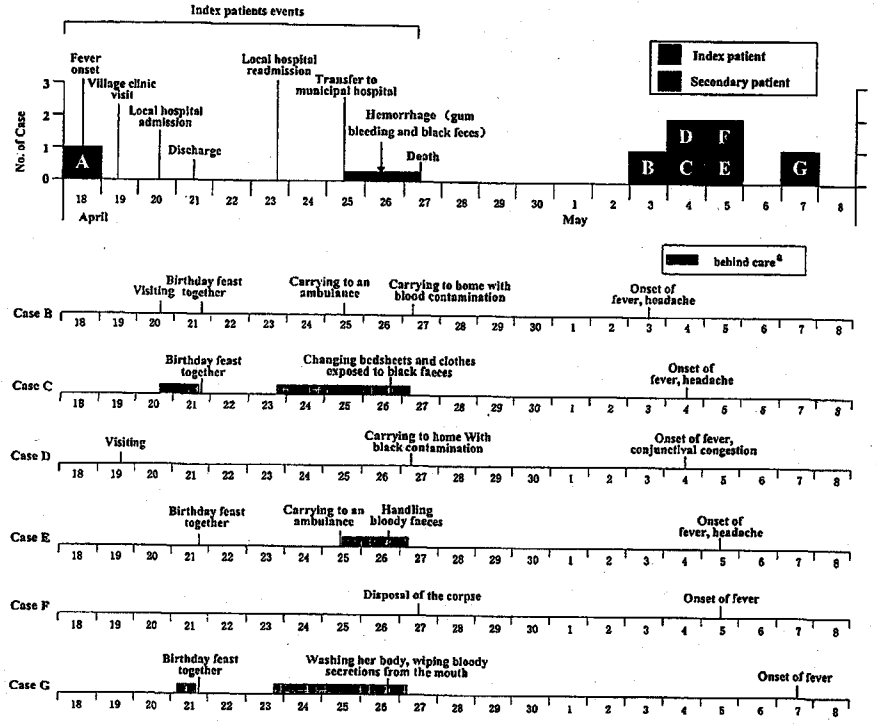

Figure 1. Epidemic curve shows progression of the family cluster and timeline of key events during the index patient's illness as well as pertinent exposure histories of secondary patients. [a]Exposure during period of providing bedside care may not have occurred continuously during the exposure period. *Capital letters designate the corresponding secondary cases in the top and bottom panels.*

By the time this family cluster was detected, the index patient had died and her body had been cremated; therefore, no specimens were available for further testing. Three serum specimens from each secondary patient were collected on 3 separate days (8 May 2007, 13 June 2007, and 17 September 2010) for a total of 18 specimens. All activities were conducted in accordance with the policies of the ethical committee of JS-CDC, and informed consent was obtained from the participants.

Laboratory Testing

In 2010, real-time reverse transcription polymerase chain reaction (RT-PCR) and serological testing were performed on blood samples (serum and EDTA blood) that had been collected from each secondary patient between days 3 and 10, after the onset of symptoms. All specimens had been stored at −70°C since initial testing in 2007.

RNA was extracted from serum or whole blood using a high pure viral RNA kit (Roche Diagnostics) according to the manufacturer's instructions. SFTS viral M and S genomic segments were amplified using specific primers and probes by RT-PCR assay. The primers and probes used in the real-time assay were synthesized according to the gene sequence of a SFTSV (GenBank accession numbers: HQ141601 to HQ141606).

Viral culture was performed on aliquots of specimens testing positive by RT-PCR. For virus isolation, 2 or 3 blind passages through Vero cells, as previously described, were performed [1, 5].

Modified microneutralization assay (MNA) and indirect immunofluorescence assays (IFA) were performed essentially as described previously for immunoglobulin G (IgG)-specific

Table 1. The Clinical Features of Patients Involved in a Family Cluster Occurred in 2007, Eastern China

	Patient A	Patient B	Patient C	Patient D	Patient E	Patient F	Patient G
General information[a]							
Age, years, and gender	80, female	53, male	53, female	54, male	53, male	72, male	53,female
Occupation	Farmer	Farmer	Farmer	Farmer	Security guard	Farmer	Farmer
Relationship with patient A	NA	Son-in-law	Elder daughter	Nephew	Son-in-law	Nephew	Younger daughter
Clinical manifestations[a]							
Date of onset	April 18	May 3	May 4	May 4	May 5	May 5	May 7
Temperature, °C	39.2 (39.3)	39.0 (40.0)	36.9 (39.0)	38.0 (39.7)	38.6 (40.0)	37.9 (38.2)	39.3 °C (39.4 °C)
Respiratory symptoms	Cough	Cough	None	Cough, hemoptysis	Cough	Cough, short of breath	None
Gastrointestinal symptoms	Diarrhea	Nausea, vomiting, diarrhea	Vomiting, diarrhea	Diarrhea	Diarrhea	Nausea, vomiting,	Diarrhea
Others	Dysphoria, coma, confusion	Myalgia, headache	Myalgia, headache	Myalgia, headache	Myalgia, conjunctival congestion	Myalgia, headache	Myalgia, headache
Complications	Bronchopneumonia	Bronchitis	None	Pneumonia	None	Pleural effusions	None
Treated with dexamethasone or hydrocortisone	Yes	Yes	No	No	Yes	Yes	Yes
Days of hospitalization	6	17	10	25	16	11	11
Blood counts[a]							
White blood cells count (×10⁹/L)	3.1 (1.6)	4.0 (2.2)	3.6 (2.9)	4.0 (2.5)	1.3 (0.9)	3.2 (2.2)	3.5 (2.6)
Lymphocytes count (×10⁹/L)	0.9 (0.3)	0.7 (0.7)	0.7 (0.7)	0.7 (0.7)	0.3 (0.3)	1.3 (0.9)	0.7 (0.7)
Platelets count (×10⁹/L)	48 (30)	91 (20)	64 (27)	67 (3)	71 (15)	22 (22)	74 (36)
Urine and stool routine tests[a]							
Proteinuria	NA	+	−	3+	+	NA	−
Hematuria	NA	+	−	+	+	NA	+
Fecal occult blood	−	−	−	+	−	−	NA
Serum biochemistry[a]							
Alanine amino transferase (U/L)	573.0	31.2 (174.9)	11.4 (52.4)	21.7 (130.8)	39.1 (255.7)	70.6 (110.0)	22.2 (124.1)
Aspartate amino transferase (U/L)	3869.0	49.0 (324.0)	24.0 (63.4)	57.0 (405.9)	93.0 (472.9)	177.3 (177.3)	93.0 (127.7)
Creatinine (U/L)	188	69 (69)	41 (87)	82 (63)	109 (109)	72 (87)	53 (87)
Lactate dehydrogenase (U/L)	3094	295 (1266)	267 (267)	382 (1334)	555 (992)	704 (704)	293 (481)
Coagulation[a]							
Prothrombin time (s)	NA	12.1	NA	12.8	12.6	NA	NA
Activated partial thromboplastic time (s)	NA	25.3	NA	23.1	25.4	NA	NA
Fibrinogen (g/L)	NA	3.48	NA	6.17	3.39	NA	NA

Abbreviation: NA, not applicable.

[a] Data are measurement at admission (peak or nadir measurement during hospitalization). For the coagulation, above-mentioned markers were measured only 1 time on 15 May 2007. NA = Not applicable or not available.

antibodies to SFTSV [6]. For MNA, briefly, 100 50% tissue culture infective dose (TCID50) units of virus were pre-incubated with serial 2-fold dilutions of serum and then used to infect Vero cells in 96-well microtiter plates. For IFA, fixed Vero cells infected with SFTSV were incubated with diluted human serum and stained with fluorescein-labeled anti-human IgG secondary antibodies. Antigens for the testing were produced from a strain isolated from a patient in 2010.

RESULTS

Patient A was in a good physical condition before the onset of illness and was known to collect tea leaves from her tea garden on a hillside around the village where she lived. Her family recalled that she did not leave the village within 1 month preceding disease onset. The village where she lived is located in a typical hilly area with a population of ~150. No obvious change in mosquito density nor rising morbidity or mortality of poultry and livestock at that time was observed through an ecological survey.

Except for patient E, the other 5 secondary patients also lived in the same village but in separate houses or in an adjacent village <3 km away. Patient E worked as a security guard for a supermarket in another county ~40 km away. After hearing of his mother-in-law's illness, he returned to the village and took part in her birthday party on April 21. He provided bedside care after she was readmitted to the local hospital.

All 6 secondary patients denied a history of insect bites, exposure to wild animals, or participation in hunting activity in the month preceding illness onset. All, however, participated in the index patient's birthday party (April 21–22). Five patients, except patient G, reported contact with bloody secretions and/or handled feces of the index patient with unprotected hands during her hospitalization. Patient G, the index patient's nephew, also reported that he touched the blood of the index patient while handling her corpse.

An additional 85 persons were identified as patient A's contacts. Only 2 individuals lived in the household of the index case: the index case and her husband. However, another 19 individuals had contact with the index case but did not acquire infection. An additional 21 relatives and 45 medical personnel (1 from the village clinic, 38 from the county hospital, 6 from the regional hospital) also had contact. It is estimated that 192 persons attended patient A's funeral. Only 2 of the 19 family members reported exposure to patient A's blood with their bare hands. Public health officials interviewed all additional contacts and found that none became ill.

RNAs extracted from serum/blood specimens from the 6 secondary patients collected during the acute phase of their illness were identified as SFTS viral RNAs using RT-PCR. Positive results were confirmed through retesting of samples at China CDC. SFTSV was isolated from 1 preserved serum collected in May 2007 from 1 secondary patient (patient E) after 3 blind passages. Complete genomic sequencing and analysis of this isolate (GenBank accession number: JF837593 to JF837595) showed that it shared 96.0%–99.7% homology in nucleotide sequence with 2 strains (JS03 and JS04) isolated from sporadic patients in Jiangsu Province, 2010. Additional sequence of SFTSV M-segments (~406bp-498bp) from 3 other secondary cases (patients B, D, and G) showed no nucleotide changes with patient E, indicating that these viruses were most likely from the same origin. Serum obtained from the 6 secondary patients collected in their acute phase were negative (the titer of neutralization antibodies was <1:10) for antibodies to SFTSV by MNA, but paired convalescent-phase specimens obtained from those patients were positive. Additionally, seroconversion was further demonstrated by IFA. A summary of the results of diagnostic testing is shown in Table 2.

DISCUSSION

Although only recently identified, SFTSV appears to have been responsible for a family cluster of fever, thrombocytopenia, and leukopenia in eastern China in May 2007. We confirmed infection in all 6 secondary patients using multiple methods. SFTS viral RNA was detected using RT-PCR in acute-phase blood specimens from patients. Serum MNA and IFA documented seroconversion of specific IgG antibody to the novel virus between acute phase and convalescence phase serum samples. Finally, SFTSV was successfully isolated from 1 of the preserved specimens through 3 blind passages. Laboratory evidence demonstrated that the 6 secondary patients had recent infections with SFTSV, in accordance with the definition of a confirmed case issued by Chinese Ministry of Health in October 2010 [7].

Infection was most probably transmitted by direct contact between the index patient and 6 family members. Person-to-person transmission has been documented with other viruses of the family *Bunyaviridae* such as CCHF virus and Andes virus [8–11]. The phenomenon of person-to-person transmission has also been observed in a more recent outbreak (October 2010) in eastern China [12]. Four pieces of epidemiological evidence gathered in this investigation indicate that the most likely transmission route of SFTS in this cluster was person-to-person. First, all subsequent patients had a history of contact with patient A's blood or feces while providing bedside care during her hospitalization or handling her corpse after death, suggesting a possible mechanism of transmission. Patient E lived and worked in another county, but he developed illness 15 days after he returned to patient A's village and participated in events there. Second, all subsequent illnesses began during a brief period (from May 3 to 7), suggesting that infection was probably due to a common, single-point exposure. This hypothesis was

Table 2. Results of Diagnostic Testing of a Family Cluster Occurred in 2007, Eastern China

				MNA[a]		IFA[b]			
Patient	Relationship	Gender, age	Outcome	AP	CP	AP	CP	2010	RT- PCR, 2007
Patient A	Mother (Index patient)	F, 80	Fatal	NT	NT	NT	NT	NT	NT
Patient B	Elder son-in-law	M, 59	survived	<10	1280	<10	1280	80	Positive
Patient C	Elder daughter	F, 59	survived	<10	1280	<10	1280	80	Positive
Patient D	Nephew 1	M, 54	survived	<10	640	<10	2560	320	Positive
Patient E[c]	Younger son-in-law	M, 53	survived	<10	640	<10	1280	80	Positive
Patient F	Nephew 2	M, 72	survived	<10	640	<10	1280	80	Positive
Patient G	Younger daughter	F, 53	survived	<10	640	<10	1280	160	Positive

Abbreviations: F, female; M, male; MNA, microneutralization assay; AP, acute phase; CP, convalescent phase; IFA, indirect immunofluorescence assay; RT-PCR, real time reverse transcription polymerase chain reaction.

[a] MNA, AP: The serum specimens were collected on 8 May 2007. CP: The serum specimens were collected on 13 June 2007.

[b] IFA, AP: The serum specimens were collected on 8 May 2007. CP: The serum specimens were collected on 13 June 2007. 2010: The serum specimens were collected in 17 September 2010.

[c] The novel virus was isolated from the patient's serum collected in May 2007.

supported by the results of SFTSV M-segments sequences alignment from 4 of the subsequent patients. Third, all 6 secondary patients seroconverted to SFTSV positivity, and antibodies against SFTSV were still detected from the serum of the secondary patients collected >3 years after recovery. In contrast, a serological survey of 180 farmers from the same village or an adjacent village in 2010 found no one with antibodies to the SFTSV. Fourth, patient A's clinical symptoms were consistent with SFTS, although her diagnosis was not laboratory confirmed because no specimens were available for testing.

By contrast, no obvious evidence thus far showed that SFTSV can transmit through inhalation or droplets based on the following reasons. First, the pulmonary symptoms of the index case and 4 of the secondary cases developed in their later phase as a complication. Second, the medical staff who provided services for the index patient and patients who stayed in the same ward with the index patient did not become ill. Third, in 2010, when another family cluster was identified, no viral RNA was detected from collected throat swab and feces specimens from patients in their acute phases (unpublished data). Two additional family members reported that they provided bedside care or handled the corpse of patient A but did not develop symptoms. It is not clear why these individuals did not develop illness, but this might be linked to the manner of interaction, frequency, or duration of contact with the acute patient, or to infectious dose, individual susceptibility, and immune status [11].

We believe it is plausible that the index patient acquired her infection from the environment. She used to pick tea leaves in a grove filled with grasses and shrubs. In 2007, a survey identified dozens of ticks belonging to the species *Haemaphysalis longicornis* in this tea grove. SFTSV has been detected from this species of ticks [1]. Unfortunately, all of the ticks collected in 2007 were discarded after testing DNA for *A. phagocytophilium*; all were negative by PCR. Although it is possible that secondary cases contracted the infection from the environment, and the timing of the disease reflects different incubation times, we believe that this hypothesis is less likely. First, except for these 6 family patients, no other cases from the same area were reported at that time. Second, 5 secondary patients except patient E lived in the same village or an adjacent village preceding their illnesses, but their daily works mainly involved raising silkworms, which does not require frequent fieldwork; all denied tick bites or exposure to wild animals before disease onset. Finally, the incubation period of most SFTSV infections ranges from 5 days to 2 weeks, according to most studies to date. If the secondary cases shared a common exposure with the index patient, we would have expected their dates of illness onset to be earlier than May 3.

Confirmation of the etiological agent for the family cluster demonstrates that the novel virus may be traced back to at least the year 2007 or earlier. The SFTSV, however, caused several outbreaks in central China with a high case fatality, inciting panic in affected and surrounding provinces since 2009 [7]. Given this situation, together with the potential for increased transmission and resulting consequences of SFTS infection, more attention to the epidemiology and biology of this pathogen is required.

Our study suggests a transmission mode not previously reported for SFTS, and the public health significance needs further evaluation. In the past 2 years, almost all SFTS patients confirmed by China CDC occurred sporadically and were geographically dispersed [1, 7]. Therefore, the emergence of person-to-person transmission, coupled with the severity of SFTS, suggests that hospitals should strengthen infection control when patients with similar clinical symptoms initially present. This should include systematic and ongoing training of hospital personnel in infection control measures, restricting visitation and provision of bedside care for suspected SFTS patients by family members, and providing appropriate personal protective equipment to all caregivers. In addition, corpses of patients who

have died from hemorrhagic disease should only be handled by trained and properly outfitted persons.

To the best of our knowledge, the SFTSV is unique to China, and no epizootic has yet been reported. Whether the novel virus also exists in other regions of the world or if it has the capacity of spread to previously unaffected areas, as RVF virus, is unknown. One death among 7 patients corresponds to the 12% fatality rate found in the 171 sporadic patients reported by Yu et al [1] is as high as that of RVF virus in humans [1, 13–15]. In addition, because it is easily cultured and associated with a high mortality rate, together with no currently available effective antiviral drugs or vaccines, the virus should be considered a potential candidate as weapons for bioterrorism, and restrictions on its distribution and handling should be established and enforced [16, 17]. It is therefore critical to establish uniform laboratory diagnostic methods and conduct surveillance on suspected viral hemorrhagic fever patients in countries that have similar geographic location and climatic conditions as China [16]. Failure to do so may result in viral identification only after a large outbreak has occurred.

Notes

Authors and Contributors. Chang-jun Bao coordinated field epidemiology, all data analysis, and manuscript writing; Xi-ling Guo did viral isolation, indirect immunofluorescence assay; Xian Qi and Zhi-feng Li did the RT-PCR and micro-neutralization testing; Jian-li Hu and Ke Xu conducted field investigation and data collection; Ming-hao Zhou and Hai-tao Yang consulted study design and implementation; Jay K. Varma and John D. Klena consulted on data analysis, interpretation, and manuscript revision; Lun-biao Cui and Jun Shan did molecular diagnosis and genomic sequencing and analysis; Yong-jun Jiao, Xian Li, Yin Chen, Zheng Zhu, Tao Wu, Hai-yan Peng and Zhi-yang Shi were involved in viral antigens preparation and serological diagnosis; Lu-xun Li and Ai-hua Shen assisted or were involved in patient identification, contact follow-up, specimen collection; Wen-yuan Tao provided clinical data; Hua Wang was the project coordinator, responsible for the project design and implementation, and supervised all aspects of fieldwork, laboratory activities and data analysis.

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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
				2012. 1. 23	該当なし	
一般的名称	人血清アルブミン					
販売名（企業名）	赤十字アルブミン20（日本赤十字社） 赤十字アルブミン25（日本赤十字社） 赤十字アルブミン5%静注12.5g/250mL（日本赤十字社） 赤十字アルブミン20%静注4g/20mL（日本赤十字社） 赤十字アルブミン20%静注10g/50mL（日本赤十字社） 赤十字アルブミン25%静注12.5g/50mL（日本赤十字社）	研究報告の公表状況	Roth WK, Busch MP, Schuller A, Satake M, et al. Vox Sang. 2012 Jan;102(1):82–90. doi: 10.1111/j.1423-0410.2011.01506.x. Epub 2011 Sep 21.	公表国 世界各国		

研究報告の概要		使用上の注意記載状況・その他参考事項等
○供血者のNATに関する国際調査 国際輸血学会（ISBT）の輸血感染症に関するワーキング・パーティ（WP-TTID）のウイルス学に関するサブグループは、核酸増幅技術（NAT）に関する国際調査を実施するため、eメールによるアンケート（各国の住民数、供血者数、初回供血者と複数回供血者の分布、スクリーニングに使用している血清学的検査及びNAT検査、検査戦略の根拠、2008年及びNAT導入後全期間におけるウイルス検査と供血者スクリーニングの数、NATが義務化されているかどうか、NATのみ陽性の供血数と陽性率、現在使用しているNATプール数、感度と特異性）を行った。質問は全血とアフェレーシス供血についてのみ回答が要請され、分画製剤のための血漿については質問から除外された。 2009年8月に59カ国77人の専門家にアンケートが送付され、37カ国50人が回答した。 この調査によって網羅される人口は合計12億人となり、2008年にNATを実行した報告した国の人口を合計すると11億6千万人であった。 ・2008年のNAT検査収率 HIV-1：3,350万人の供血者に関する完全なNAT検査データのうち、2,189供血がHIV-1 NAT陽性であった（HIV-1抗体陽性、陰性含む）。陽性結果の約2/3は南アフリカからで、1,331症例が報告された。南アフリカでの陽性初回供血者の割合は残りの報告国と比べて125倍。陽性複数回供血者の割合は33倍高かった。 HCV：2,660万人の供血者に関する完全なNAT検査データのうち、4,586供血がHCV NAT陽性であった（HCV抗体陽性、陰性含む）。エストニアは初回供血者の陽性率が最も高い国であった（7336.2/100万人）。エストニアとギリシャはマレーシアとともに複数回供血者の陽性率も高かった（それぞれ165.2/100万人、134.2/100万人、193.3/100万人）。 HBV：902万人の供血者に関する完全なNAT検査データのうち、3,081供血がHBV NAT陽性であった（HBV抗体陽性、陰性含む）。ギリシャ及びマレーシアの陽性供血数は合計1,517件であり、両国の陽性初回供血者の割合は他の報告国の8.4倍、陽性複数回供血者の割合は15.6倍高かった。 この調査は、供血者の感染症スクリーニングに関してこれまで公表された中で最も大きなデータを含み、最も広範な研究である。各国のスクリーニング数の拡大及び技術と自動化の件筆すべき進歩が示された。	赤十字アルブミン20 赤十字アルブミン25 赤十字アルブミン5%静注12.5g/250mL 赤十字アルブミン20%静注4g/20mL 赤十字アルブミン20%静注10g/50mL 赤十字アルブミン25%静注12.5g/20mL 血液を原料とすることに由来する感染症伝播等
報告企業の意見	**今後の対応**	
国際輸血学会（ISBT）のサブグループにより国際的なNATに関する調査が行われ、37カ国からのデータが報告されたとのことである。日本赤十字社のNAT検査は国際的に見ても最前線に位置することが示された。日本では血漿分画製剤の原料血漿基準は輸血用血液製剤と同じであること、6カ月間貯留保管をしていることから、高い安全性が確保されていると考える。	特別な対応を必要としないが、今後も引き続き情報の収集に努める。	

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Vox Sanguinis

International survey on NAT testing of blood donations: expanding implementation and yield from 1999 to 2009

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Introduction

Thirteen years ago, blood centres voluntarily started testing whole blood and platelet apheresis donors using nucleic acid amplification technology (NAT). The first reports on the feasibility of routine blood donation NAT screening were published in 1998/99, followed by wide-scale implementation or mandated NAT testing in multiple countries over the subsequent several years [1–5]. Although studies demonstrated that NAT testing could efficiently detect serologically negative donors who were infected with all three major transfusion-transmitted viruses (HCV, HIV-1 and HBV), most countries initially mandated NAT testing only for HCV or for HCV and HIV-1. Thus, despite the cost and logistical challenges to blood banks that tried to establish NAT testing on a routine basis, HCV and HIV-1 NAT testing expanded rapidly in the early 90s in many countries. The main reasons for the delayed implementation of HBV NAT were that the predicted yield and clinical value of interdiction of sero-negative HBV infections were low, commercial tests for routine NAT donor screening targeting all three viruses were not available at that time, and only a small number of blood banks had access to sensitive and reliable in-house HBV NAT tests.

An International Forum of Vox Sanguinis was organized in 2002 based on an eight-question survey entitled 'Implementation of donor screening for infectious agents transmitted by blood by nucleic acid technology'[6]. The experts in the field who responded to the survey did not necessarily represent countries where NAT testing had already been introduced. Questions referred to the viruses for which NAT testing was being performed or considered; the technology used; the pool size employed; the sensitivity, specificity and robustness of the assays; the degree of automation; the yield of NAT testing; the role of HCV core antigen testing; and the status of implementation of HAV and Parvovirus B19 NAT testing.

Because NAT screening was expanding and evolving very rapidly at that time, an updated survey was conducted in 2004 and published in 2005. This survey, which employed a standardized questionnaire based on similar questions to the 2002 survey, was sent to experts in 26 countries, with 18 countries contributed to this second International Forum on NAT testing [7].

Since the 2005 International Forum NAT screening has been introduced in many additional countries worldwide, hundreds of papers on sensitivity, specificity and yield of NAT testing have been published from many countries with diverse epidemiological situations resulting in dramatic differences in yield of window phase and occult infections. Assays and testing platforms have improved, with recent development of high-throughput automation enabling NAT testing of small pools or individual donations with reduced technical expertise and manpower. This important new technical approach to blood donation testing has significantly contributed to blood safety and provided new insights into the early dynamics of viral replication and infectivity of acute and chronic infections.

Methods

The Working Party on Transfusion Transmitted Infectious Diseases (WP-TTID) of the International Society of Blood Transfusion (ISBT) is dedicated to advancing blood safety in the world with specific focus on infectious risks. This goal is accomplished through gathering and analysing relevant data and developing and coordinating international studies. In order to provide a convenient means to perform complex international surveys, the ISBT WP-TTID subgroup on virology developed an electronic questionnaire that can easily be distributed by e-mail and the responses readily compiled and analysed. It was decided to first use this tool to update the preceding International Fora on NAT testing. Although the survey included questions on overall yield since NAT testing was implemented, it was designed to focus on obtaining detailed cross-sectional data for one complete year of testing, rather than comprehensive longitudinal data over the whole testing period since the introduction of NAT in each individual country. This was because we wanted to compile and analyse data generated from current NAT technologies that are more sensitive and reliable than previous methods. These data, derived primarily using commercialized NAT systems, were expected to be more consistent such that results from different countries, including testing approaches, yield and epidemiology, could be compared.

The questionnaire contained detailed questions on the number of inhabitants and the donor populations in each country, the distributions of first-time and repeat donations, serological and NAT tests employed for screening, confirmation testing strategies, the viruses tested for by NAT and number of donations screened in 2008 and over the entire period since the date of introduction of NAT testing, whether NAT is mandated or not, the number of NAT-only-positive donations and the NAT yield rates, the NAT technology currently employed including pool size, sensitivity and specificity. Data were requested for whole blood and apheresis donations. Plasma for fractionation was excluded. The survey itself is available as supplemental material to this manuscript on the ISBT website (add link).

Results

The questionnaire was sent by e-mail in August 2009 to 77 experts from 59 countries. This distribution date seemed appropriate to allow each participant to compile and submit completed data including all confirmatory results for donations given in the 2008 calendar year. Most completed questionnaires were received between September and November 2009. A first electronic reminder was sent in November 2009, and a second personal reminder was sent to non-responding experts in May 2010. Seventy-three experts from 55 countries received the survey based on valid e-mail addresses, and 50 experts from 37 countries responded. Thirty-seven questionnaires from 25 countries were filled out completely and contained valid data for all questions including yield of NAT screening. In addition, 6 countries that did not perform NAT testing in 2008 sent back their serological data. Incomplete NAT data were received from seven other countries. The population covered by our survey (including the countries that did not perform NAT testing in 2008) totalled 1·2 billion. The population of all reporting countries in which NAT testing had been performed in 2008 totalled 1·16 billion. The exact numbers of donations with complete data sets for evaluation varied by virus as indicated in the respective sections that follow.

Implementation of NAT testing by country

Germany was the first country to introduce NAT screening of whole blood and apheresis donations on a routine basis with required negative NAT results prior to release of components (Fig. 1). Initially, this testing was voluntary and employed in-house NAT tests for HCV, HBV and HIV-1. HCV and HIV-1 NAT tests were mandated in Germany late in 1999 and 2004, respectively. Several other countries started NAT testing in 1999, primarily for HCV. Over the subsequent several years, NAT testing for HCV was mandated and began to be performed with commercial diagnostic assays that were more or less adapted to the needs of blood banks. Some countries started HIV-1 NAT testing as well. As seen in the Fig. 1, the number of countries per year that entered into HCV NAT testing was highest in 1999 and declined over time, whereas the number of countries that initiated HIV-1 NAT testing each year was relatively constant with small peaks in 2001–2003 and again in 2008. After the mandated introduction of HBV NAT in Japan and voluntary introduction of HBV NAT in Austria in 1999, there was a gap until 2004 followed by a peak in global implementation of HBV NAT in 2007–2008. In 2002, voluntary NAT for HBV was also introduced in several US blood centres (located in Sacramento California and Seattle Washington, with limited numbers of donations); broader implementation of HBV NAT did not occur in the United States until 2009 when multiplexed commercial NAT assays that included HBV detection were licensed in the United States.

As of 2010, 33 countries reported that they had introduced or would soon introduce HCV and HIV-1 NAT, and 27 of these reported current or planned introduction of HBV NAT. HCV NAT is mandated in 23 and HIV-1 NAT in 20 of the 33 countries assessed. HBV NAT is mandated in 12 and voluntarily performed in 15 countries.

NAT test systems and pool sizes employed in 2008

As technology improved significantly, early semi-automated and in-house-developed testing approaches have been generally replaced by fully automated commercial platforms and assays. Data on test systems employed were consequently requested for 2008 only (Table S1). The largest number of responding countries that have introduced NAT testing are in Europe. Thirteen of these

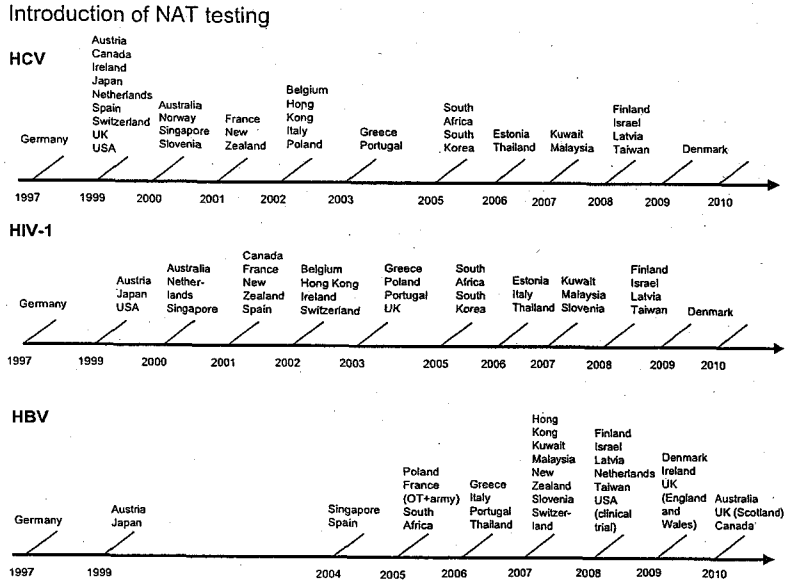

Fig. 1 Introduction of nucleic acid amplification technology (NAT) testing.

are using Roche assays and testing platforms, and 11 are using Novartis/Chiron assays/systems. Four countries [Germany, Austria, the Netherlands and UK (Scotland)] were using in-house testing approaches for the majority of their donations. Rest of UK were using Chiron. Germany, Austria and UK (Scotland) were the only countries in which in-house NAT tests were still in use in 2008 [UK (Scotland) subsequently converted to use of Roche's commercial assay]; the Netherlands performed commercial NAT assays with modified (in-house) extraction methods in 2008 but subsequently implemented the Roche extraction system.

In Africa, Asia-Pacific and North America, Novartis systems are the most prevalent (nine countries), followed by Roche (seven countries). None of these countries use in-house tests. Although there is no nationwide NAT testing in Brazil (it is planned for 2011 in the public system of hemocentres), one private hospital blood bank reported in-house NAT testing for HCV and HIV-1 since 1998, using an in-house system. It is estimated that ~5%–10% of donations in Brazil are now tested by NAT in private hospital blood banks for HIV-1, HCV and HBV using commercial or in-house adapted NAT assays.

In total, 22 countries perform Novartis/Gen-Probe NAT tests, 18 perform Roche NAT tests and only four perform in-house tests. In seven of the reporting countries, testing platforms from both commercial suppliers are approved and installed and operating in multiple testing laboratories. The numbers of donations tested by the respective testing systems do not reflect the distribution of numbers of tests applied per country due to variable penetrance of the different commercial NAT systems within countries. Some countries have blood centres that have introduced both commercial suppliers NAT assays/systems and centres that use in-house tests alone or in addition to commercial tests (Germany and Austria). Overall, a similar number of donations were tested by Roche (13 475 731) and Novartis (12 183 446) test systems in 2008, followed by in-house tests (3 258 028).

The majority of countries still performed NAT on mini-pools rather than individual donation testing in 2008, although there has been a clear progression towards smaller pools (e.g. 24 donations to six donations per minipool for Roche's system) and to individual donation testing (for Novartis/Gen-Probe's system). Discriminating numbers of donations tested and NAT yield data by pool size was not

feasible due to low numbers of donations tested in pools ≥ 48. Although there was a tendency to a lower yield of NAT positives among serologically confirmed positives for countries with high pool size especially for HCV and HBV, data were not conclusive. Germany with pools of 96 donations has reported comparable rates of virus detection (among both sero-negative and sero-positive donations) to those observed in epidemiologically similar countries performing HIV, HCV and HBV NAT on small pool sizes or even individual donations. This finding serves to emphasize the importance of sample processing and extraction techniques (e.g. ultracentrifugation of large volumes of pooled plasma as performed in Germany prior to extraction) in addition to pool size and analytical sensitivity of NAT assays on overall sensitivity and yield of NAT screening.

Yield of NAT testing in 2008

HIV-1

In total, 37·2 million donations were tested by HIV-1 NAT (Table S2). Complete data were received for 33·5 million donations (Table S3a). Of these 2189 donations (65·3/million) tested HIV-1 NAT positive, including RNA-positive donations with and without HIV-1 antibodies. The rate for first-time donations (295·5/million) was 11·2 times that for repeat donations (26·5/million). Almost two-thirds of the HIV-1 positives were reported from South Africa with 1331 cases (1796·7/million); the remaining 858 HIV-1 NAT positives were derived from the rest of the reporting countries with a composite rate of 26·2/million.

First-time donations from South Africa had a 22-fold higher HIV-1 NAT positivity rate (11 037·9/million) than repeat donations (503·2/million), whereas first-time donations (88·9/million) from the rest of the reporting countries had only a sixfold higher rate than repeat donations (15·4/million). It is anticipated that most of the HIV-1 NAT-positive repeat donors were acutely infected or incident infections, dependent on the interdonation intervals, whereas positive first-time donors reflect predominantly long-standing or prevalent infections. The rate (prevalence) of HIV-1-positive first-time donations in South Africa exceeded that from the rest of the reporting countries by a factor of 125, whereas the rate (incidence) of HIV-1-positive repeat donations in South Africa exceeded that from the rest of the reporting countries by a factor of 33. There are several countries in Asia and southern/eastern Europe with relatively high HIV-1 prevalence and incidence rates, reflected by overall NAT yield rates in first-time and repeat donors, respectively, compared with the majority of the remaining countries, but at far lower rates than for South Africa.

HIV-1 NAT-only positives. Compiled data from 2008 were reported for 37·4 million screened donations, of which 72 (1·9/million) were HIV-1 NAT-only positive (Tables 1 and S4a). Thirty-five of the 72 HIV-1 NAT-only positives reported in 2008 were from South Africa, with eight HIV-1 NAT-only positives identified in Spain and Thailand, five in the USA and four in Germany. All other countries reported no more than two HIV-1 NAT-only yield donations in 2008.

Data sorted by first-time and repeat donation status were provided for 35·3 million donations, with 4·9 million first-time and 30·4 million repeat donations. A total of 56 HIV-1 NAT-only positives were detected in these countries (overall rate of 1·6/million) with 19 HIV-1 NAT-only positive first-time donations (3·9/million) and 37 HIV-1 NAT-only positive repeat donations (1·2/million). Of these, 35 were reported from South Africa, with an HIV-1 NAT-only positive rate of 131·9/million for first-time donations and 35·4/million for repeat donations.

HIV-1 NAT positives among serologically confirmed positives. A total of 4917 HIV-1 serologically confirmed positive donations were reported out of 39·0 million donations tested (126·1/million) (Table S5a). Of these, 2174 sero-positive donations derived from 33·5 million screened (64·9/million) donations had corresponding HIV-1 NAT data assessable (Taiwan and Thailand reported serological data only) (Tables 2 and S6a). 2130 of 2174 (97·98%) sero-positive donations also tested HIV-1 NAT positive (Table 2). There was no major difference in the proportions of sero-positive donations with confirmed viraemia by NAT between first-time (97·84%) and repeat (98·24%) donations. Taking out the USA data that had lower rates of concordance of NAT with seropositivity (only 86·59% and 93·81% of HIV-1 sero-positive first-time and repeat donations tested NAT positive, respectively), the percentage of HIV-1 NAT positives among serologically confirmed positives increased to 99·29% and 98·91%, respectively, for the rest of the reporting countries. Very high rates of serologically positive donations were reported from Taiwan and Thailand without reporting NAT data.

The difference in the rates of HIV-1 serologically confirmed positive and HIV-1 NAT-only positives per million was a factor of 65·3. Taking out South Africa, a total of 3613 HIV-1 serologically confirmed positive donations were reported out of 38·2 million donations tested (94·5/million); restricting the analysis to these countries increased the ratio of serologically confirmed positive donations to HIV-1 NAT-only positives (1·01/million) to 93·6.

HCV

In total, 37·2 million donations were tested by HCV NAT (Table S2). Complete data were received for 26·6 million donations (no HCV NAT data were reported from Spain, and only combined data for first-time and repeat donations were reported from Japan) (Table S3b). Of these, 4586 (172·4/million) were HCV NAT positive, including RNA-positive donations with or without antibodies. The rate for first-time donations (995·2/million) was 35-fold that for

Table 1 NAT-only positives in 2008

Region/ country	Virus	First-time donations: Number of tested donations	First-time donations: NAT-only positive	First-time donations: Rate/ 1 000 000 donations	Repeat donations: Number of tested donations	Repeat donations: NAT-only positive	Repeat donations: Rate/ 1 000 000 donations	Total (first-time and repeat donations): Number of tested donations	Total (first-time and repeat donations): NAT-only positive	Total (first-time and repeat donations): Rate/ 1 000 000 donations
Africa	HIV-1	90 959	12	131·93	649 864	23	35·39	740 823	35	47·24
	HCV	90 959	0	0·00	649 864	1	1·54	740 823	1	1·35
	HBV	90 959	11	120·93	649 864	21	32·31	740 823	32	43·20
Asia/Pacific	HIV-1[a]	1 362 593	1	0·73	8 195 982	2	0·24	10 053 686	11	1·09
	HCV[b]	811 646	9	11·09	3 369 691	4	1·19	9 753 686	19	1·95
	HBV[c]	185 979	10	53·82	542 805	12	22·11	5 805 840	101	17·40
Europe	HIV-1[a]	1 743 371	5	2·87	13 128 773	8	0·61	16 431 874	21	1·28
	HCV[b]	1 753 371	4	2·28	13 228 457	11	0·83	16 541 558	18	1·09
	HBV[c]	913 025	3	3·29	7 243 954	20	2·76	9 438 036	28	2·97
North America	HIV-1	1 678 862	1	0·60	8 408 812	4	0·48	10 087 674	5	0·50
	HCV	1 706 556	14	8·20	8 300 902	17	2·05	10 007 458	31	3·10
	HBV	702 533	1	1·42	3 200 417	7	2·19	3 902 950	8	2·05
South America	HIV-1	31 020	0	0·00	20 680	0	0·00	51 700	0	0·00
	HCV	31 020	0	0·00	20 680	0	0·00	51 700	0	0·00
	HBV	No NAT testing								
Total (all countries)	HIV-1[a]	4 906 805	19	3·87	30 404 111	37	1·22	37 365 757	72	1·93
	HCV[b]	4 362 532	27	6·19	25 569 594	33	1·29	37 095 225	69	1·86
	HBV[c]	1 892 314	25	13·21	11 637 040	60	5·16	19 887 649	169	8·50

NAT, nucleic acid amplification technology.

[a]Cumulative data only (first-time + repeat donations) were received from Thailand and Spain.

[b]Cumulative data only (first-time + repeat donations) were received from Kuwait, Thailand and Spain.

[c]Cumulative data only (first-time + repeat donations) were received from Japan and Spain.

repeat donations (28·3/million). Estonia and Greece reported the highest prevalence in first-time donations with 7336·2/million and 4249·6/million, respectively, followed by Poland with 2886·3/million and Malaysia with 2858·3/million. Estonia and Greece also share the highest infection rates in repeat donations with Malaysia, 165·2, 134·2 and 193·3 per million, respectively; the HCV infection rate of donations from Polish repeat donors was about six-fold lower, with 23·9 HCV-RNA-positive donations/million. Taking out the data from Estonia, Greece, Malaysia and Poland resulted in a rate of 778·8/million HCV NAT-positive first-time donations and a rate of 25·2/million HCV NAT-positive repeat donations for the rest of the reporting countries. The difference between first-time and repeat donations decreased to a factor of 30·9 with the remaining countries, whereas for the indicated high-prevalence countries alone, the factor increases to a factor of 44·2.

HCV NAT-only positives. Compiled data were reported for 37·1 million donations in 2008, of which 69 (1·9/million) were HCV NAT-only positive (Tables 1 and S4b). Data sorted by first-time and repeat donation status were reported for a total of 29·9 million donations, including 4·4 million first-time and 25·6 million repeat donations. A total of 60 donations were HCV NAT-only positive (2·0/million). These HCV NAT yield donations sorted into 27 HCV NAT-only-positive first-time donations (6·2/million) and 33 HCV NAT-only-positive repeat donations (1·3/million). Due to low numbers of HCV NAT-only positives (see Tables 1 and S4b), further calculations were not made as they would not provide significant insights into the underlying epidemiology.

HCV NAT positives among serologically confirmed positives. A total of 13 903 HCV serologically confirmed positive donations were reported out of 38·6 million donations tested, for a rate of 360·3/million (Table S5b). Out of these, 6706 of 26·2 million (249·2/million) donations were assessable for comparison with NAT data and analysis of rates of nucleic acid detection among serologically confirmed positive donations by donation type (Taiwan and Thailand reported high numbers/rates of serologically positive donations without reporting HCV NAT data, and Japan reported only combined data for first-time and repeat donations). Of 6706 serologically confirmed positive donations, 4723 (70·4%) were also HCV NAT positive (Tables 2 and S6b).

Table 2 NAT positives among serologically positives in 2008

Region	Virus	First-time donations: Number of tested donations	First-time donations: NAT positives among serologically positives	Repeat donations: Number of tested donations	Repeat donations: NAT positives among serologically positives	Total (first-time and repeat donations): Number of tested donations	Total (first-time and repeat donations): NAT positives among serologically positives
Africa	HIV-1	90 959	992/998 = 99·40%	649 864	304/306 = 99·35%	740 823	1296/1304 = 99·39%
	HCV	90 959	33/50 = 66·00%	649 864	16/29 = 55·17%	740 823	49/79 = 62·03%
	HBV	90 959	642/664 = 96·69%	649 864	68/68 = 100%	740 823	710/732 = 96·99%
Asia/Pacific	HIV-1	1 362 593	95/95 = 100%	8 195 982	138/138 = 100%	9 558 575	233/233 = 100%
	HCV[a]	734 314	425/566 = 75·09%	3 404 049	71/148 = 47·97%	4 138 363	496/714 = 69·47%
	HBV	110 352	709/818 = 86·67%	308 246	59/76 = 77·63%	418 598	768/894 = 85·91%
Europe – total	HIV-1	1 673 478	161/163 = 98·77%	11 698 433	181/185 = 97·84%	13 371 911	342/348 = 98·28%
	HCV	1 674 459	1523/2016 = 75·55%	10 390 293	125/180 = 69·44%	12 064 752	1648/2169 = 75·05%
	HBV	397 829	1132/1525 = 74·23%	4 259 078	172/293 = 58·70%	4 656 907	1304/1818 = 71·73%
Northern Europe	HIV-1	22 572	1/1 = 100%	255 425	1/1 = 100%	277 997	2/2 = 100%
	HCV	22 572	8/14 = 57·14%	255 425	2/3 = 66·67%	277 997	10/17 = 58·82%
	HBV	22 572	3/3 = 100%	255 425	2/2 = 100%	277 997	5/5 = 100%
Middle/Western Europe	HIV-1	1 065 791	40/42 = 95·24%	8 447 951	62/66 = 93·94%	9 513 742	102/108 = 94·44%
	HCV	1 065 791	306/455 = 67·25%	8 447 951	32/71 = 45·07%	9 513 742	338/526 = 64·26%
	HBV	266 153	376/442 = 85·07%	3 224 325	18/20 = 90·00%	3 490 478	394/462 = 85·28%
Eastern Europe	HIV-1	301 839	47/47 = 100%	1 491 574	39/39 = 100%	1 793 413	86/86 = 100%
	HCV	302 820	951/1202 = 79·12%	1 496 750	88/103 = 85·44%	1 799 570	1039/1305 = 79·62%
	HBV	72 242	688/1013 = 67·92%	589 161	151/270 = 55·93%	661 403	839/1283 = 65·39%
Southern Europe	HIV-1	283 276	73/73 = 100%	1 503 483	79/79 = 100%	1 786 759	152/152 = 100%
	HCV	283 276	258/345 = 74·78%	190 167	3/3 = 100%	473 443	261/348 = 75·00%
	HBV	36 862	65/67 = 97·01%	190 167	1/1 = 100%	227 029	66/68 = 97·06%
North America	HIV-1	1 652 996	144/166 = 86·75%	8 120 466	93/99 = 93·94%	9 773 462	237/265 = 89·43%
	HCV	1 627 850	2118/3049 = 69·47%	7 602 930	362/609 = 59·44%	9 230 780	2480/3658 = 67·80%
	HBV	1 250 280	145/217 = 66·82%	5 643 853	16/76 = 21·05%	6 894 133	161/293 = 54·95%
South America	HIV-1	31 020	13/14 = 92·86%	20 680	9/10 = 90·00%	51 700	22/24 = 91·67%
	HCV	31 020	30/35 = 85·71%	20 680	20/24 = 83·33%	51 700	50/59 = 84·75%
	HBV	No NAT testing					
Total (all countries)	HIV-1	4 811 046	1405/1438 = 97·84%	28 685 425	725/738 = 98·24%	33 496 471	2130/2174 = 97·98%
	HCV	4 158 602	4129/5718 = 72·74%	22 067 816	594/990 = 60·00%	26 226 418	4723/6706 = 70·43%
	HBV	1 849 420	2628/3224 = 81·51%	10 861 041	315/513 = 61·4%	12 710 461	2 943/3737 = 78·75%

NAT, nucleic acid amplification technology.

[a]HCV Asia/Pacific: data from Israel and Japan excluded (see Table S6b).

There was a great difference between rates of HCV RNA detection by NAT among sero-positive first-time (71·63%) and repeat (57·96%) donations. The ratio of serologically confirmed to NAT-only HCV positive donations was 193·7, which is approximately two times the respective factor for HIV-1.

HBV

In total, 20·9 million donations in 2008 were reported to have been tested by HBV NAT (Table S2). Complete HBV NAT data were received for 9·02 million donations (no HBV NAT data were reported from Spain, and only combined HBV NAT data from first-time and repeat donations were reported from Japan) (Table S3c). Of these, 3081 (341·70/million) were HBV NAT positive, including both serologically reactive and non-reactive donations. The rate for first-time donations (2151·4/million) was 42·6 times that for repeat donations (50·5/million). HBV NAT-positive donations from Greece and Malaysia accounted for 1517 of the 3081 cases, with a combined HBV DNA detection rate of 2169·8/million; when data from these countries were removed, there were 1564 HBV NAT positives among the remaining reporting countries, with a combined rate of 188·0/million.

First-time donations from Malaysia and Greece had 27·4-fold higher rates of HBV DNA detection (10 372·9/million) than repeat donations (378·2/million), whereas first-time donations (1 235·2/million) from the rest of the reporting countries had a 50·8-fold higher rate than repeat donations (24·3/million). The infection rates of Malaysian and Greek HBV-positive first-time donations exceed that from the rest of the reporting countries by a factor of 8·4, and the infection rates of HBV-positive repeat donations from these countries exceed that from the rest of the reporting countries by a factor of 15·6.

HBV NAT-only positives. Compiled data were reported for 19·9 million donations, of which 169 (8·5/million) were HBV NAT-only positive (Tables 1 and S4c). Split data for first-time and repeat donations were reported for a total of 13·5 million donations including 1·9 million first-time and 11·6 million repeat donations. A total of 85 donations were HBV NAT-only positive (HBV DNA-positive with negative HBsAg and anti-HBc, if performed), for a rate of 6·3/million. These sorted into 25 HBV NAT-only-positive first-time donations (13·2/million) and 60 HBV NAT-only-positive repeat donations (5·2/million).

Thirty-two HBV NAT yield donations were reported from South Africa, with a HBV NAT-only-positive rate of 120·9/million for first-time donations and 32·3/million for repeat donations, while 10 HBV NAT-only positives were identified in Hong Kong (47·0/million) and 9 (68·7/million) in Malaysia (Table S4c). It is important to recognize that these three countries, as well as many others, did not perform routine anti-HBc testing, and consequently, their yield numbers/rates include occult HBV infections (HBsAg negative, HBV NAT and anti-HBc positive), in addition to window period cases.

The above numbers do not include 79 HBV NAT yield donations (out of 5 077 238 combined first-time and repeat donations for a rate of 15·6/million) reported from Japan where anti-HBc screening is performed.

HBV NAT positives among HBsAg-confirmed positives. A total of 20 515 HBsAg-confirmed positive donations were reported out of 31·7 million donations tested for a rate of 647·3/million (no serological data were reported from Japan) (Table S5c). Out of these, 3737 HBsAg-positive donations detected following screening of 12·7 million donations (294·0/million) were assessable for comparison with HBV NAT data (Tables 2 and S6c). Of the 3737 HBsAg-positive donations, 2943 (78·75%) were HBV NAT positive. When sorted by donation status, 2628 of 3224 (81·51%) HBsAg-confirmed positive first-time donations and 315 of 513 (61·40%) HBsAg-confirmed positive repeat donations were HBV NAT positive (Tables 2 and S6c). Taking out the data from South Africa, Greece and Malaysia that had high HBV infection rates, HBV DNA was detected in 743 out of 899 (82·65%) HBsAg-confirmed positive first-time donations and 41 out of 106 (38·68%) HBsAg-confirmed positive repeat donations for the rest of the reporting countries.

The ratio of HBsAg-confirmed positives to HBV NAT-only positives was 121·4, similar to the NAT-only/serology yield ratio for HIV-1 and lower than the ratio for HCV. Taking out South Africa, Malaysia and Greece, a total of 16 021 HBsAg-confirmed positives were reported out of 28·7 million donations tested (557·7/million). This decreases the ratio of HBsAg-confirmed positives to HBV NAT-only-positive donations (6·0/million) to a factor of 92·95.

Anti-HBc screening was performed in 2008 by 12 of the 37 responding countries and partially by one more country (Table S7). However, data sets were often incomplete precluding rigorous evaluation. Focusing on the data on HBV NAT-only positives and HBV NAT and anti-HBc positives from those counties that reported data, either from anti-HBc screening or from anti-HBc confirmatory testing, there were a total of 75 HBV NAT-only-positive window period donations and 250 HBV NAT and anti-HBc-positive occult HBV infections detected (reported as HBsAg negative) out of 10 987 306 donations; this yields rates of 6·8/million for window period and 22·8/million for occult HBV infections, respectively. Although the yield numbers and ratios for individual countries were quite variable (Table S7), the data indicate that HBV NAT yield rates for occult HBV cases (HBV NAT and anti-HBc positive) generally exceeded the rates for acute window period infections (HBV NAT-only positives) by a factor of 3·35.

Yield of NAT testing since introduction

NAT yield data since introduction of NAT screening are summarized in Tables 3 and S8c. A total of 272 520 696 donations were screened by HIV-1 NAT, 303 196 074 were screened by HCV NAT, and 114 286 214 were screened by HBV NAT. Of these, 244 (0·9/million) were NAT-only positive for HIV-1, 680 (2·2/million) were NAT-only positive for HCV, and 1884 (16·5/million) were NAT-only positive for HBV DNA.

The number of screened donations and NAT yield data per country are also compiled in Table S8a. Compared with the NAT yield cases per number of donations tested in 2008 (Tables 1 and S4 a–c), there are no major differences in the total NAT yield rates since introduction of NAT testing for HIV-1, HCV and HBV, both regarding individual countries and geographic regions.

Additional findings

The survey requested that countries report the distribution of genotypes for HIV, HCV and HBV, both among the NAT yield donations and for the countries based on other epidemiological surveillance data. Although incomplete data were reported for a proportion of countries, the findings supported the conclusion that the genotypes present in blood donors are consistent with those reported from other studies in each country and region (Table S9).

In addition, the survey requested information on the HIV, HCV and HBV serological assays employed in donor screening, so the yield of NAT-only donations and the proportion of NAT positives among sero-positive donations could be evaluated in the context of the generation and manufacturer of respective serological screening tests (Table S10).

The survey asked participants to list the type and source of run controls used to validate and track performance of NAT assays (Table S11). Most countries performing NAT include such controls, generally manufactured by commercial vendors and supplied by the NAT vendors.

Finally, the survey requested information on the status of testing for other infectious agents, including HTLV-I/II, and NAT screening for Hepatitis A Virus, Parvo B19 Virus, West Nile Virus, Hepatitis E Virus and dengue virus (Table S12), as well as HBV vaccination programmes (Table S13).

Table 3 NAT-only positives since introduction of NAT testing

Region/ country	Virus	Screened donations since implementation of NAT	NAT-only positives	NAT-only positives/ million
Africa	HIV-1	2 202 295	81	36·78
	HCV	2 202 295	4	1·82
	HBV	2 202 295	232	105·34
Asia/ Pacific	HIV-1	71 458 330	44	0·62
	HCV	71 458 330	169	2·37
	HBV	50 679 100	1091	21·53
Europe	HIV-1	110 860 111	73	0·66
	HCV	139 474 595	206	1·48
	HBV	56 342 555	550	9·76
North America	HIV-1	87 652 586	45	0·51
	HCV	89 652 687	299	3·34
	HBV	5 062 264	11	2·17
South America	HIV-1	347 374	1	2·88
	HCV	408 167	2	4·9
	HBV	No NAT testing		
Total (all countries)	HIV-1	272 520 696	244	0·9
	HCV	303 196 074	680	2·24
	HBV	114 286 214	1884	16·48

NAT, nucleic acid amplification technology.

Conclusions

This survey included 37 countries that reported results from NAT screening of approximately 300 million donations for HIV and HCV and over 100 million donations for HBV over the 10-year period extending from 1998 to 2008. To our knowledge, this is the largest data set ever compiled and most expansive study ever published on blood donor infectious disease screening.

The findings indicate remarkable progress in the implementation of molecular amplification techniques, with consequent interdiction of approximately 3000 viraemic donations that would have been missed by serological screening methods. The analysis demonstrated the successful expansion of NAT screening over the 10-year period, both in terms of numbers of countries screening and in terms of advances in technology and automation. As documented by the more detailed analysis of practices in 2008, the vast majority of NAT screening is now conducted with multiplexed HIV/HCV/HBV assay performed on highly automated instrument platforms that ensure reliable results with excellent sensitivity and specificity.

We believe that international bodies and regulators should more strongly encourage and preferably require NAT testing to improve blood safety (current recommendations and regulations from the World Health Organization, Council of Europe, Pan American Health Organization, etc., are not yet definitive in their recommendations for NAT testing). Although as our analysis confirms that the yield of NAT-only units is modest relative to the yield of serological screening, the infectivity of viraemic donations detected by NAT (with or without detectable serological markers) is very high. Hence, the relative impact of NAT screening is arguably greater than that of serological screening, although the existence of sero-positive but NAT-negative donations indicates that serological screening must be maintained even with the most sensitive NAT testing performed on individual donations. Consequently, the incremental cost-effectiveness of NAT is marginal since the safety benefits used in these calculations are restricted to the prevention of transmission of NAT-only yields and the cost of NAT testing remains relatively expensive.

The ISBT WP-TTID believes that collection and analysis of NAT and serological infectious disease donor screening data should be performed on a more regular basis in the future, given the expanded number of countries performing NAT, changing epidemiology of infectious diseases and donor selection practices and the likely evolution of NAT testing to target additional agents. Although this task is an appropriate one for the ISBT TTID working group, ongoing funding will be required to execute electronic surveys and rigorously analyse compiled data on a regular basis (every 3–5 years). The authors of this International Forum are grateful to the companies who have supported the working party to date and encourage their ongoing support, as well as funding from other sources (WHO, EU, NIH or CDC). Finally, we thank all of the

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Supporting information

Additional Supporting Information may be found in the online version of this article:
Data S1 Questionnaire on NAT testing of blood donations.
Table S1 NAT test systems and pool size applied in 2008
Table S2 Total number of donations tested by NAT in 2008
Table S3 (a) HIV-1 NAT, (b) HCV NAT and (c) HBV NAT positives in 2008
Table S4 (a) HIV-1 NAT-only, (b) HCV NAT-only and (c) HBV NAT-only positives in 2008
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販売名（企業名）	赤十字アルブミン20（日本赤十字社） 赤十字アルブミン25（日本赤十字社） 赤十字アルブミン5%静注12.5g/250mL（日本赤十字社） 赤十字アルブミン20%静注4g/20mL（日本赤十字社） 赤十字アルブミン20%静注10g/50mL（日本赤十字社） 赤十字アルブミン25%静注12.5g/50mL（日本赤十字社）	研究報告の公表状況	Thiele T, Krüger W, Zimmermann K, Ittermann T, Wessel A, Steinmetz I, Dölken G, Greinacher A. Transfusion. 2011 Dec;51(12):2620–6. doi: 10.1111/j.1537-2995.2011.03203.x. Epub 2011 Jun 3.	公表国 ドイツ	
研究報告の概要	○サイトメガロウイルス（CMV）抗体検査未実施の白血球除去血液製剤によるCMV感染：同種造血幹細胞移植を受けているハイリスク患者を対象とした単一施設での前方視的研究 背景：造血幹細胞移植（HSCT）後の輸血関連CMV感染（TT-CMV）を防ぐため、輸血用血液製剤に抗CMV抗体陰性血液や白血球除去血液を選択する方法がある。CMV抗体陰性HSCTを実施されたCMV抗体陰性レシピエントを対象に、抗CMV抗体検査未実施の白血球除去血液製剤の投与によるTT-CMVについて評価した。 研究方法：1999～2009年の前方視的研究において、全てのHSCT患者に抗CMV抗体検査未実施の白血球除去血液製剤を輸血した。患者のCMV抗体をスクリーニングした。CMV抗体陰性HSCTを受けたCMV抗体陰性レシピエントの臨床的なTT-CMVを監視し、CMV NATによって検査した。 結果：同種HSCTを受けた142患者において、23組のCMV抗体陰性造血幹細胞ドナーレシピエントを確認した。23患者は3,180供血者由来の1,847の血液製剤を輸血された。TT-CMV検査の結果、全ての患者はCMV DNAが陰性で、CMV関連の臨床的な合併症は発生しなかったため、供血者1人当たりのTT-CMVリスクは0%という結果になる（95%信頼区間、0.0%－0.12%）。しかしながら23患者のうち17患者に抗CMV IgG抗体の陽転化が起こった。抗CMV IgM抗体の陽転化が起こった者はいなかった。抗CMV IgG抗体が陽転した者は、陽転しなかった者に比べて1週間当たりの輸血量が顕著に多かった。 結論：抗CMV抗体検査未実施の白血球除去血液製剤を輸血されたCMV抗体陰性HSCT患者において、TT-CMVのリスクは低い。抗CMV IgG抗体陽転化の原因は、血液製剤中の移行抗体である可能性が最も高い。				使用上の注意記載状況・その他参考事項等 赤十字アルブミン20 赤十字アルブミン25 赤十字アルブミン5%静注12.5g/250mL 赤十字アルブミン20%静注4g/20mL 赤十字アルブミン20%静注10g/50mL 赤十字アルブミン25%静注12.5g/20mL 血液を原料とすることに由来する感染症伝播等
報告企業の意見	抗サイトメガロウイルス（CMV）抗体検査未実施の白血球除去血液製剤を輸血されたCMV抗体陰性造血幹細胞移植レシピエントにおいて輸血関連CMV感染のリスクを調査したところ、そのリスクは低いことが示されたとの報告である。 CMVは脂質膜を持つ大型のDNAウイルスである。これまで、本製剤によるCMV感染の報告はない。本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれていることから、本製剤の安全性は確保されていると考える。	今後の対応	特別な対応を必要としないが、念のため今後も情報収集に努める。		

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CME

Transmission of cytomegalovirus (CMV) infection by leukoreduced blood products not tested for CMV antibodies: a single-center prospective study in high-risk patients undergoing allogeneic hematopoietic stem cell transplantation

Thomas Thiele, William Krüger, Kathrin Zimmermann, Till Ittermann, Antje Wessel, Ivo Steinmetz, Gottfried Dölken, and Andreas Greinacher

BACKGROUND: Measures to prevent transfusion-transmitted cytomegalovirus (TT-CMV) infection after hematopoietic stem cell transplantation (HSCT) include transfusion of CMV antibody–negative blood units and/or transfusion of leukoreduced cellular blood products. We assessed the incidence of TT-CMV in CMV-seronegative patients receiving CMV-seronegative HSC transplants, who were transfused with leukoreduced cellular blood products not tested for anti-CMV.

STUDY DESIGN AND METHODS: In a prospective observational study between 1999 and 2009, all HSCT patients received leukoreduced cellular blood products not tested for anti-CMV. Patients were screened for CMV serostatus and CMV-negative recipients of CMV-negative transplants were systematically monitored for TT-CMV clinically and by CMV nucleic acid testing. Anti-CMV antibodies (immunoglobulin [Ig]G and IgM) were assessed after three time intervals (Interval 1, study inclusion to Day +30 after HSCT; Interval 2, Day +30-Day +100; Interval 3, after Day +100).

RESULTS: Among 142 patients treated with allogeneic HSCT, 23 CMV-negative donor-patient pairs were identified. These 23 patients received 1847 blood products from 3180 donors. All patients remained negative for CMV DNA and none developed CMV-associated clinical complications. This results in a risk for TT-CMV per donor exposure of 0% (95% confidence interval, 0.0%-0.12%). However, 17 of 23 patients seroconverted for anti-CMV IgG, but none for anti-CMV IgM. CMV IgG seroconverters received significantly more transfusions per week than nonconverters.

CONCLUSION: The risk of TT-CMV is low in high-risk $CMV^{neg/neg}$ HSCT patients transfused with leukoreduced blood products not tested for anti-CMV. The cause of anti-CMV IgG seroconversion is most likely passive antibody transmission by blood products.

Cytomegalovirus (CMV) infection is a severe complication in patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT),[1,2] and prevention of transfusion-transmitted CMV (TT-CMV) infection is a major issue in transfusion medicine. The best approach how to prevent TT-CMV is still controversial.[3]

Two main strategies are used to reduce the risk of TT-CMV by cellular blood components. One is to use only blood from donors seronegative for anti-CMV, and the other is in-line leukoreduction during preparation of cellular blood components because CMV is highly associated with white blood cells (WBCs). While both strategies are applied since the pivotal study of Bowden and colleagues in 1995,[4] several authors still favor the use of blood from anti-CMV–negative donors over transfusion of leukoreduced nontested blood based on observational studies.[5,6] As a consequence, current practice in managing patients at high risk for TT-CMV differs largely between transfusion services.[3]

We implemented in-line leukoreduction in our blood center and omitted CMV antibody testing. Our rationale was the high seroprevalence of anti-CMV in blood donors of our population (50%-80% seropositivity rate[7,8]) and the rather long window phase between CMV infection and seroconversion[9] (which could negate the value of screening for anti-CMV). We hypothesized that exclusive transfusion of blood components from anti-CMV IgG–negative blood donors paradoxically could increase the risk for transfusing a blood product obtained during the diagnostic window period. Within the diagnostic window, CMV viremia occurs and the virus can be transmitted even by leukoreduced blood products thereby conferring a high risk for TT-CMV.[9]

Concomitantly, we started a quality assurance program monitoring all patients undergoing allogeneic HSCT for new CMV infection by CMV DNA nucleic acid testing (CMV NAT) and anti-CMV testing. The analysis of CMV-seronegative recipients receiving stem cells from CMV-seronegative stem cell donors allowed risk assessment of TT-CMV.

Here, we report our experience over one decade with the use of in-line leukoreduced blood products not tested for anti-CMV in this high-risk patient population at a single institution. We found no case of TT-CMV and provide data that the occurrence of anti-CMV IgG after allogeneic HSCT is a marker for multiple transfusions, but not for true CMV seroconversion in matched $CMV^{neg/neg}$ HSCT recipients.

ABBREVIATIONS: HSCT = hematopoietic stem cell transplantation; PC(s) = platelet concentrate(s); TT-CMV = transfusion-transmitted cytomegalovirus.

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TT and AG designed the study. KZ and IS performed CMV assays; TI and AW performed statistical analyses and made the figures and tables; WK and GD treated the patients; TT, WK, GD, and AG analyzed results and wrote the manuscript; and all authors reviewed and approved the final version of the manuscript.

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MATERIALS AND METHODS

Patients and study design

This prospective, observational study at the University Hospital Greifswald (Greifswald, Germany) was performed between January 1999 and December 2009. All consecutive patients designated for allogeneic stem cell transplantation and their stem cell donors were assessed for anti-CMV IgG and IgM as initial serostatus. CMV-seronegative (anti-CMV IgG– and IgM–negative) patients were assigned for the selection of stem cell allografts from CMV-seronegative donors and only those who received a seronegative transplant between 1999 and 2009 were included in this analysis.

From Day +1 after allogeneic HSCT, CMV DNA monitoring was performed twice a week and in addition, if clinical symptoms suspicious for CMV infection were observed during daily physical examination. After discharge, CMV DNA tests were carried out at each outpatient visit at least weekly until Day +100 after transplantation. The interval was extended based on the risk state, for example, in the case of graft-versus-host disease. Anti-CMV IgG and IgM were assessed at the end of three consecutive time intervals: Interval 1, from study inclusion until Day +30 after HSCT; Interval 2, from Day +30 until Day +100; and Interval 3, after Day +100 at outpatient follow-ups or at readmission.

Type and number of blood products and transfusion dates were obtained retrospectively from the blood bank information system. Transfusion data were assigned to the three investigated time intervals. Weekly transfusion rates were calculated for plasma-poor red blood cells (RBCs; containing approx. 30 mL of plasma) and for plasma-rich fresh-frozen plasma (FFP) and platelet concentrates (PCs; both contain approx. 250 mL of plasma) to allow comparisons between transfusion rates and anti-CMV levels after the time intervals.

A subgroup analysis was performed for anti-CMV IgG kinetics in patients meeting the following criteria: 1) seroconversion for anti-CMV IgG at the end of Interval 1; 2) available follow-up data throughout all three intervals; 3) low transfusion rate during Interval 3 (<0.4 RBC units per week and <0.2 PCs and FFPs per week); 4) absence of intravenous immunoglobulin (IVIG) administration.

Furthermore, the anti-CMV IgG levels in our study cohort were compared with those of women who tested positive for anti-CMV IgG during pregnancy screening. These women did not receive transfusions, and anti-CMV IgG antibodies were most likely the result of previous CMV infections.

Recruitment of stem cell donors

Stem cell donors were chosen according to the German consensus:[10] preference was given to HLA-identical siblings upon availability. In case of HLA-identical sibling transplantation, any constellation of CMV serology was accepted. Matched unrelated donors with identical CMV serostatus and up to one HLA mismatch were accepted. The transplantation of stem cells from a CMV-seronegative donor to a CMV-seropositive patient was avoided, whenever possible.

Production and provision of blood components

Blood products were produced at the Department for Transfusion Medicine by standard blood bank procedures using top and bottom blood bags (MacoPharma, Tourcoing, France). RBCs were buffy coat reduced, in-line leukoreduced, and stored in additive solution. The majority of PCs were produced by the buffy coat pool method.[11] Until 2001, five to six single PCs prepared from the buffy coat of one whole blood donation each were pooled and leukoreduced in-line during pooling to

produce one therapeutic unit.[12] After 2001, four buffy coats and one plasma unit were pooled before a second spin for producing one in-line leukoreduced therapeutic unit.[11] Alloimmunized patients or patients requiring PCs of blood group AB received HLA-compatible and/or AB apheresis PCs (AS-104 cell separator, C4F and S4F apheresis sets, Fresenius, Bad Homburg, Germany), all other patients were treated with pooled buffy coat–derived PCs. In case of shortage of buffy coat–derived PCs, patients also received apheresis PCs. Whenever possible, PCs were transfused ABO identical. Leukoreduction was performed using filters (BioR-plus BBS for RBCs and BioP-plus BBS PF filters for pool PCs, Fresenius) between 1999 and 2001. After 2001, RBCs and pooled PCs were leukoreduced by filters (for RBCs, Leukoflex LCR-5, Macopharma; and for pooled PCs, Sepacell PLX-5 filters, ASAI Medical, Tokyo, Japan). Apheresis PCs were leukoreduced with in-line filters integrated in the apheresis sets. All applied leukoreduction filters reached a residual WBC count of less than 5×10^6 WBCs per unit.[13-16] The internal quality control cutoff for residual WBCs was 1×10^6 WBCs per unit for RBCs and PCs. All quality controls performed throughout the study period were below this cutoff. FFP was prepared by processing whole blood according to standard methods. All cellular blood products were gamma irradiated with 30 Gy before transfusion. IVIG was administered in case of bacterial infections in patients with severe hypogammaglobulinemia. Anti-CMV testing of blood donors was omitted throughout the entire study period.

CMV NAT

CMV NAT was performed using ethylenediaminetetraacetate blood or serum samples (Vacutainer, BD, Plymouth, UK). CMV DNA was extracted by a DNA blood kit (QIAamp, Qiagen, Hilden, Germany) according to the manufacturer's instructions. From 1999 to 2001, CMV NAT was carried out by standard polymerase chain reaction (PCR).[17] Since 2001, CMV NAT was tested by real-time PCR[18] using a real-time PCR detection system (iCycler, Bio-Rad, Munich, Germany; or MX3000P, Agilent Technologies, Waldbronn, Germany).

CMV antibody testing

For anti-CMV IgG and IgM measurements, plasma samples were collected, separated within 24 hours, and stored below –30°C until testing by enzyme immunoassay (Enzygnost, anti-CMV IgG or IgM, BEP III; Siemens Healthcare Diagnostics, Eschborn, Germany). Anti-CMV IgG levels were expressed in (U/L) and interpreted as positive, if a gray zone (230-550 U/L) or higher level was detected. Cutoff for negative values was 230 U/L.

Statistical analyses

Comparisons were performed for transfusion rates between anti-CMV IgG–positive and –negative patients after each interval and for anti-CMV IgG levels between the study cohort and the reference cohort using the Wilcoxon-Mann-Whitney test (SAS, Version 9.1, SAS Instituted, Cary, NC). All values are given as median and range unless indicated otherwise. Confidence intervals (CIs) for the risk of TT-CMV were calculated as described.[19]

RESULTS

Patient characteristics and clinical course

In total, 150 allogeneic HSCTs were performed in 142 patients between 1999 and 2009 with 36 related HSCT donors (24.0%) and 114 unrelated stem cell donations (76.0%). Indications for allogeneic HSCT involved acute leukemia (acute lymphoblastic leukemia, acute myelogenous leukemia), chronic leukemia (chronic myelogenous leukemia, chronic lymphocytic leukemia), lymphomas (Hodgkin's lymphoma, non-Hodgkin's lymphoma), and myelodysplastic/myeloproliferative disorders (myelodysplastic syndrome).

A total of 104 patients (73.2%) presented initially with a positive CMV serostatus (anti-CMV IgG positive, anti-CMV IgM negative) with 71 patients (50.0%) receiving allografts from CMV-seropositive and 31 (21.8%) from CMV-seronegative stem cell donors, respectively.

From 38 patients (26.8%) initially presenting with CMV-negative serostatus, 23 individuals (16.2%) received a CMV-seronegative allograft and were thus included in the study. None of these 23 patients developed CMV disease or CMV-associated complications after HSCT.

Characteristics of transfused blood units

In total, 1847 blood units were transfused in 23 patients within the study period with a median of 55 (range, 3-313) units per patient. The blood products were obtained from 3180 donors, resulting in a median donor exposure of 125 (range, 3-318) donors per patient. Patients were most often transfused with RBCs (36 units; range, 2-136 units), followed by PCs (25 units; range, 0-84 units) and FFPs (0 units; range, 0-182 units); 64.1% of transfused PCs were pooled PCs and 35.9% were apheresis PCs.

The three time intervals investigated for antibody status (see section "Incidence, kinetics, and levels of anti-CMV") are characterized by different transfusion rates. Interval 1 (<Day +30) comprised 50.0% of all transfusions, whereas 29.8% of the administered blood products were transfused during Interval 2 (Day +30-Day +100) and 20.2% during Interval 3 (>Day +100), respectively. Interval 3 differed in length between patients, due to different observational periods, with a median of 275 days (25 and 75% quartiles, 234 and 456 days, respectively).

CMV NAT and risk of TT-CMV

None of the serial CMV NATs performed in 23 patients throughout the observation period became positive. Together with the lack of clinical symptoms of CMV infection, this strongly indicates that there was no case of TT-CMV in our cohort, resulting in a risk of TT-CMV of 0% (95% CI, 0.0%-0.12%) per donor exposure and 0% (95% CI, 0.0%-14.31%) per patient.

Incidence, kinetics, and levels of anti-CMV

Anti-CMV status was assessable in 22 patients and all antibody analyses were concomitantly performed for anti-CMV IgM and IgG. In accordance with the clinical presentation and the negative CMV-NAT, no patient seroconverted for anti-CMV IgM. In contrast, a high incidence of anti-CMV IgG was observed: 17 patients (77.3%) developed positive anti-CMV IgG serum levels. Five patients (22.7%) remained CMV seronegative throughout the study (Table 1).

We found different transfusion rates for anti-CMV IgG–negative and anti-CMV IgG–positive patients (Fig. 1): Interval 1, FFPs plus PCs 0.2 (range, 0-2.5) versus 2.0 (range, 1.0-6.3) units per week ($p = 0.0009$) and RBCs 1.0 (range, 0-3.2) versus 2.0 (range, 0.7-6.4) units per week ($p = 0.0392$); Interval 2, FFPs plus PCs 0.0 (range, 0-0.1) versus 0.3 (range, 0-26.5) units per week ($p = 0.0033$) and RBCs 0.2 (range, 0-0.7) versus 1.6 (range, 0-9.7) units per week ($p = 0.0079$); and Interval 3, FFPs plus PCs 0.0 (range, 0-0.3) versus 1.1 (range, 0.3-10.5) units per week ($p = 0.0011$) and RBCs 0.0 (range, 0-0.5) versus 1.7 (range, 0.8-3.4) units per week ($p = 0.0021$). All patients who received IVIG tested positive for anti-CMV IgG after the respective interval and showed the highest anti-CMV IgG levels (Table 1).

Furthermore, in a subgroup of four patients testing positive for anti-CMV IgG after the first interval, who had a low transfusion rate during Interval 3 (<0.4 RBC units and <0.2 PC and FFP units/per week) and in whom follow-up data throughout all three intervals were available, the anti-CMV IgG levels decreased over time leading to a negative CMV serostatus (Fig. 2A). Finally, the levels of anti-CMV IgG in our cohort (1200 U/L; range, 270-4700 U/L) were significantly lower compared to a cohort of actively immunized individuals (11,000 U/L; range, 690-37,000 U/L; $p < 0.0001$; Fig. 2B).

DISCUSSION

This prospective study provides further evidence of a minor risk of CMV transmission by leukoreduced non-CMV antibody–tested blood products in a high-risk group for TT-CMV infection, that is, CMV seronegative recipients of CMV-seronegative HSCT-allografts. We systematically applied highly sensitive CMV NAT to assess early viral replication in these patients throughout allogeneic HSCT until at least Day +100. We did not observe either any positive CMV NAT or a positive test for anti-CMV IgM. This matched with the patients' clinical courses where no manifestations of CMV infection were observed.

In contrast, we observed a high incidence of anti-CMV IgG in the study cohort, which, however, showed much lower levels compared to actively immunized individuals (Fig. 2B). This suggests that anti-CMV IgG were passively transferred by the blood products in these multiple transfused patients. Accordingly, the weekly transfusion rates of patients testing positive for anti-CMV IgG were significantly higher compared to anti-CMV IgG–negative patients at all three intervals (Table 1, Fig. 1). These findings are especially remarkable, given that in Intervals 2 and 3, some carryover of passively transfused anti-CMV IgG should have weakened the chance of finding an association, because not all anti-CMV IgGs passively transfused in Interval 1 had been cleared.

Our hypothesis of passively transferred anti-CMV IgG is further corroborated by the decrease of their levels during intervals in which only few blood products were transfused (Fig. 2A). In active immunization, stable or even increasing levels of anti-CMV IgG would have been expected. Taken together, these findings strongly support our hypothesis of passively transferred anti-CMV IgG by blood products as the cause for anti-CMV IgG seroconversion in our study.

Our findings are in line with earlier reports,[20,21] which also concluded that passive IgG transfer by transfused blood products might be a likely cause for CMV seroconversion, but these studies did not systematically address this hypothesis by CMV NAT screening for early CMV replication. CMV NAT has superior specificity and sensitivity in detecting CMV replication in HSCT patients[22] compared to culture-based antigen tests. We found no case of positive CMV NAT, which implicates that no case of CMV replication after transfusion was evident in our study.

This study has the limitation of a relatively small number of patients. Based on the 1847 transfused blood products (obtained from 3180 donors) and no observed case of TT-CMV, the 95% CIs for the risk of TT-CMV in our cohort was 0% to 14% per patient and 0% to 0.21% per donor exposure. This is in the range of the estimated risk for TT-CMV of 95% CIs of 1% to 18% per patient and 0.06% to 0.62% per donor exposure previously reported[23] for universal leukoreduction of cellular blood products.

Based on our greater than 10-year experience omitting CMV antibody screening, we recommend against

TABLE 1. Weekly transfusion rates, IVIG-administration, and anti-CMV IgG levels at the end of each interval*

		Interval 1 (<Day +30)					Interval 2 (Day +30-Day +100)					Interval 3 (>Day +100)				
		Units per week					Units per week					Units per week				
Case	Year of HSCT	PC	FFP	RBCs	IVIG	CMV IgG (U/L)	PC	FFP	RBCs	IVIG	CMV IgG (U/L)	PC	FFP	RBCs	IVIG	CMV IgG (U/L)
1	1999	1.2	0.0	0.1	+	Positive†	0.0	0.0	0.1	–	1800	0.0	0.0	0.0	–	Negative
2	2007	4.2	0.0	0.3	–	2200	0.3	0.0	0.1	–	870	0.0	0.0	0.0	–	Negative
3	2004	1.4	0.0	0.3	–	1500	0.4	0.0	0.0	–	gz	0.1	0.0	0.0	–	Negative
4	2008	4.8	0.0	0.4	–	1000	0.1	0.0	0.1	–	Negative	0.0	0.0	0.0	–	Negative
5	2001	1.0	0.0	0.1	–	590	0.0	0.0	0.0	–	Negative	0.0	0.0	0.0	–	Negative
6	2003	1.7	0.0	0.4	–	gz	0.0	0.0	0.1	–	Negative	0.0	0.0	0.0	–	Negative
7	2006	0.9	0.0	0.1	–	Negative	0.0	0.0	0.0	–	Negative	0.0	0.0	0.0	–	Negative
8	2007	0.2	0.0	0.0	–	Negative	0.0	0.0	0.0	–	Negative	0.0	0.0	0.0	–	Negative
9	2007	0.0	0.0	0.3	–	Negative	0.0	0.0	0.0	–	Negative	0.0	0.0	0.0	–	Negative
10	2007	0.6	0.0	0.2	–	Negative	0.0	0.0	0.1	–	Negative	0.0	0.0	0.1	–	Negative
11	2009	0.0	0.0	0.0	–	Negative	0.0	0.0	0.0	–	Negative	0.3	0.0	0.1	–	Negative
12	2005	2.2	0.0	0.5	–	1300	0.0	0.0	0.0	–	gz	2.2	0.0	0.5	+	2200.0
13	2009	0.2	0.0	0.1	–	Negative	0.0	0.0	0.1	–	Negative	4.3	6.2	0.5	+	1600.0
14	2007	2.5	0.0	0.2	–	1000	0.0	0.0	0.1	–	gz	0.3	0.0	0.1	–	1200.0
15	2005	1.9	0.0	0.3	–	650	0.0	0.0	0.0	–	Negative	1.1	0.0	0.2	–	gz
16	2008	0.2	0.0	0.2	–	Negative	0.0	0.0	0.0	–	Negative	0.4	0.2	0.1	–	gz
17	2002	0.7	0.0	0.1	–	Negative	0.2	0.0	0.3	+	4700	ND	ND	ND	ND	ND
18	2003	2.5	0.0	0.5	–	Negative	1.6	0.4	0.4	+	3800	ND	ND	ND	ND	ND
19	2002	3.2	0.0	0.9	–	790	2.7	8.0	0.7	+	2500	ND	ND	ND	ND	ND
20	1999	0.4	0.0	0.1	+	ND	3.3	23.2	1.4	–	1700	ND	ND	ND	ND	ND
21	2008	1.8	0.1	0.1	–	1200	15.2	0.0	1.0	–	1500	ND	ND	ND	ND	ND
22	2000	4.1	0.3	0.9	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
23	1999	6.3	0.0	0.6	–	2100	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

* Cutoff for negative values was 230 U/L.
† Level not available.
gz = gray zone anti-CMV IgG level, 230-550 U/L. ND = not done.

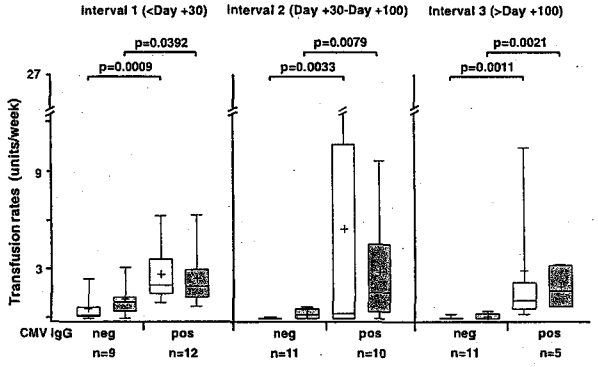

Fig. 1. Comparison of transfusion rates between anti-CMV IgG antibody–negative and –positive patients after three time intervals. Displayed are box plots of weekly transfusion rates for plasma-rich blood units (PCs and FFPs combined, □) and plasma-poor RBCs (▣). The median is given as line in the box plots, the mean is indicated by "+." Patients testing positive for anti-CMV IgG antibodies at the end of an interval received significantly more transfusions than CMV-seronegative patients.

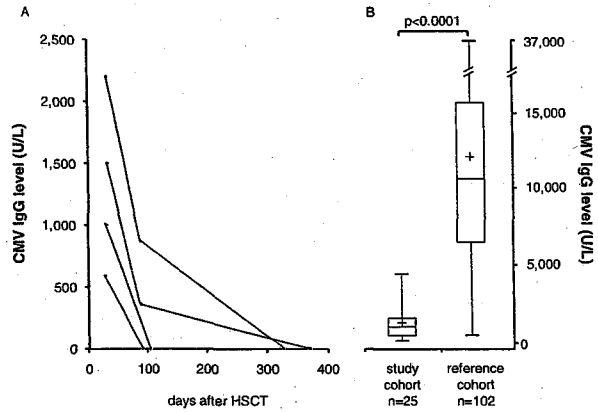

Fig. 2. Evidence for passive transfer of anti-CMV IgG by blood products. (A) Anti-CMV IgG levels of four representative patients (Cases 2-5, Table 1), who received multiple transfusions (but no IVIG) in Interval 1 and only few transfusions during Interval 3. The decrease of anti-CMV IgG levels indicates that CMV IgG seroconversion after allogeneic HSCT is caused by passive transfer of CMV IgG antibodies with the blood products. (B) Anti-CMV IgG levels in our study cohort were significantly lower compared with those in women actively immunized against CMV (tested during pregnancy screening) also indicating passively transferred anti-CMV IgG rather than active immunization.

testing blood donors for CMV antibodies. The prevalence of non–cell-bound CMV DNA in the circulation of blood donors, as a marker for free virus, is highly associated with CMV seroconversion of recipients whereas no CMV DNA-emia was observed in donors who had a positive CMV serostatus for at least 1 year.[9] This suggests that active viral replication in immunocompetent blood donors latently infected with CMV occurs less often than in CMV-seroconverting donors. In this context transfusion of blood from window-phase donations would bear the highest risk for TT-CMV infection. Consequently the use of blood components exclusively from CMV-seronegative donors might increase the risk for window-phase donations. This may in fact even paradoxically increase the probability for TT-CMV infection. The second argument is the appropriate location of resources in times of budget restrictions. With approximately 25,000 donations per year at our transfusion service, CMV antibody testing would have created costs of at least €600,000 (assumed rate of 50% of donors to test and costs of €5.0 per test) and provision of pooled PCs would not have been possible for logistic reasons adding additional costs of approximately €100,000 for apheresis PCs.

We conclude that the risk of TT-CMV by leukoreduced blood products from donors not tested for CMV antibodies is low in $CMV^{neg/neg}$ HSCT recipients. From our perspective, anti-CMV testing does not contribute to a major increase in transfusion safety in these patients. More efficient for increasing transfusion safety might be to use the resources needed for CMV antibody testing to establish measures reducing transfusion rates, for example, by less toxic conditioning therapies and implementation of lower transfusion triggers.

CONFLICT OF INTEREST

None of the authors declares any conflicts of interest.

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1

別紙様式第２－１

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日 2011年12月16日	新医薬品等の区分 該当なし。	総合機構処理欄
一般的名称	別紙のとおり。		研究報告の公表状況	共同通信（2011年12月15日配信）	公表国 日本	
販売名（企業名）	別紙のとおり。					使用上の注意記載状況・その他参考事項等 記載なし。

研究報告の概要

問題点：日本で腸管出血性大腸菌O157がウシ肝臓の内部より初めて検出された。

厚生労働省の調査で、牛の肝臓の内部から腸管出血性大腸菌O157が初めて確認されたことが分かった。全国16自治体の食肉衛生検査所などで調査した結果、約150頭の牛のうち2頭の肝臓の内部から生きている状態のO157が見つかった。厚労省は生レバーの提供を禁止するかどうか検討しており、20日の審議会に調査結果を報告する。

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

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INF2011-005-1

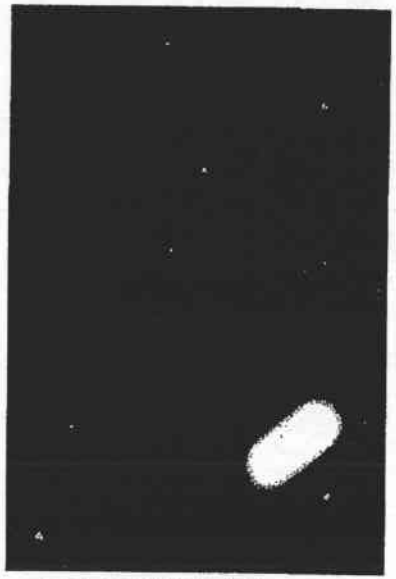
牛の肝臓内にO157初確認　生レバー禁止の可能性

厚生労働省の調査で、牛の肝臓の内部から腸管出血性大腸菌O157が初めて確認されたことが15日、分かった。

厚労省は生レバーの提供を禁止するかどうか検討しているが、O157による食中毒は死亡の危険もある上、肝臓内の菌を死滅させるには十分な加熱が必要で、提供禁止となる可能性が高まった。同省は20日の審議会に調査結果を報告する。

厚労省などによると夏以降、全国16自治体の食肉衛生検査所などで調査。約150頭の牛のうち2頭の肝臓の内部から生きている状態のO157が見つかった。

2011/12/15 10:57　【共同通信】



「O157」の電子顕微鏡写真(国立感染症研究所提供)

別紙様式第2−1

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日 2011年12月20日	新医薬品等の区分 該当なし。		総合機構処理欄
一般的名称	別紙のとおり。		研究報告の公表状況	薬事・食品衛生審議会食品衛生分科会乳肉水産食品部会資料(2011年12月20日開催)		公表国 日本	
販売名(企業名)	別紙のとおり。						

研究報告の概要

問題点：日本で腸管出血性大腸菌O157がウシ肝臓の内部より初めて検出された。

平成23年12月20日開催の薬事・食品衛生審議会食品衛生分科会乳肉水産食品部会において、ウシ肝臓内部における腸管出血性大腸菌の汚染実態に関し、日本全国16か所の食肉衛生検査所における調査及び文献調査の結果が発表された。糞便、胆汁、肝臓内部又は肝臓表面から検体を増菌培養の後、分離培養および遺伝子検出が行われた結果、初めて肝臓内部由来の検体からも腸管出血性大腸菌O157が検出された。

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

使用上の注意記載状況・その他参考事項等

記載なし。

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MedDRA/J ver. 14.1

資料 2

牛レバー内部における腸管出血性大腸菌等の汚染実態調査（概要）

岩手大学　特任教授・名誉教授
品川邦汎

○内容

牛レバーの腸管出血性大腸菌の汚染実態状況について、全国16か所の食肉衛生検査所における調査及び文献調査を行った。

- 調査項目：
  1. 同一牛の糞便、胆嚢胆汁、肝臓表面（拭き取り）及び肝臓内部について、腸管出血性大腸菌の分離培養及び遺伝子検査（一部の機関で大腸菌、大腸菌群の検査も実施）
  2. 胆汁中及び肝臓表面の大腸菌群の汚染実態調査（追加試験）
  3. 牛胆汁における腸管出血性大腸菌の増殖性試験
- 調査期間：８～11月

○調査協力機関

秋田県、山形県、埼玉県、さいたま市、東京都、神奈川県、静岡県、岐阜県、大阪市、兵庫県、岡山県、鳥取県、徳島県、愛媛県、大分県及び宮崎県の食肉衛生検査所

汚染実態調査

○ サンプリング方法
- 糞便：肛門もしくは直腸より採取
- 肝臓：内臓摘出時に滅菌トレイで衛生的に採取した検体、通常の内臓検査前後、もしくは内臓業者から購入したものを使用
- 肝臓表面は拭き取り、肝臓内部は左葉を中心に採取（アルコール綿で表面の清拭、火炎殺菌等実施し、交差汚染のないよう採取）
- 胆汁は注射器により採取

○ 供試検体量

糞便（1g）、胆汁（5ml）、肝臓内部（25g）又は肝臓表面（100cm^{2}以上）を増菌培養後、分離培養又は遺伝子検出を実施

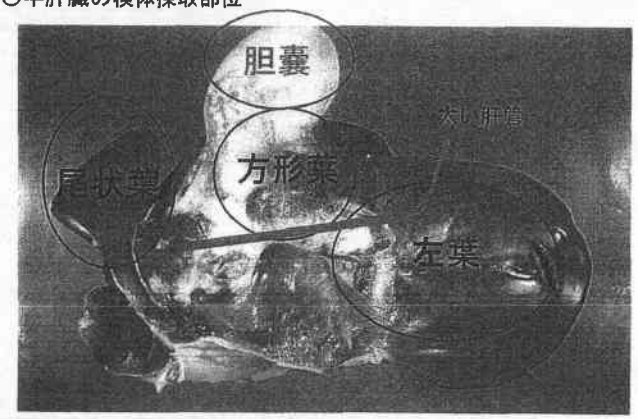
○ 使用培地
- 増菌用培地：　ノボビオシン加mEC培地
- O157分離培養：O157（CT-SMAC、クロモアガー）　等

○ 遺伝子検出
- O157、VT-1、VT-2の混合プライマー

（O157＆ベロ毒素遺伝子同時検出キット、O157 VT1/2 One Shot PCR Typing Kit Ver.2（いずれもタカラバイオ社製）等）

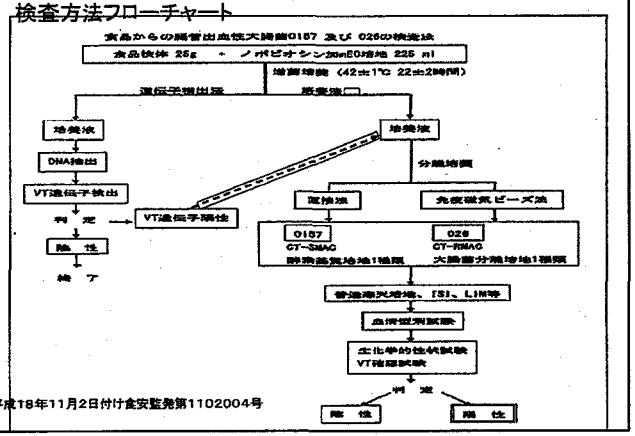
汚染実態調査

○牛肝臓の検体採取部位



汚染実態調査

○ 検査方法フローチャート



平成18年11月2日付け食安監発第1102004号

汚染実態調査

○ 結果（大腸菌）（速報値）

実施機関	検体数	陽性数			
		糞便	胆汁	肝臓表面	肝臓内部
4	50	35	9	28	11

○ 追加試験結果（胆汁及び肝臓表面の大腸菌群数）（速報値）

	胆汁	肝臓表面
検体数	159	140
検出件数	16	93
菌数	$<10^2$(/ml) :7検体 10^3～10^4(/ml) :3検体 $10^5<$(/ml) :6検体	5×10^{-2}－8.4×10^3 (/cm^2)

注）菌数は陽性検体における菌数の範囲

汚染実態調査

○ 結果（腸管出血性大腸菌EHEC）（速報値）

		糞便	胆汁	肝臓表面	肝臓内部
分離培養	検体数	173	186	193	173
	EHEC	20	0	13	3
	うちO157	11	0	5	2
遺伝子	検体数	155	168	178	157
	検出数	64	1	35	10
	うちVT1	5	1	5	0
	うちVT2	35	0	20	4
	うちVT1 or 2	13	0	9	1
	うちVT1＆2	11	0	1	5

VT: ベロ毒素

牛胆汁における腸管出血性大腸菌の増殖性①

○ 調査概要
供試検体：牛胆汁
調査期間：平成23年9月下旬

○ 調査概要
採取した10頭分の牛胆嚢胆汁のうち菌未発育の6頭分の胆汁を用いて、以下の①、②の腸管出血性大腸菌の増殖試験を実施。

① プール胆汁における腸管出血性大腸菌の増殖態度
6頭分の胆汁全てを混合したプール胆汁に3種類の菌液（A、B、C）を接種し、37℃で一晩培養。

② 胆汁の違いによる腸管出血性大腸菌の増殖態度
各胆汁（6頭分）に菌液Aを接種し、37℃で一晩培養。

＜増殖試験に用いた菌液＞
菌液A：O157VT1&2、 菌液B：O157VT2、 菌液C：O26VT1

牛胆汁における腸管出血性大腸菌の増殖性②

○ 結果

① プール胆汁における腸管出血性大腸菌の増殖態度

	スタート時菌量(/ml)	培養後菌量(/ml)
①プール胆汁10ml＋菌液A 0.1ml	1.9×10^2	$>10^6$
②プール胆汁10ml＋菌液B 0.1ml	2.3×10^2	$>10^6$
③プール胆汁10ml＋菌液C 0.1ml	1.5×10^2	$>10^6$
④プール胆汁10ml＋生理食塩水0.1ml	0	0

② 胆汁の違いによる腸管出血性大腸菌の増殖態度

	スタート時菌量(/ml)	培養後菌量(/ml)
⑤胆汁No.2 10ml＋菌液A 0.1ml	1.9×10^2	$>10^6$
⑥胆汁No.4 10ml＋菌液A 0.1ml	1.9×10^2	$>10^6$
⑦胆汁No.5 10ml＋菌液A 0.1ml	1.9×10^2	$>10^6$
⑧胆汁No.7 10ml＋菌液A 0.1ml	1.9×10^2	$>10^6$
⑨胆汁No.8 10ml＋菌液A 0.1ml	1.9×10^2	$>10^6$
⑩胆汁No.9 10ml＋菌液A 0.1ml	1.9×10^2	$>10^6$
⑪生理食塩水10ml＋菌液A 0.1ml	1.9×10^2	50

文献調査：国内文献①

食肉処理場での腸管出血性大腸菌汚染実態

検体	検体数	菌株分離数	分離率(%)	血清型	stx型	stx遺伝子検出数	検出率(%)	検体採取時期	備考	
胆嚢胆汁	548	0	0.0	−	−	2	0.4	2001年9月−2005年3月		*1
胆嚢胆汁	119	0	0.0	−	−	1	0.8	2005年4月−2006年3月(12月、2月を除く)		
肝臓中心部(尾状葉)	102	4	3.9	OUT:H11 O157:H21 OUT:HUT OUT:H21	2 1,2 1,2 2	5	4.9	2005年5月−2006年1月(12月除く)		*2
胆嚢胆汁	318	1	0.3	O91:HUT	1,2	−	−	2004年6月−2007年1月		
肝臓中心部(尾状葉)	165	7	4.2	OUT:H11 O157:H21 OUT:HUT OUT:H21 O28:HUT O91:HUT OUT:HUT	2 1,2 1,2 2 2 1,2 1,2	−	−	2005年5月−2007年1月	胆汁及び肝臓のO91:HUTの菌株は、同一牛個体から分離。	*3

*1~3： 参考資料6を参照

文献調査：国内文献②

市販流通品の腸管出血性大腸菌汚染実態

検体	検体数	菌株分離数	分離率(%)	血清型	stx型	stx遺伝子検出数	検出率(%)	検体採取時期	備考	
肝臓(生食用)	10	1	10.0	O127a:H−	−	−	−	1994年(6月、7月、9月)		*4
肝臓(生食用)	24	0	0.0	−	−	−	−	1998年8月−12月		*5
肝臓(生食用)	16	0	0.0	−	−	−	−	1998年度		*6
肝臓(生食用)	50(肝臓と挽肉合計)	0	0.0	−	−	1	−	1999年9月−2000年1月	菌株分離数、stx遺伝子検出数は肝臓のデータ	*7
肝臓(生食用)	10	0	0.0	−	−	−	−	1999年度		*8
肝臓	24	2	8.3	O157	1,2	−	−	2000−2004年(各年7−9月の間)	2分離菌株ともにO157、stx1,2産生	*9
肝臓	15	0	0.0	−	−	−	−	2007年9月−11月		*10
肝臓	15	0	0.0	−	−	−	−	2008年9月−2009年1月		*11
肝臓	36	0	0.0	−	−	5	13.9	2010年7月−11月	stx遺伝子を検出した5検体のうち1検体はO157遺伝子	*12

*4~12： 参考資料6を参照

文献調査：海外文献①

肉牛の糞便、胆嚢からの腸管出血性大腸菌O157

	検体数	陽性検体数（%）
直腸便	933	66（7.1）
胆嚢粘膜スワブ	933	1（0.1）
胆嚢粘膜組織	933	4（0.4）

USA:2か所の食肉処理場での調査(2005年5〜7月)

Reinstein, S., et al. Prevalence of Escherichia coli O157:H7 in Gallbladders of Beef Cattle. Applied and Environmental Microbiology Feb. 2007: 1002-1004.

文献調査：海外文献②

感染実験牛からの糞便、第一胃、胆汁からの腸管出血性大腸菌 O157

感染牛	1グループ 8頭（雄仔牛：投与菌数10^6cfu，36日後）	
	糞便（結腸）	7頭陽性（菌数10^2～10^3cfu）
	第一胃	2頭陽性
	胆汁	5頭陽性
感染牛	2グループ 7頭（雄仔牛：投与菌数10^6cfu，15日後）	
	糞便（結腸）	5頭陽性（菌数10^2～10^3cfu）
	第一胃	4頭陽性
	胆汁	全て陰性（－）
感染牛	3グループ 8頭（雄仔牛：投与菌数10^6cfu，9日後）	
	糞便（結腸）	8頭陽性（菌数10^2～10^6cfu）
	第一胃	8頭陽性
	胆汁	

Jeong, K.C. et al. Isolation of Escherichia coli O157:H7 from the gall bladder of inoculated and naturally-infected cattle. Veterinary Microbiology 119(2007): 339-345.

7

別紙様式第 2-1
番号 13

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2011 年 12 月 6 日	新医薬品等の区分	厚生労働省処理欄
一般的名称	①②③④⑤ポリエチレングリコール処理人免疫グロブリン ⑥⑦人免疫グロブリン		研究報告の公表状況		公表国 アメリカ	
販売名（企業名）	①献血ヴェノグロブリン IH5%静注 0.5g/10mL	（ベネシス）		Canada Communicable Disease Report (CCDR) weekly Issue 47/2011/11/25		
	②献血ヴェノグロブリン IH5%静注 1g/20mL	（ベネシス）				
	③献血ヴェノグロブリン IH5%静注 2.5g/50mL	（ベネシス）				
	④献血ヴェノグロブリン IH5%静注 5g/100mL	（ベネシス）				
	⑤献血ヴェノグロブリン-IH ヨシトミ	（ベネシス）				
	⑥グロブリン筋注 450mg/3mL「ベネシス」	（ベネシス）				
	⑦グロブリン筋注 1500mg/10mL「ベネシス」	（ベネシス）				

研究報告の概要

梅毒が 4 年間で米国において 36%増加：

2006 年～2010 年まで、米国での報告された梅毒症例数は 36%増加した。疾病管理予防センター（CDC）によって出版された報告によると、若い、アフリカ系アメリカ人男性の間で、率が 135%増加した。著者は、性感染症（STD）は米国における巨大な健康と経済の結果に隠れた伝染病であると説明した。感染した人々の相当数が公然と進み出ることがなく、その上この種の疾患の社会的及び生物学的特徴のため、STD は隠れた伝染病と呼ばれている。
著者は書いた：「全てのコミュニティーが STD に影響を受けるので、全てのアメリカ人が性感染症予防に関心がある、そして全ての個人は直接、或いは間接的にこれらの疾患のコストを払う。」
CDC は、男性パートナーと性的に行動的な男性は、1 年に 1 回よりはむしろ 3 箇月に 1 回、STD のためにスクリーニングされなければならないと考えている。

淋病－
淋病の報告された症例は、4 年の期間の間で、これまでで最も低いレベルまで、16%低下した。しかしながら、最後の年に、淋病は僅かに増加した。2010 年に 300,000 以上の報告された症例があった。幾つかの CDC の調査システムによれば、淋病はこの疾患のために利用できる唯一の薬物に対して耐性を示すようになっている。
クラミジアー
検査の増加により、報告された症例の数は24%増加した。2010年に約130万症例の報告があった。クラミジアをもつアメリカの大多数の人々は診断未確定である－彼らはクラミジアであるということを知らない。CDCは全ての性的に行動的な若い女性は毎年検査することを勧める。
梅毒－
増加した率の長い期間の後、梅毒の発生率は2009年以降1.6低下した。若い、アフリカ系アメリカ人男性の間の率は、2006年から2010年まで134%増加した。アフリカ系アメリカ人のMSM（男性間性的接触者）の間の率はかなり増加した、そして報告されたものが加わった。STDの1900万例の新しい症例が、米国において毎年診断される。STDは健康管理システムに1年につき170億ドルを要した。ハイリスク群のそれらの内、半分だけが検査を受けていると、著者は書いた。彼らは症状がないため、感染した個々の多くは気づいていない。

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリン IH5%静注 0.5g/10mL の記載を示す。
2. 重要な基本的注意
1)本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-I 抗体陰性で、かつ ALT（GPT）値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分からポリエチレングリコール 4000 処理、DEAE セファデックス処理等により人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及びろ過膜処理（ナノフィルトレーション）及び pH3.9～4.4 の条件下での液状インキュベーション処理を施しているが、投与に際し次の点に十分注意すること。

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STD未治療のままの結果
性的に行動的なアメリカ人の4分の1は若者である。しかしながら、若者は全ての新しく報告されたSTDのほぼ半分を代表する。未治療のままの場合、性感染症は長期的な転帰をもたらす可能性がある：
・淋病とクラミジアー
未治療のままの場合、これらの2つのSTDによって女性で永続的な不妊性が起きることがありえる。24,000人の米国女性は、STDのため毎年生殖力がなくなる。
・梅毒ー
未治療のままの場合、合併症は脳、心血管及び器官損傷に帰着することがありえる。感染している妊婦は、先天梅毒で新生児を出産する。先天梅毒は、死産、周産期死亡、生存する人々における神経性合併症と身体的な奇形に帰着することがありえる。母が未治療の梅毒を有する新生児の40%は死亡する。未治療の梅毒は深刻な皮膚の問題につながることがある。
・HIVリスクー
最近の研究によれば、クラミジア、梅毒、或いは淋病を有する人々は、HIVに感染する大きなリスクがある。

報告企業の意見	今後の対応
梅毒トレポネーマ（Treponema pallidum）はスピロヘータ科トレポネーマ属の一種で、大きさは0.10～0.18×6～20μm（平均0.13～0.15×10～13μm）のラセン状の細菌で、低温保管や凍結乾燥、加熱処理により死滅するとされている。そのため、万一原料血漿に梅毒トレポネーマが混入したとしても、製造工程において不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

グロブリン

MN medicalnewstoday.com

Syphilis Rises 36% In USA In Four Years

17 Nov 2011 Click to Print

From 2006 to 2010, the number of reported syphilis cases in the USA rose 36%. Among young, African-American males the rate rose by 135%, according to a report issued by the Centers for Disease Control and Prevention (CDC).

The authors explained that sexually transmitted diseases (STDs) are hidden epidemics of huge health and economic consequences in the USA. STDs are called hidden epidemics because a considerable number of infected people are unwilling to come forward openly, and also because of the social and biologic characteristics of these types of diseases.

The authors wrote:

"All Americans have an interest in STD prevention because all communities are impacted by STDs and all individuals directly or indirectly pay for the costs of these diseases."

The CDC believes that sexually active males with male partners should be screened for STDs once every three months, rather than yearly.

Gonorrhea - reported cases of gonorrhea fell 16% over the four-year period, down to their lowest levels ever. However, over the last year they have risen slightly. In 2010 there were over 300,000 reported cases. According to some CDC surveillance systems, gonorrhea is becoming resistant to the only medication available for this disease.

Chlamydia - the number of reported cases rose 24%, due to an increase in screenings. There were approximately 1.3 million cases reported in 2010. The majority of people in America with Chlamydia are undiagnosed - they don't know they have it. The CDC recommends that all sexually active young women be screened annually; less than half of them do so.

Syphilis - after a long period of increased rates, the incidence of syphilis dropped 1.6 since 2009. The rate among young, African-American males rose 134% from 2006 to 2010. The rate among African-American MSM (men who have sex with men) rose considerably, the reported added.

Nineteen million new cases of STDs are diagnosed annually in the USA. STDs cost the health-care system $17 billion a year.

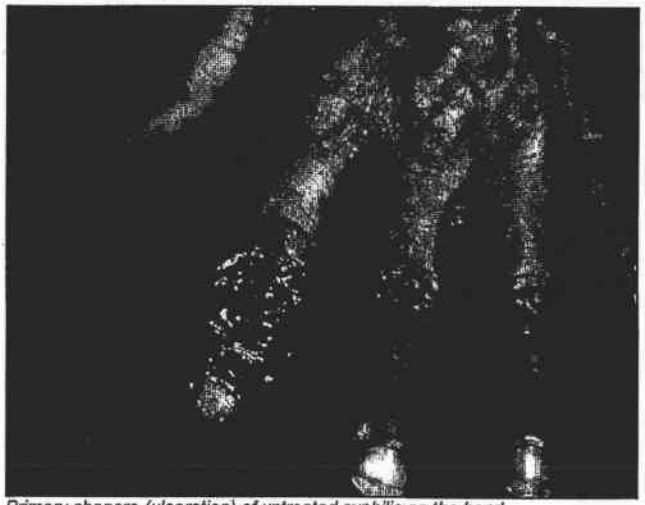
Of those in high risk groups, only half are being tested, the authors wrote. A significant number of infected individuals are unaware, because they have no symptoms.

Consequences of leaving an STD untreated

One quarter of sexually active Americans are young people. However, young people represent nearly half of all new reported STDs. Sexually transmitted diseases, if left untreated, can have long-term consequences:

- **Gonorrhea and Chlamydia** - if left untreated, these two STDs can cause permanent infertility in a woman. 24,000 US women become infertile each year because of STDs.
- **Syphilis** - if left untreated, complications can result in brain, cardiovascular and organ damage. A pregnant woman who is infected can give birth to a newborn with congenital syphilis. Congenital syphilis can result in stillbirth, perinatal death, neurological complications in those who survive, and physical deformity. 40% of newborns whose mothers have untreated syphilis die.

Untreated syphilis can lead to serious skin problems, as seen below:



Primary chancre (ulceration) of untreated syphilis on the hand

- **HIV risk** - individuals with Chlamydia, syphilis or gonorrhea have a higher risk of becoming infected with HIV, according to recent studies.

The authors wrote:

> "STDs are public health problems that lack easy solutions because they are rooted in human behavior and fundamental societal problems. Indeed, there are many obstacles to effective prevention efforts. The first hurdle will be to confront the reluctance of American society to openly confront issues surrounding sexuality and STDs. Despite the barriers, there are existing individual- and community-based interventions that are effective and can be implemented immediately. That is why a multifaceted approach is necessary to both the individual and community levels.
>
> To successfully prevent STDs, many stakeholders need to redefine their mission, refocus their efforts, modify how they deliver services, and accept new responsibilities. In this process, *strong leadership*, innovative thinking, partnerships, and adequate resources will be required. The additional investment required to effectively prevent STDs may be considerable, but it is negligible when compared with the likely return on the investment.
>
> The process of preventing STDs must be a collaborative one. No one agency, organization, or sector can effectively do it alone; all members of the community must do their part. A successful national initiative to confront and prevent STDs requires widespread public awareness and participation and bold national leadership from the highest levels."

Written by Christian Nordqvist

Article URL: http://www.medicalnewstoday.com/articles/237886.php

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. 1. 5	該当なし	
一般的名称	人血清アルブミン				公表国	
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン5%静注12.5g/250mL(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)		研究報告の公表状況	ProMED 20120104.0027.2012-01-04	英国、韓国など	

研究報告の概要

○プリオン疾患最新情報
・英国:国立CJDサーベイランスユニット 月次統計、2012年1月4日現在
英国のCJDサーベイランスユニットから公表されたプリオン病の患者数に関する最新情報である。
2012年1月4日現在、vCJD確定または疑い症例数は176件である(生存者0名/死亡者176名)。英国のvCJD発生は全体的に減少しているという見解は変わっていない。
全てのCJDについては、2011年の1年間で照会された症例数は全部で148件あり、死亡者数は、弧発性CJD:74件、GSS:2件、家族性CJD:9件、vCJD:5件、医原性CJD:3件であった。
1990年に記録を開始して以来では2,881件の照会があり、死亡者数は、弧発性CJD:1,289件、vCJD:176件、家族性CJD:92件、医原性CJD:68件、GSS:45件となっている。
・韓国で第1、2症例目の医原性CJD
韓国疾病管理予防センターによると、2011年11月、韓国で初めての医原性CJDによる死亡者が確認された。翌月(12月)には2例目の死亡者が確認された。これらの患者はそれぞれ1987年と1988年に脳外科手術を受け、その際、ドイツで生産された人工硬膜であるLyoduraが使用されていた。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン5%静注12.5g/250mL
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/20mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
英国CJDサーベイランスユニットの統計によると、2012年1月4日現在、vCJD確定例または疑い例の総数は176名であり、2011年の死亡者数は5名であったとの報告である。 プリオン病の原因とされる異常プリオンがコーン分画工程で効果的に除去されるとの成績と併せて、これまでの疫学研究ではいかなるプリオン病も、アルブミンを介して伝播したとの証拠は無い。また本製剤の使用は一時的かつ限定的であることから伝播のリスクは非常に低いものと考える。	輸血あるいは第Ⅷ因子製剤によりvCJDに感染する可能性が示唆されていることから、今後も引き続き情報の収集に努める。なお、日本赤十字社は、CJD、vCJDの血液を介する感染防止の目的から、献血時に過去の海外滞在歴(旅行及び居住)、CJDの既往歴(本人、血縁者)、hGH製剤投与の有無を確認し、該当するドナーを無期限に献血延期としている。

MedDRA/J Ver.14.1J

Published Date: 2012-01-04 17:44:07
Subject: PRO/AH/EDR> Prion Disease update 2012 (01)
Archive Number: 20120104.0027

PRION DISEASE UPDATE 2012 (01)
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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

[With the continuing decline in the number of cases in the human population of variant Creutzfeldt-Jakob disease -- abbreviated previously as vCJD or CJD (new var.) in ProMED-mail -- the scope of the occasional ProMED-mail updates has been broadened to include other prion-related diseases. In addition to vCJD, data on other forms of CJD: sporadic, iatrogenic, familial, and GSS (Gerstmann-Straussler-Scheinker disease) are included also since they may have some relevance to the incidence and etiology of vCJD. - Mod.CP]

In this update:
[1] UK: National CJD Surveillance Unit - monthly statistics as of Wed 4 Jan 2012 - no new vCJD cases.
[2] UK: caprine BSE
[3] South Korea: 1st case iCJD
[4] South Korea: 2nd case iCJD
[5] Switzerland: BSE

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[1] UK: National CJD Surveillance Unit - monthly statistics as of Wed 4 Jan 2012 - no new vCJD cases.
Date: Wed 4 Jan 2012
Source: UK National CJD Surveillance Unit, monthly statistics [edited]
http://www.cjd.ed.ac.uk/figures.htm

vCJD statistics

The number of deaths due to definite or probable vCJD as of Wed 4 Jan 2012 remains 176. No definite/probable patient remains alive, so the total number of definite or probable vCJD cases (dead and alive) remains 176.

The overall picture remains consistent with the view that the vCJD outbreak in the UK is in decline, albeit now with a pronounced tail. The 1st cases were observed in 1995, and the peak number of deaths was 28 in the year 2000, followed by 20 in 2001, 17 in 2002, 18 in 2003, 9 in 2004, 5 in 2005, 5 in 2006, 5 in 2007, 1 in 2008, 3 in 2009, 3 in 2010, and 5 in 2011.

Totals for all types of CJD cases in the UK in the year 2011

During 2011, there were 148 referrals, 74 fatal cases of sporadic CJD, 2 cases of GSS, 9 cases of familial CJD, 5 cases of vCJD, and 3 cases of iatrogenic CJD.

Since records began in 1990, there have been 2881 referrals, 1289 fatal cases of sporadic CJD, 176 cases of vCJD, 92 cases of familial CJD, 68 cases of iatrogenic CJD, and 45 cases of GSS.

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

[See http://healthmap.org/r/1INY for the interactive HealthMap/ProMED map of the UK>. - Mod.MPP]

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[2]
UK: caprine BSE Date: Sat 3 Dec 2011 Source: Emerging Infectious Diseases 17(12) 12 [edited]
http://wwwnc.cdc.gov/eid/article/17/12/11-0333_article.htm

Isolation of prion with BSE properties from farmed goat

[Authors: Spiropoulos J, Lockey R, Sallis RE, Terry LA, Thorne L, Holder TM, et al. Animal Health and Veterinary Laboratories Agency, Weybridge, Surrey, UK]

Abstract

Transmissible spongiform encephalopathies are fatal neurodegenerative diseases that include variant Creutzfeldt-Jakob disease in humans, scrapie in small ruminants, and bovine spongiform encephalopathy (BSE) in cattle. Scrapie is not considered a public health risk, but BSE has been linked to variant Creutzfeldt-Jakob disease. Small ruminants are susceptible to BSE, and in 2005 BSE was identified in a farmed goat in France. We confirm another BSE case in a goat in which scrapie was originally diagnosed and retrospectively identified as suspected BSE. The prion strain in this case was further characterized by mouse bioassay after extraction from formaldehyde-fixed brain tissue embedded in paraffin blocks. Our data show that BSE can infect small ruminants under natural conditions and could be misdiagnosed as scrapie. Surveillance should continue so that another outbreak of this zoonotic transmissible spongiform encephalopathy can be prevented and public health safeguarded.

Transmissible spongiform encephalopathies (TSEs) are fatal diseases characterized by neurodegenerative changes in the central nervous system that include vacuolation, gliosis, and accumulation of an abnormal isoform (PrPSc) of a naturally occurring host-encoded protein (PrPC) (1). According to the prion hypothesis, PrPSc is the major or the sole infectious agent (1). Although this hypothesis has not received universal acceptance, PrPScis ubiquitous in all known naturally occurring TSEs, and its detection is widely used for their diagnosis.

Bovine spongiform encephalopathy (BSE), a TSE of cattle, was first detected in 1986 (2) and has since been linked with emerging TSEs in other species (3,4) including humans (5,6). Because of its ability to cross species barriers and particularly its zoonotic potential, BSE is considered a public health risk, and extensive measures have been established to detect and eliminate the disease.

Scrapie, a naturally occurring TSE affecting small ruminants, has been known for centuries (7) and is not considered to pose a public health risk (8). Under experimental conditions, however, small ruminants are susceptible to BSE, with pathogenesis and clinical signs that are not readily distinguishable from scrapie (9-12). Additionally, the fact that small ruminants were exposed to BSE-contaminated food before the exclusion of meat and bone meal from ruminant feedstuffs led to the possibility that sheep and goats on commercial farms could be affected by BSE that could be misdiagnosed as scrapie (13,14). The response to this potential risk was the implementation of extensive statutory active surveillance, elimination, and breeding for resistance programs in the European Union (EU).

In 2005, as part of a review of historical TSE-positive cases of sheep and goats in France, a specimen from a goat slaughtered for human consumption in 2002 was reported to be "indistinguishable from a BSE isolate on the basis of all identification criteria available." (15). In response to this report, 2 retrospective studies were initiated in the United Kingdom to analyze archived samples from goat cases that were initially diagnosed as scrapie (16,17). Because only fixed material was available, both studies had to use differential immunohistochemical analysis (D-IHC), a technique that can discriminate scrapie from experimentally induced BSE in sheep (18). These studies identified a single case, originally diagnosed in 1990 as scrapie, that had a D-IHC signature indistinguishable from BSE (16).

Given the wide phenotypic variance of scrapie in sheep and our limited knowledge of this variance in goats, the D-IHC result on its own was insufficient for an unequivocal diagnosis. In accordance with EU regulation 36/2005 (19), the case was referred to the EU Reference Laboratory Strain Typing Expert Group, which recommended further investigation by bioassay.

Bioassay is conventionally undertaken by using unfixed tissues to prepare inocula. Much historical tissue is available only as formalin fixed or formalin fixed and paraffin wax embedded. TSE infectivity persists in such material but with a lower infectious titer than with unfixed frozen tissue (20). However, the potential effects on biological activity, and therefore strain characterization, of fixation and processing are unknown. Thus, further investigation of this case required an extensive panel of controls. We report the results of the bioassay analysis and confirm the diagnosis of BSE in a goat in the United Kingdom.

-- Communicated by: Terry S Singeltary Sr

[Interested readers should access the original text via the source URL above to view the full text an the references cited. The following has been extracted from the Discussion.

"The 2 cases of naturally occurring BSE in small ruminants, the one reported here and the one identified in France (15), occurred in different countries, during different time periods, and before strict BSE control measures were fully implemented. Therefore, the most likely origin of these 2 cases would be exposure to BSE-contaminated food supplements. Although in France goats constitute 14.3 percent of the small ruminant population, in the United Kingdom they account for only 0.3 percent of small ruminants. It is intriguing, therefore, that the only naturally occurring BSE cases in small ruminants in France and particularly in the United Kingdom were detected in goats and not in sheep, although they have also been exposed to contaminated food supplements. A possible explanation could be that goats are generally managed more intensively than sheep and thus might have been exposed to higher doses of the infectious agent because of the more frequent use of concentrates in intensive dairy farming. Similar observations have been reported in cattle, in which the incidence of BSE was significantly higher in dairy herds and in which management is much more intensive than in beef herds (34). In the United Kingdom, most of the commercial goat herds are kept for milk production in a typically

intensive production system, similar to dairy cattle.

The BSE case we have confirmed was 1 of 26 historic goat samples examined in the United Kingdom collected during 1984 -2002 (16,17). Since 1993, scrapie in goats has been a notifiable disease in the United Kingdom, and since 2005, samples from all suspected cases of TSE in small ruminants are required to be tested for BSE-like features by using Western blotting (WB) (19). No BSE cases have been identified, although an intermediate case in a goat was reported and is under investigation by bioassay for final resolution (35,36). This screening of brain samples from all small ruminant cases offers reassurance that BSE is not present in the contemporary small ruminant population. However, application of WB to sheep experimentally co-infected with BSE and scrapie detected only the scrapie agent (37). Also, in contrast to BSE, where infectivity is mainly confined to the nervous system, in small ruminants the BSE agent is widely distributed in peripheral tissues and can be transmitted horizontally (11,38). Therefore, feed ban measures alone would be inadequate to control a BSE outbreak in small ruminants. Also, it would be impossible to prevent BSE from entering the human food chain through consumption of food products derived from small ruminants.

Because TSEs in goats are still a problem, particularly in Mediterranean countries, our data suggest that extensive surveillance and breeding schemes must remain in place to prevent a BSE outbreak in small ruminants and to safeguard public health. This report also highlights several issues regarding the use of mouse bioassay to identify TSE strains. As governing bodies seek confirmation of equivocal cases that are identified worldwide, they must be aware of the limitations, cost, and timescale demands of confirming such cases." - Mod.CP]

[See http://healthmap.org/r/1INY for the interactive HealthMap/ProMED map of the United Kingdom. - Mod.MPP]

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[3]
South Korea: 1st case iCJD Date: Wed 30 Nov 2011 Source: The Chosun Ilbo [edited]
http://english.chosun.com/site/data/html_dir/2011/11/30/2011113001421.html

A woman has died of iatrogenic Creutzfeldt-Jakob disease (iCJD), becoming the 1st confirmed victim of the terminal disorder in Korea. She is believed to have been infected during a subdural transplant surgery in the mid-1980s. The disease is not to be confused with the similarly named variant Creutzfeldt-Jakob disease, the human form of mad cow disease, though both lead to disintegration of the brain.

iCJD occurs when the prion-infected tissue of one person infects another. It has nothing to do with variant CJD, which infects humans who have eaten beef from infected cattle, health authorities said. According to the Korea Centers for Disease Control and Prevention [KCDC] on Tuesday, [29 Dec 2011] the 54-year-old woman suffered from meningioma, a disease that causes water to fill a certain area of the brain. In 1987 surgery was performed to remove tumors and the dura mater that surrounded them. She had a subdural transplant over the surface of her brain with Lyodura, a German-manufactured piece of artificial dura mater that was produced in the 1980s, [see comment below] to cover the affected area.

She had since lived without any special symptoms. But since 2010 she suffered from sensory disorders in the face and feet, movement disorder, and brain disease symptoms like seizures. She rapidly deteriorated and died in November last year [2010], 5 months after the iCJD symptoms began, the KCDC added. CJD normally has an incubation period of 20 to 30 years.

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

[See http://healthmap.org/r/1BIE for the interactive HealthMap/ProMED map of South Korea. - Mod.MPP]

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[4]
South Korea: 2nd case iCJD Date: Fri 8 Dec 2011 Source: Yonhap News Agency [edited]
http://english.yonhapnews.co.kr/business/2011/12/08/51/0502000000AEN20111208002700320F.HTML

South Korea's health authorities on Thursday [7 Dec 2011] confirmed the country's 2nd case of Creutzfeldt-Jakob disease (CJD), a degenerative neurological disorder. A 48-year-old man was diagnosed with iatrogenic CJD (iCJD) on Wednesday [6 Dec 2011], according to the Center for Disease Control [KCDC].

The report of the country's 2nd-ever iCJD case comes after a 54-year-old woman was found last month [November 2011] to have died from the same disease that is often transmitted by the use of defective prion proteins found in surgical tissue graft products. The woman had received brain surgery using Lyodura, a tissue graft product, some 23 years ago. The KCDC said the man in the latest case had also received Lyodura during brain surgery in 1988.

This form of CJD [iatrogenic CJD] has an incubation period of more than 20 years but once symptoms occur, death usually takes place within a year. CJD is the most common of the so-called human prion diseases with one person in every 1 million diagnosed each year worldwide. It is an invariably fatal illness with death occurring after the onset of dementia, hallucinations, coordination dysfunction, and seizures.

The animal form of the disease is called bovine spongiform encephalopathy (BSE) which is commonly called mad cow disease. BSE also leaves holes in the brain that resemble a sponge.

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

[These 2 deaths have been attributed to the use of Lyodura, a medical product derived from cadavers and used in

neurosurgery. Lyodura was introduced in 1969 as a product of B Braun Melsungen AG, a leading hospital supply company based in Germany. The product was used as a quick and effective patch material for surgery on the brain. It was a section of freeze-dried tissue which could be stored for extended periods on hospital shelves and could be made ready for use simply by soaking it in water for a few minutes.

The raw material for Lyodura was the dura mater of a human cadaver. The tissue would usually be harvested during an autopsy and then sold to the manufacturer. After neurological diseases were linked to use of Lyodura, an investigation determined that the manufacturer had obtained the donor tissue by black market methods. Autopsy staff would remove the tissue from cadavers, regardless of whether the deceased's family had agreed to an autopsy or not, and sell it in quantity to representatives of the manufacturer. Due to this illegal method of collection, no record of patient history accompanied the tissue to production.

Large quantities of the harvested tissue would be mass sterilized in a heated vat. The tissue would then be freeze dried and packaged for purchase. The manufacturer believed that its sterilization procedure was sufficiently powerful to render any tissue harmless and was therefore unconcerned about cross-contamination from CJD-containing tissue to other tissue in the same sterilization vat. It is now believed that almost all Lyodura product was tainted with Creutzfeldt-Jakob disease [prion] through this process. More than 70 CJD-related deaths in Japan occurred as a consequence of use of the Lyodura product. (From http://en.wikipedia.org/wiki/Lyodura.

An account of Lyodura use and the risk of iatrogenic Creutzfeldt-Jakob disease in Australia, by FJ Brooke, S Boyd, GM Klug, CL Masters, and SJ Collins has been published in the Medical Journal of Australia; 180(4), 177-81, 2004. The following information has been extracted from the Introduction to this publication:

"Although infectiousness is a feature of Creutzfeldt-Jakob disease (CJD), only a small proportion of cases are linked to transmission through healthcare provision. As of January 2003, over 120 cases of CJD associated with use of human cadaveric dura mater had been recognised worldwide; almost all were associated with the commercial product Lyodura. Most cases (97) have occurred in Japan, giving an overall risk estimate of around 1 per 2268 patients treated with Lyodura (0.04 percent) in that country. In Australia, 5 cases of CJD have so far been linked to Lyodura, but, given the protracted tails of previous epidemics of transmissible spongiform encephalopathies, further cases are possible. Results of surveys of Lyodura use in Australia are incomplete, but information from the manufacturer suggests that 2208-2478 sheets of Lyodura may have been used here. This use translates to a relatively high incidence of Lyodura-associated CJD, with current overall rates appearing around 5 times higher than those reported in Japan; reasons for this difference are unclear.

Creutzfeldt-Jakob disease (CJD) is a fatal, transmissible, neurodegenerative disorder belonging to the group known as the transmissible spongiform encephalopathies (TSEs). CJD can occur without explanation (sporadic), secondary to mutations in the prion protein gene (PRNP), or as a complication of medical treatment using contaminated therapeutic agents or equipment (iatrogenic). Although corneal grafts and neurosurgical equipment have been associated with disease transmission, the most common causes of iatrogenic CJD have been treatments involving human-derived cadaveric pituitary hormones or dura mater.

The 1st identified case of CJD in a dura mater recipient was reported in the United States in early 1987. In response, the US Food and Drug Authority issued a safety alert in April 1987, seeking immediate discontinuation of use of the identified dura mater batch (Lyodura batch no. 2105). A 2nd patient with CJD linked to Lyodura was detected in New Zealand in 1988, but the specific batch could not be identified. This has remained a frequent difficulty when tracing contamination sources.

As of January 2003, over 120 CJD cases related to dura mater use had been detected globally, with 97 in Japan. These cases were predominantly associated with Lyodura, a commercial product produced since 1969 by B Braun Melsungen AG Only a few reports suggest the possibility of CJD after use of dura mater from other commercial or non-commercial sources.

Lyodura consists of lyophilised, irradiated human dura mater sourced post mortem. Additional processing with immersion in a solution of 1M sodium hydroxide was instituted in 1987, with a noticeable reduction in Lyodura-related cases thereafter. Lyodura has been used in a number of countries, including Australia, Japan, Canada, the United States, and the United Kingdom, mainly in neurosurgery, but also in orthopaedic, otological, dental, urological, gynaecological, and cardiac procedures.

Most cases of CJD associated with dura mater have occurred in Japan. In 1996, in response to the growing incidence, a survey was undertaken of Lyodura use in almost 3000 Japanese healthcare institutions. This estimated that up to 100 000 people received Lyodura grafts between 1983 and 1987, and up to 220 000 between 1979 and 1991 (out of a total of 260 000 who received dura mater grafts). Use of these grafts greatly declined after 1991 but may have continued until 1997. Assuming that all cases of CJD associated with dura mater were a consequence of Lyodura use, the overall risk of Lyodura-associated CJD in Japan is approximately 0.04 percent." (For further information, go to http://www.mja.com.au/public/issues/180_04_160204/bro10566_fm.html).

The extreme resistance of the CJD infectious agent to inactivation by irradiation and severe chemical treatments in the Lyodura process confirms the singular nature of prions. - Mod.CP]

[See http://healthmap.org/r/1BIE for the interactive HealthMap/ProMED map of South Korea. - Mod.MPP]

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[5]
Switzerland: BSE Date: Fri 16 Dec 2011 Source: Prionics AG, e-scope newsletter [edited]
http://escope.prionics.com/issue/2011-december-4/

In spring 2011, 2 new cases of BSE were discovered in Switzerland [see ProMED-mail posting Prion disease update 2011 (10) 20111107.3317]. Both cases were detected using the Prionics(R)-Check BSE tests. A report has now been published showing that these cases represent a novel type of BSE. What are the consequences of these new BSE cases?

After a period of 4 years without BSE positive cows, in spring this year [2011] Switzerland was shaken by the discovery of 2 new BSE cases detected only one month apart from each other. The cases appeared in different areas of Switzerland and involved animals aged 8 and 15 years, which were tested with the Prionics(R)-Check BSE tests as part of the active disease surveillance program. Bettina Bernhard, Head of the Prionics diagnostic laboratory reported that: "It was the 1st time in 4.5 years that we had found a BSE positive sample in our laboratory. Based on the results from the Prionics(R)-Check WESTERN, we immediately saw that the fingerprint of the prion protein was not that of the classical BSE cases we have detected before. We then informed the Swiss National Reference Laboratory and veterinary authorities and the positive result was confirmed with the Prionics(R)-Check PrioSTRIP."

Novel type of BSE?

BSE cases that differ from the classical BSE strain have been detected before, however, with low incidence. These atypical strains, designated BASE/L-BSE and H-BSE, were first reported in 2004 in Italy and France. Both strains were detected as part of routine surveillance using the Prionics(R)-Check WESTERN and ELISA tests. The recent publication by Torsten Seuberlich of the Swiss National and OIE [World Organisation for Animal Health] Reference Laboratories for BSE and Scrapie and his colleagues, is showing that these 2 Swiss cases not only differ from classical BSE, but also from the atypical BSE cases found in other countries. It appears that the 2 BSE cases detected in Switzerland seem to represent a novel type of atypical BSE. Dr Seuberlich explains: "We are now undertaking further investigations into these 2 cases and until there is more clarity, surveillance should continue to be carried out at a high level and disease awareness should be increased. Furthermore, we have to ensure that diagnostic techniques are applied that identify such cases."

Continued vigilance needed

Whereas consumption of meat from cows affected by classical BSE has been associated with vCJD, the public health hazard from atypical BSE is unclear. Little is known about its origin and whether it can be transmitted to other animals. These cases show, however, that BSE has not been completely eradicated and that the disease can continue to occur even with current preventive measures (such as the meat-and-bone meal ban) in place. The appearance of new strains of the prion protein could also indicate that BSE is still evolving. Continuous monitoring will be needed to keep these new strains under surveillance.

-- Communicated by: Terry S Singeltary.Sr

[[See http://healthmap.org/r/1AFv for the interactive HealthMap/ProMED map of Switzerland. - Mod.MPP]

See Also

2011

Prion disease update 2011 (11) 20111207.3543
Prion disease update 2011 (10) 20111107.3317
Prion disease update 2011 (09) 20111003.2983
Prion disease update 2011 (08) 20110905.2710
Prion disease update 2011 (07) 20110810.2423
Prion disease update 2011 (06) 20110607.1736
Prion disease update 2011 (05) 20110505.1393
Prion disease update 2011 (04) 20110406.1066
Prion disease update 2011 (03) 20110309.0764
Prion disease update 2011 (02) 20110211.0473
Prion disease update 2011 (01): correction 20110112.0140
Prion disease update 2011 (01) 20110110.0119
2010

Prion disease update 2010 (11) 20101206.4364
Prion disease update 2010 (10) 20101105.4008
Prion disease update 2010 (09) 20101006.3622
Prion disease update 2010 (08) 20100911.3285
Prion disease update 2010 (07) 20100809.2720
Prion disease update 2010 (06) 20100706.2248
Prion disease update 2010 (05) 20100507.1488
Prion disease update 2010 (04) 20100405.1091
Prion disease update 2010 (03) 20100304.0709
Prion disease update 2010 (02) 20100205.0386
Prion disease update 2010 20100107.0076]
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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	Molecular Psychiatry advance online publication 4 October 2011; doi: 10.1038/mp.2011.120	公表国：米国	
販売名（企業名）	－					

研究報告の概要

最も一般的な老年認知症であるアルツハイマー病（以下「AD」）は、折り畳み構造の異常なアミロイドベータ（以下「Aβ」）の脳内蓄積と関連している。Aβの折り畳み異常やオリゴマー形成がADにおける誘発イベントであるという有力な証拠があるものの、Aβ蓄積が始まるメカニズムは分かっていない。
本研究では、ADがプリオン様のメカニズムで伝播可能かどうかを検討するため、全く突然変異のないヒトアミロイド前駆体蛋白質を発現する遺伝子を導入された、ヒト蛋白配列を持つAβは産生するが、Aβ沈着が出現することはないマウスを用いた。
臨床的及び病理学的に確認されたAD患者（90歳）由来の脳抽出液を165日齢マウスの海馬内（両側）に脳内接種し、対照としてアミロイド沈着のなかった若年者（163日齢）の脳抽出物を接種した。
接種後285、450、585日目に屠殺し観察したところ、AD脳抽出液を注射したマウスでは抗Aβ抗体による免疫染色でAβ凝集体がみられ、Aβ沈着物は接種後に時間とともに次第に増加し、接種部位から遠く離れた脳領域でAβ病変が認められたが、対照マウスではAβ沈着がみられなかった。
このことにより、Aβ凝集体を含む脳抽出液を接種することにより、接種しなければその自然のままの一生でこのような変化を起こすことのないマウスでADの神経病理学的特徴の一部を誘発できることが証明された。
今回の結果は、ADに関連する典型的な脳異常の一部は、折り畳み構造の異常な蛋白質の増殖を介したプリオン様の疾病伝播メカニズムによって引き起こされている可能性を示唆している。
これらの研究成果は、AD発症に関与する分子レベルのメカニズムの理解に大きく影響し、本症の新たな予防法や介入法の開発に寄与する可能性がある。

使用上の注意記載状況・その他参考事項等

20

報告企業の意見	今後の対応
マウスを用いた脳内接種実験から折り畳み構造の異常なアミロイドβによりアルツハイマー病が伝播する可能性があるとの報告である。アルツハイマー病は最も一般的な老年認知症で90％以上が散発的に発症していることから原因は不明であり、今後の究明に留意したい。	今後ともアミロイドβによるアルツハイマー病伝播に関する情報に留意していく。

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ORIGINAL ARTICLE

De novo induction of amyloid-β deposition *in vivo*

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Alzheimer's disease (AD), the most common type of senile dementia, is associated to the build-up of misfolded amyloid-β (Aβ) in the brain. Although competing evidences indicate that the misfolding and oligomerization of Aβ is the triggering event in AD, the mechanisms responsible for the initiation of Aβ accumulation are unknown. In this study, we show that Aβ deposition can be induced by injection of AD brain extracts into animals, which, without exposure to this material, will never develop these alterations. The accumulation of Aβ deposits increased progressively with the time after inoculation, and the Aβ lesions were observed in brain areas far from the injection site. Our results suggest that some of the typical brain abnormalities associated with AD can be induced by a prion-like mechanism of disease transmission through propagation of protein misfolding. These findings may have broad implications for understanding the molecular mechanisms responsible for the initiation of AD, and may contribute to the development of new strategies for disease prevention and intervention.
Molecular Psychiatry advance online publication, 4 October 2011; doi:10.1038/mp.2011.120

Keywords: amyloid; prion; protein misfolding; disease transmission

Introduction

Alzheimer's disease (AD) is the most common type of senile dementia, mainly affecting individuals over 65 years old. Disease manifestation is characterized by progressive impairment of memory and cognition, principally produced by synaptic dysfunction and neuronal loss. The etiology of the disease is currently unknown and it is a matter of great interest, as more than 90% of AD cases arise sporadically.

Cerebral accumulation of misfolded aggregates composed of the amyloid-β (Aβ) protein and hyperphosphorylated tau have long been associated to the disease.[1,2] Compelling evidence suggest that misfolding and aggregation of Aβ might be the triggering event, which is responsible for inducing the subsequent brain abnormalities.[3–4] However, it is currently unknown why Aβ, which is a naturally produced protein, begin to misfold and aggregate in the brain. Interestingly, brain accumulation of misfolded protein aggregates is a common feature of several neurodegenerative diseases, including, besides AD, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and prion disorders.[4] These diseases can have sporadic or inherited origin, except in case of prion diseases, in which the pathology can be transmitted by infection.[5] Strikingly, the infectious agent responsible for prion diseases is composed exclusively by the misfolded and aggregated form of the prion protein that has the surprising ability to propagate the disease through an infection process, which involve the auto-catalytic conversion of the normal host prion protein.[6]

Extensive *in vitro* studies have shown that disease-associated misfolding and aggregation of proteins follow a seeding-nucleation model in which the formation of oligomeric seeds is a slow and rate-determining event.[7,8] In this model, protein misfolding and aggregation is greatly accelerated by addition of pre-formed seeds. The seeding/nucleation mechanism offers a plausible explanation for the transmissibility of prion diseases by infectious misfolded prion protein and predicts that other misfolded proteins have the potential to transmit the disease through a prion-like infectious process.[9]

Exciting recent studies have reported that Aβ deposition can be accelerated *in vivo* by injecting AD brain homogenates carrying Aβ aggregates.[9–11] These studies have been done in transgenic mice expressing a mutant form of the human amyloid precursor protein (APP), which spontaneously develop AD-like neuropathological alterations. Thus, the

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induction of Aβ deposition observed in these studies only represents an acceleration of few months of the spontaneous process that was set to occur by introduction of the mutant gene. This is drastically different from the *bona fide* infectious process produced by prions in animals, which, without inoculation with the misfolded protein, would not spontaneously develop the disease. The goal of this study is to demonstrate that similar to prion diseases, an AD-like pathology can be induced in animal models that naturally do not develop amyloid aggregates during their lifespan.

Materials and methods

Preparation of human brain homogenates
The AD brain sample used in this study was obtained from the cerebral cortex of a 90-year-old woman with clinical diagnosis of AD, which was confirmed by a post-mortem histopathological analysis. Control sample was obtained from a 163-day-old male, who did not have any detectable amyloid deposits in the brain (data not shown). Because of the existence of misfolded Aβ aggregates in the brain of elderly people, a young control was chosen. Samples were taken within 2 h post-mortem and frozen at −70 °C. Brain samples (cortex) were homogenized at 10% in phosphate-buffered saline plus a protease inhibitor cocktail (Roche, Mannheim, Germany), vortexed for 2 min and sonicated for 3 s at 60 units of amplitude in a Sonics—Vibra Cell manual sonicator (Newtown, CT, USA). Resulting homogenates were vortexed again, and centrifuged at 3000 *g* for 5 min to remove tissue debris. Supernatant was aliquoted and stored at −70 °C until use.

Intracerebral inoculations into mice
For our studies, we used mice that express the human wild-type *APP* gene (*HuAPPwt*).[12] Heterozygous transgenic mice were used in these experiments. Groups of six mice were injected when 165 days old, with 2 µl of 10% brain homogenates. Injections were performed stereotaxically in the hippocampus (both hemispheres), using the following coordinates: anterioposterior (AP) = −1.8 mm; mediolateral (ML) = ± 1.8 mm; dorsoventral (DV) = −1.8 mm. Animals were killed at 30, 90, 285, 450 or 585 days-post injection (dpi).

Immunohistochemistry
Paraffinized tissues were deparaffinized with three 5-min changes of xylene. Samples were later rehydrated and heated for 10 min at 95 °C in citrate buffer. Cold samples were covered with 3% H_2O_2 for 5 min. Tissue sections were covered with M.O.M. blocking reagent working solution (Vector Labs, Burlingame, CA, USA) and 200 µl of 4G8 antibody (1:1000) were used to cover each slide overnight at 4 °C. Slides were treated with biotinylated anti-mouse secondary antibody (1:200) and incubated for 30 min at room temperature. Later, samples were rinsed and incubated with Vectastain ABC Reagent (Vector Labs) for 30 min. Tissue sections were covered with diaminobenzidine at room temperature, and after gentle washing with water, they were covered with Mayer's Hematoxylin. Finally, samples were dehydrated with ethanol and xylene and mounted with Super Mount (Innogenex, San Ramon, CA, USA) and cover slips. Reactive astrocytes were measured by a similar procedure, but using anti-glial fibrillary acidic protein (GFAP) antibody. Samples were analyzed using a Leica DMI6000 B microscope (Wetzlar, Germany) and subjected to image analysis using the ImagePro software (Silver Spring, MD, USA).

Thioflavin S staining
Dewaxed and hydrated tissue slides were placed in 0.1% aqueous Thioflavin S (ThioS) solution. Samples were later dehydrated with 95% ethanol, followed sby two changes of 100% ethanol, and cleared with xylene. Tissue slides were finally covered with resinous mounting medium. Final specimens were stored in a dark place at 4 °C until visualized. Samples were analyzed using a Leica DMI6000 B microscope.

Western blot
Proteins were fractionated by electrophoresis using 4–12% sodium dodecyl sulphate-polyacrylamide gels (SDS-PAGE; Invitrogen, Carlsbad, CA, USA), electroblotted into Hybond-ECL nitrocellulose membrane (Amersham Biosciences, Little Chalfont, UK) and probed with the 4G8 antibody (1:3000) (Covance, Princeton, NJ, USA). The immunoreactive bands were visualized by ECL Plus Western blotting detection system (GE Healthcare, Little Chalfont, UK), using a UVP Bioimaging system EC3 apparatus (UVP, Upland, CA, USA).

Statistical analysis
Results of GFAP burden were analyzed by one-way analysis of variance followed by the Tukey's multiple comparison post-test using the Graph Pad Prism 5.0 software (La Jolla, CA, USA).

Results

To test whether AD can be transmissible by a prion-like mechanism, we used a transgenic mice that expresses human APP without any mutation (here termed HuAPPwt).[12] These animals produce Aβ bearing the human sequence of the protein, but do not develop Aβ deposits during their whole lifespan (Figure 1a). This is significantly different from other AD transgenic mice models such as the widely-used tg2576, which expresses the human APP harboring the Swedish mutation; as a result, these mice show detectable Aβ aggregates (starting at approximately 9 months), which become very prominent by 15 months of age (Figure 1b).[13]

We injected intracerebrally the brain extracts from a clinically and pathologically confirmed AD patient (Figure 2) into the hippocampus (bilaterally) of the

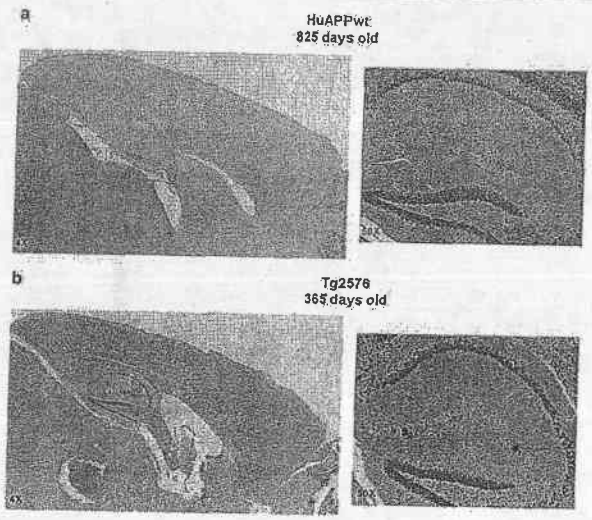

Figure 1 Human wild-type amyloid precursor protein (APP) gene (*HuAPPwt*) mice do not develop amyloid-β (Aβ) deposits spontaneously. (a) Brain coronal sections from an 825-day-old HuAPPwt mouse were analyzed by immunohistochemistry using an anti-Aβ antibody. The pictures were taken with two different magnifications (× 4 and × 50) to show the entire slide and a more magnified section of the hippocampal area. (b) Equivalent brain sections of a 365-day-old tg2576, harboring substantial amyloid deposits. Image magnification is noted at the bottom-left corner of each picture.

165-day-old HuAPPwt mice. As control, we injected brain preparations of a young individual that did not carry amyloid deposits (data not shown). The amount and aggregation state of Aβ in each of the inoculated samples were considerably different as analyzed by histology and Western blotting (Figure 2). To follow protein deposition over time, we killed HuAPPwt mice injected with AD and control brain extracts at 285, 450 and 585 dpi. Interestingly, only HuAPPwt mice injected with AD brain homogenates showed detectable Aβ aggregates by immunostaining with an anti-Aβ antibody (4G8; Figure 3a). In contrast, none of the mice injected with control brain homogenate presented any Aβ deposit at the same ages and times after injection (Figure 3a, left panels). Staining with ThioS, a dye that specifically binds to amyloid structures,[14] only showed positive reactivity in some of the brains (3/7) from the group inoculated with AD material, which was killed at 585 dpi (Figure 3a, right panels). None of the samples in any other group were ThioS positive. These data suggest that 4G8-positive signals in mice killed at 285 and 450 dpi correspond to diffuse Aβ aggregates, which are considered precursors of mature ThioS-positive AD-senile plaques.[15] To rule out that the aggregates observed do not correspond to the original inocula, but to *de novo* deposition of endogenous Aβ, we injected AD brain extracts into HuAPPwt mice and we killed them at 30 and 90 dpi. Aβ deposits were not detected in any of these mice by immunohistochemical analysis (Figure 3b).

Then, we compared the morphological and staining features of Aβ deposits induced by inoculation of AD brain homogenates into HuAPPwt with those observed spontaneously in the brain of tg2576 mice, which is probably the best characterized and most widely used transgenic mouse model of AD. When comparing the aggregates generated in HuAPPwt mice with those in tg2576, we observed a similar distribution of deposits, mostly located in hippocampus and cortex. Morphologically the Aβ deposits were also similar (Figure 4), albeit a bit smaller and less compact in HuAPPwt mice, suggesting again that they are in the way to grow into large mature plaques. Aggregates were also similar in ThioS staining, and in both cases, they are associated with an extensive astroglial proliferation (Figure 4). Tau staining with AT8 anti-phospho-Tau was negative in all samples (data not shown).

Finally, to assess the progression of Aβ deposition in injected mice, we subjected 4G8-stained slides to image analysis. The percentage of mice harboring detectable Aβ aggregates increased with time, reaching 100% at 585 dpi (Figure 5a). We also measured the

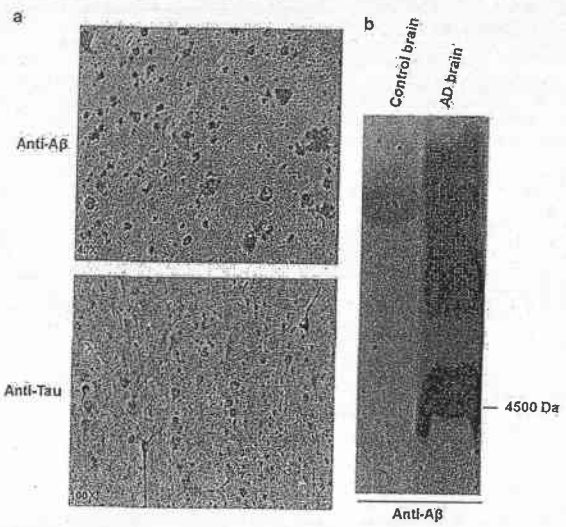

Figure 2 Histological and biochemical characterization of brain used as inocula. (a) Sections of the entorhinal cortex of the Alzheimer's disease (AD) brain used to inject human wild-type amyloid precursor protein (APP) gene (*HuAPPwt*) mice was analyzed by immunohistochemistry with 4G8 anti-amyloid-β (Aβ) antibody and 4855 anti-phospho-Tau antibody. Pictures showing Aβ and tau staining were obtained at ×40 and ×100 magnifications, respectively. (b) Western blot showing Aβ burden in control and AD brain homogenates used for inoculation in HuAPPwt mice. Each line represents the signal equivalent to 10 µl of a 10% brain homogenate.

amyloid load by counting the number of Aβ aggregates (Figure 5b) and measuring the area occupied by amyloid deposits (Figure 5c) in hippocampus (site of injection) and cortex. Both the number and area of Aβ deposits increased significantly with time in the two brain regions analyzed (Figures 5b and c). The fact that Aβ deposits were also detected far from the injection site (cortex) suggests that the seeding activity can diffuse in the brain. The extent of astrogliosis measured by the area stained by GFAP antibody was also higher in the animals injected with AD brain homogenate (Figure 5d).

Discussion

Recent studies have proposed that other protein-misfolding disorders besides prion diseases could be transmissible *in vivo*, following the principles posited by the heretical prion mechanism.[2,16–20] If this hypothesis proves to be correct, it will open a novel view on the biology of misfolded protein aggregates and the origin of protein-misfolding disorders, which will have broad-ranging implications for understanding the disease mechanisms and development of new strategies for disease prevention and intervention. In this scenario, our results demonstrate that the administration of brain homogenates containing Aβ aggregates can induce some of the neuropathological characteristics of AD in animals, which, without inoculation, will not develop these alterations during their natural lifespan. This experimental paradigm mimic, at least with respect to Aβ aggregation, a situation in which a normal person will live his entire life without developing AD abnormalities, unless the process is induced by exposure to material containing seeds of preformed Aβ aggregates.

Our findings suggest that in an experimental setting, misfolded Aβ aggregates can behave in a similar way as infectious prions. Indeed, as in prion diseases, our data shows that the quantity and degree of maturation of the protein deposits increases with age and that the seeding activity can spread to areas other than the site of injection. At this time, we do not know which of the various Aβ-misfolded species are most efficient in triggering Aβ aggregation, but *in vitro* experiments suggest that soluble, small oligomers may be the key factors in seeding Aβ misfolding and aggregation (Supplementary Figure 1). Long incubation periods are a key feature of infectious prion diseases. In fact, incubation periods in Kuru, a human prion disease, has been reported to reach more than

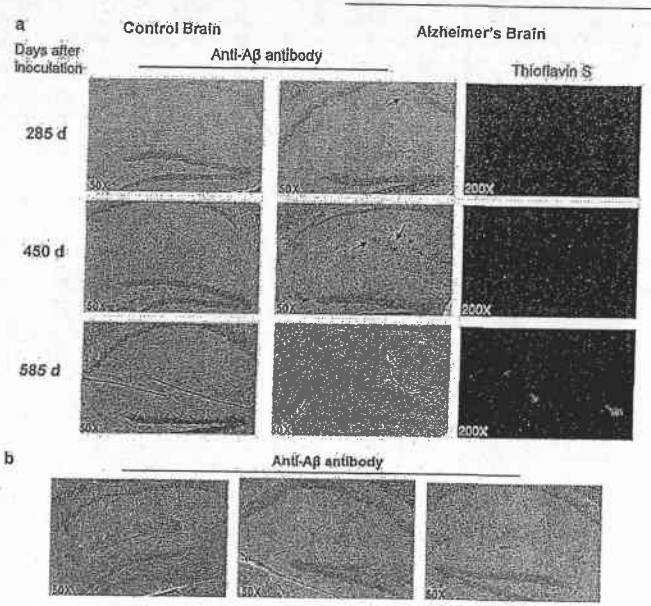

Figure 3 Human wild-type amyloid precursor protein (APP) gene (*HuAPPwt*) mice inoculated with Alzheimer's disease (AD) brain extracts develop cerebral amyloid-β (Aβ) deposits. (a) HuAPPwt mice injected with brain homogenates from a healthy individual (left panel) or an AD patient (middle and right panels) were killed at different times after injection. Brain slides were stained with anti-Aβ (4G8) antibody (left and middle panels) or Thioflavin S (ThioS; right panels). Pictures correspond to representative slides of all animals analyzed. Arrows point to Aβ deposits typically observed in the inoculated mice. (b) Representative slides of the hippocampus of three different mice killed 30 days-post injection (dpi), where no Aβ deposits were detected by immunohistochemistry (4G8). Similar results were obtained in animals killed 90 dpi (data not shown). Picture magnification is indicated in the bottom-left corner.

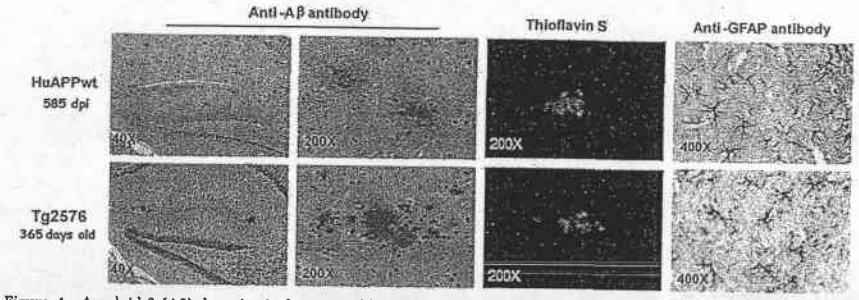

Figure 4 Amyloid-β (Aβ) deposits in human wild-type amyloid precursor protein (APP) gene (*HuAPPwt*; Alzheimer's disease (AD)-injected 585 days post-injection (dpi)) and Tg2576 (365 days old) mice. The figure shows immunohistochemical staining with the 4G8 monoclonal anti-Aβ antibody at two different magnifications (×40 and ×200), Thioflavin S (ThioS) staining (×200) and astrogliosis detected by anti-GFAP staining (×400). The images correspond to representative pictures from the hippocampus (Aβ and ThioS staining) or cortex (GFAP staining) of the HuAPPwt group inoculated 585 days before, with AD brain homogenate and 12-month-old tg2576 mice that spontaneously developed the lesions.

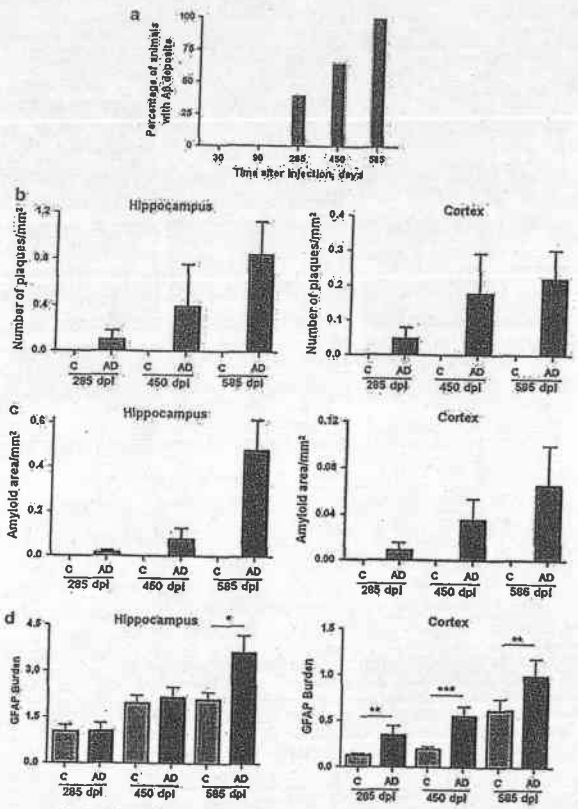

Figure 5 Progressive accumulation of amyloid-β (Aβ) deposits in the brain of HuAPPwt mice. Brain slides from HuAPPwt mice injected with normal (C) or AD brain homogenate (AD) were analyzed for Aβ deposition after 4G8 immunostaining in hippocampus (including the dentate girus, subiculum and hippocampal regions CA1, CA2 and CA3). (a) Percentage of HuAPPwt mice exhibiting Aβ deposits in groups killed at different times after injection. (b) The number of Aβ aggregates in hippocampus and cortex, was estimated by counting number of 4G8-positive deposits per slide, and is expressed as number of Aβ deposits per mm². (c) The load of amyloid was estimated by the area stained by 4G8 antibody in relation with the total area analyzed. (d) The extent of astrogliosis was estimated by calculating the area stained by GFAP antibody in relation with the total area analyzed. All groups consisted of six animals, except the control in the 285 days-post injection (dpi) in which one animal died from an unrelated problem. Data was analyzed by one way analysis of variance, and in all cases, the differences between animals injected with control or AD brain homogenate were significant with $P<0.001$. For the GFAP burden results, individual differences were evaluated by using the Tukey's multiple comparison post test (*$P<0.05$; **$P<0.01$; ***$P<0.001$).

four decades.[21] These long incubation periods complicate epidemiological tracking of an infectious process. Our findings added to recent experimental evidences suggesting prion-like propagation of neuropathological abnormalities in various common neurodegenerative diseases,[9–11,22–24] may shed new light regarding the etiology of diseases associated with accumulation of misfolded protein aggregates. It remains to be studied whether at least a proportion of AD cases could be initiated through a transmissible prion-like mechanism under natural conditions in humans.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)

B　個別症例報告概要

○　総括一覧表
○　報告リスト

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（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18回	18- 1	10021881/ 感染症および寄生虫症 /Infections and infestations	10020161/ HIV感染 /HIV infection	イギリス	男性	不明	不明	不明	症例報告	外国製品	11000037	2011/9/20	14.0	
第18回	18- 1	10021881/ 感染症および寄生虫症 /Infections and infestations	10019744/ C型肝炎 /Hepatitis C	イギリス	男性	不明	不明	不明	症例報告	外国製品	11000037	2011/9/20	14.0	
第18回	18- 1	10021881/ 感染症および寄生虫症 /Infections and infestations	10019731/ B型肝炎 /Hepatitis B	イギリス	男性	不明	不明	不明	症例報告	外国製品	11000037	2011/9/20	14.0	

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
100505	16-Mar-12	111001	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	無	有	無
100506	16-Mar-12	111002	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	無	有	無

資料３－１

供血者からの遡及調査の進捗状況について
（目次）

事　務　連　絡
平成２４年４月６日

日本赤十字社血液事業本部　御中

薬事・食品衛生審議会血液事業部会事務局
厚生労働省医薬食品局血液対策課

供血者からの遡及調査の進捗状況について

標記につきましては、平成２４年２月７日付け血安第４１号にて貴社血液事業本部長より資料の提出があり、これを平成２３年度第４回血液事業部会運営委員会に提出したところです。今般、平成２４年５月２８日（月）に平成２４年度第１回血液事業部会運営委員会が開催されることとなりましたので、下記の事項について改めて資料を作成いただき、平成２４年４月２０日（金）までに当事務局あて御提出いただきますようお願いいたします。

記

１．「供血者の供血歴の確認等の徹底について」（平成１５年６月１２日付け医薬血発第 0612001 号）に基づく遡及調査に係る以下の事項
（１）遡及調査実施内容
①　調査の対象とした献血件数
②　上記①のうち、調査の対象とした輸血用血液製剤の本数
③　上記②のうち、医療機関に情報提供を行った本数
（２）個別 NAT 関連情報
①　（１）①のうち、個別 NAT の結果が陽性となった献血件数
②　上記①のうち、医療機関へ供給された製剤に関する報告件数
③　上記②のうち、受血者情報が判明した件数
④　上記③のうち、医薬品副作用感染症報告を行った件数

２．資料の作成に当たっての留意事項
①　本数又は件数については、病原体別及びその合計を明らかにすること。また、上記（１）の③及び（２）の①～③については、対象期間ごとに本数又は件数を記載すること。
②　本数又は件数については、平成２４年２月７日付け血安第４１号の提出時において判明したものに、その後の遡及調査の進展状況を反映させて記載すること。

血安第１７６号
平成２４年４月２０日

厚生労働省医薬食品局血液対策課長 様

日本赤十字社
血液事業本部長

供血者からの遡及調査の進捗状況について（回答）

平成24年4月6日付事務連絡によりご連絡のありました標記の件について、別紙により報告いたします。

別紙

供血者から始まる遡及調査実施状況

平成24年2月29日現在

対象期間	平成21年4月1日 ～ 平成22年3月31日			平成22年4月1日 ～ 平成23年3月31日			平成23年4月1日 ～ 平成24年2月29日		
	HBV	HCV	HIV	HBV	HCV	HIV	HBV	HCV	HIV
(1)遡及調査実施内容									
① 調査の対象とした献血件数(個別NAT実施件数)									
1)総数	1,806			1,852			2,378		
2)個別件数	1,688	69	49	1,730	74	48	2,299	54	25
② 上記①のうち、調査の対象とした輸血用血液製剤の本数									
1)総数	2,014			2,072			2,626		
2)個別本数	1,877	84	53	1,934	82	56	2,541	62	23
③ 上記②のうち、医療機関に情報提供を行った本数									
1)総数	2,014			2,072			2,268		
2)個別本数	1,877	84	53	1,934	82	56	2,194	54	20
(2)個別NAT関連情報									
① 遡及調査実施対象[(1)①]のうち、個別NATの結果が陽性となった献血件数									
1)総数	144			100			108		
2)個別件数	144	0	0	100	0	0	108	0	0
② 上記①のうち、医療機関へ供給された製剤に関する報告件数									
1)使用された本数	140	0	0	98	0	0	113	0	0
2)医療機関調査中	0	0	0	0	0	0	0	0	0
3)院内で廃棄	6	0	0	5	0	0	2	0	0
4)不明	6	0	0	3	0	0	0	0	0
計	152	0	0	106	0	0	115	0	0
③ 上記②のうち、受血者情報が判明した件数									
1)陽転事例	1	0	0	5	0	0	6*	0	0
2)非陽転事例	55	0	0	28	0	0	42	0	0
3)死亡	55	0	0	44	0	0	52	0	0
4)退院・未検査	19	0	0	15	0	0	10	0	0
5)陽性だが輸血前不明	10	0	0	6	0	0	3	0	0
計	140	0	0	98	0	0	113	0	0
④ 上記③のうち、医薬品副作用感染症報告を行った件数									
報告件数	1	0	0	5	0	0	4	0	0

＊6例中2例はHBs抗体のみの陽転であり、輸血血液からの移行抗体等と医療機関において判断された事例である。

※血液製剤等に係る遡及調査ガイドライン(平成20年12月26日一部改正)に基づく遡及調査対応基準を適用。

HBV ：HBs抗原CLEIA法確認試験（中和試験）又は個別NAT陽性の場合は遡及調査を行う。
：HBc抗体CLEIA法陽転の場合は遡及調査を行う。
HCV ：HCV抗体CLEIA法陽転の血液及び前回の血液について個別NATを実施し、いずれかが陽性の場合は遡及調査を行う。
HIV ：HIV抗体CLEIA法で陽転し、確認試験（WB法）又は個別NAT陽性の場合は遡及調査を行う。
共通 ：スクリーニングNAT陽転の場合は遡及調査を行う。

〈参考〉

供血者から始まる遡及調査実施状況

対象期間		平成11年4月1日～平成18年3月31日			平成18年4月1日～平成19年3月31日			平成19年4月1日～平成20年3月31日			平成20年4月1日～平成21年3月31日		
		HBV	HCV	HIV	HBV	HCV	HIV	HBV	HCV	HIV	HBV	HCV	HIV
① 調査の対象とした献血件数													
	1）遡及調査の対象件数	23,104			2,193			2,694			5,219		
② 上記①のうち、個別NAT検査を実施した本数（検体数）													
	1）本数（検体数）	23,104			2,193			2,694			5,219		
	2）実施率	100%			100%			100%			100%		
③ 上記②のうち陽性が判明した本数													
	本数	311	3	1	60	1	0	25	0	0	118	0	0
④ 上記①のうち医療機関に情報提供を行った件数													
	1）血液製剤数（総数）	33,114			2,408			2,867			4,034		
	個別本数				2,062	288	58	2,444	345	78	3,552	417	65
	2）情報提供数	33,114			2,408			2,708			3,469		
	個別件数				2,062	288	58	2,319	317	72	3,150	254	65
	＊平成11年4月1日～平成17年3月31日までの情報提供数には、医療機関の廃院等による追跡不能数930件を含む												
⑤ 上記③のうち医療機関へ供給された製剤に関する報告件数													
	1）使用された本数	326	3	1	51	2	0	26	0	0	94	0	0
	2）医療機関調査中	0	0	0	0	0	0	0	0	0	0	0	0
	3）院内で廃棄	16	0	0	2	0	0	2	0	0	5	0	0
	4）不明	7	1	0	0	0	0	0	0	0	0	0	0
	計	349	4	1	53	2	0	28	0	0	99	0	0
⑥ 上記⑤のうち、受血者情報が判明した件数													
	1）陽転事例	17	1	1	4	1	0	4	0	0	3	0	0
	2）非陽転事例	69	0	0	11	0	0	9	0	0	30	0	0
	3）死亡	118	2	0	31	1	0	10	0	0	42	0	0
	4）退院・未検査	15	0	0	0	0	0	0	0	0	0	0	0
	5）陽性だが輸血前不明	7	0	0	1	0	0	0	0	0	0	0	0
	計	226	3	1	47	2	0	23	0	0	75	0	0
	＊個別NAT陰性（NATウインドウピリオド）の遡及調査対象血液の輸血により、受血者が陽転した例を含む												
⑦ 上記⑥のうち、医薬品副作用感染症報告を行った件数													
	報告件数	16*	1	1	5	1	0	4	0	0	3	0	0
	ウイルス別合計	HBV:28　HCV:2　HIV:1											

＊受血者情報の陽転事例のうち医薬品感染症報告が行われていない平成12年3月の事例は、献血血液が遡及調査の対象（個別HBV-NAT陽性）となり、受血者の陽転化情報が得られたが、患者は原疾患により死亡した事例である。

＊平成20年度は、遡及調査対応基準を改定した。（同年10月29日開催「薬事・食品衛生審議会血液事業部会運営委員会」にて了承済）

薬事法第７７条の４の３に基づく回収報告状況

○平成２４年２月～平成２４年４月

報告日	回収開始年月日	回収対象製品	製造番号	対象本数
平成24年4月4日	平成24年4月3日	照射濃厚血小板HLA-LR「日赤」	13-1034-0532	1
平成24年3月30日	平成24年3月29日	赤血球濃厚液-LR「日赤」400mL由来	50-0520-0516	1
平成24年3月29日	平成24年3月28日	照射赤血球濃厚液-LR「日赤」400mL由来	23-3124-7325	1
平成24年3月22日	平成24年3月19日	照射赤血球濃厚液-LR「日赤」400mL由来	01-2220-5437	1
平成24年3月19日	平成24年3月14日	新鮮凍結血漿-LR「日赤」成分採血由来	37-2137-2148	1
平成24年3月19日	平成24年3月16日	新鮮凍結血漿-LR「日赤」400mL由来	55-8022-9812	1
平成24年3月7日	平成24年2月24日	照射赤血球濃厚液-LR「日赤」400mL由来	01-5124-1310	1
平成24年2月1日	平成24年1月31日	新鮮凍結血漿-LR「日赤」400mL由来	01-0725-2432	1

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

輸血用血液製剤で HCV（C 型肝炎ウイルス）感染が疑われた事例（平成 24 年 2 月 8 日報告）について

1 経緯

平成 24 年 2 月 8 日、日本赤十字社から輸血（照射赤血球濃厚液-LR、新鮮凍結血漿-LR）による HCV 感染疑い事例で、患者が死亡した症例の報告があった。

2 事例

・患者は 70 歳代の女性。原疾患は膀胱癌、糖尿病及び閉塞性動脈硬化症。
・平成 23 年 10 月 28 日、膀胱全摘出術、子宮卵巣拡大切除術及び尿管皮膚瘻造設術の施行時の出血に伴い輸血（赤血球濃厚液 14 単位、新鮮凍結血漿 6 単位）を受ける。
・平成 23 年 11 月 4 日に AST 51、ALT 45。11 月 7 日に AST 103、ALT 139。11 月 28 日に AST 245、ALT 156。12 月 20 日に肝機能障害にて転院し、AST 515、ALT 337、HCV 抗体陽性。
・平成 24 年 1 月 27 日に消化管出血、出血性ショックにて死亡。
・輸血前（平成 23 年 9 月 8 日）の HCV 抗体検査は陰性であったが、輸血後の HCV 抗体検査は陽性（平成 23 年 12 月 20 日）、HCV 核酸増幅検査は陽性（平成 23 年 12 月 21 日）であった。

3 状況

（1）輸血された血液製剤について
・当該患者には、11 人の供血者から採血された 8 本の赤血球濃厚液及び 3 本の新鮮凍結血漿を輸血。
・当該製剤と同一供血者から 7 本の原料血漿、1 本の新鮮凍結血漿及び 3 本の赤血球濃厚液を製造。原料血漿と新鮮凍結血漿は全て確保済み。3 本の赤血球濃厚液は全て医療機関供給済み。

（2）検体検査等の状況
・日本赤十字社において保管検体の個別 NAT を実施し、11 本全て陰性。
・11 人の供血者のうち 7 人が献血又は事後検査に来訪し、HCV 関連検査は全て陰性。

（3）担当医の見解
・「C 型急性肝炎の程度は重篤であり、本剤との関連性は可能性が大きい」、「輸血と死亡との関連性あり」とのコメント。

4 今後の対応

・再来していない供血者 4 例のフォローアップを引き続き行う予定。

輸血用血液製剤で感染が疑われる事例について

（平成２４年４月２０日時点。過去５年間分）

【ＨＢＶ感染が疑われた事例】

報告日	輸血された血液製剤	供血者数	供血者検査結果等	同一血液由来の他製剤等について	新規報告
H21.11.20	新鮮凍結血漿 血小板製剤 赤血球製剤	45 人	保管検体個別 NAT 全て陰性 感染が疑われる輸血時の製剤の供血者 23 人中 21 人来訪 HBV 関連検査陰性：21 人	原料血漿：20 本中 2 本確保。18 本使用済み。 新鮮凍結血漿：3 本全て供給済み。 赤血球製剤：22 本全て供給済み。	平成 23 年 6 月 27 日以降、残る 2 人の来訪なし。

【ＨＣＶ感染が疑われた事例】

報告日	輸血された血液製剤	供血者数	供血者検査結果等	同一血液由来の他製剤等について	新規報告
H24.2.8	新鮮凍結血漿 赤血球製剤	11 人	保管検体個別 NAT 全て陰性 供血者 11 人中 7 人来訪 HCV 関連検査陰性：7 人	原料血漿：7 本全て確保。 新鮮凍結血漿：1 本全て確保。 赤血球製剤：3 本全て供給済み。	残る 4 人の来訪なし。

感染症報告事例のまとめについて

（平成 23 年 12 月～平成 24 年 4 月報告分）

1　平成 23 年 12 月から 24 年 4 月までに報告（新規及び追加）があった感染症報告（疑い事例を含む。供血者からの情報により開始した遡及調査によるものを除く）は、輸血用血液製剤 20 件である。

輸血用血液製剤の内訳は、

（１）HBV 感染報告事例：　9 件
（２）HCV 感染報告事例：　9 件
（３）HIV 感染報告事例：　0 件
（４）その他の感染症報告例：　2 件（細菌感染報告事例）

2　HBV 感染報告事例

（１）輸血前後に感染症検査で HBV-DNA、HBs 抗原等が陽転した事例は 8 件。
輸血後 NAT で陰性又は輸血前後で陽性は 1 件。
（２）使用された輸血用血液製剤を提供した献血者の保管検体の個別 NAT 陽性の事例は 1 件。
（３）劇症化又は輸血後に死亡（原疾患又は他の原因による死亡を除く）したとの報告を受けた事例は 0 件。

3　HCV 感染報告事例

（１）輸血前後に抗体検査（又は HCV-RNA）等が陽転した事例は 8 件。輸血後 NAT で陰性又は輸血前後で陽性は 1 件。
（２）使用された輸血用血液製剤を提供した献血者の保管検体の個別 NAT 陽性事例は 0 件。
（３）劇症化又は輸血後に死亡（原疾患又は他の原因による死亡を除く）したとの報告を受けた事例は 1 件。

4　HIV 感染報告事例

（１）輸血前後に抗体検査等が陽転した事例は 0 件。
（２）使用された輸血用血液製剤を提供した献血者の保管検体の個別 NAT 陽性事例は 0 件。
（３）輸血後に死亡（原疾患又は他の原因による死亡を除く）したとの報告を受けた事例は 0 件。

5　その他の感染症報告事例

（１）B 型肝炎及び C 型肝炎以外の肝障害報告事例は 0 件。
（２）細菌等感染報告事例において、使用された輸血用血液製剤を提供した献血者の保管検体の無菌試験陽性事例は 0 件。
（３）輸血後に死亡（原疾患又は他の原因による死亡を除く）したとの報告を受けた事例は 0 件。

日赤番号	識別番号	FAX受付日	報告受領日	販売名(一般名)	患者性別	年代	原疾患	感染症名	投与年月	投与前検査(年月)	投与後検査(年月)	日赤投与前検査	日赤投与後検査	受血者個別NAT	献血者個別NAT	併用血液製剤等	備考	使用単位数	供血者再献血※	同一供血者製剤確保※	同一供血者製剤使用※	感染症等転帰	転帰	供血者発遡及の場合の供血者保管検体(抗原、抗体、NAT)(投与時点)	供血者発遡及の場合の供血者の検査値
				輸血によるHBV感染報告例(疑い例を含む。)																					
供血者陽性事例																									
3-1200011	A-11000103	2012/2/8	2012/2/22	新鮮凍結血漿-LR(新鮮凍結人血漿) 照射赤血球濃厚液ーLR(人赤血球濃厚液(放射線照射)) 照射濃厚血小板-LR(人血小板濃厚液(放射線照射))	男	30	肝硬変 食道静脈瘤 汎血球減少症	B型肝炎	11/06	HBV-DNA(−) HBsAb(−)(−) HBcAb(−)(−) IgM-HBcAb(−) HBeAg(−) HBeAb(−) (11/06)	HBV-DNA(+) HBsAg(−) HBcAb(+) IgM-HBcAb(+) HBeAg(+) HBeAb(−) (12/01)	-	HBV-DNA(+) HBsAg(+) HBsAb(-) HBcAb(+) (12/2)	陽性(輸血後)	保管検体13本についてHBV-DNA(−)、1本HBV-DNA(+)		※HBV-DNA陽性輸血用血液(献血者)についての情報 ①同一採血製造番号:赤血球濃厚液-LRを1本製造。医療機関へ供給済み。 ②再来献血:3回献血に再来。(保管検体3本についてHBV-NAT実施予定) 2本の原料血漿、1本の新鮮凍結血漿-LR、3本の赤血球濃厚液-LRを製造。原料血漿は全て使用済み。新鮮凍結血漿-LRは確保済み。赤血球濃厚液-LRは1本確保済みで、2本は医療機関へ供給済み。 ③当該以前の献血:可能な限り過去に遡り、保管検体の個別NATが陰性と判定されるまで全ての輸血用血液、原料血漿を遡及する	20単位 6単位 10単位	7/14(HBV関連検査陰性)	2本の原料血漿、2本の新鮮凍結血漿-LR、10本の赤血球濃厚液-LRを製造。原料血漿は1本確保済み。新鮮凍結血漿-LRは全て確保済み。赤血球濃厚液-LRは1本廃棄済み。	原料血漿は1本使用済み。赤血球濃厚液-LRは9本は医療機関へ供給済み。	重篤	未回復		献血者検体(当該採血検体はウイルス量が少なくPCR増幅できなかったため、2012年2月16日採血検体を用いた)と患者検体とでS領域193bpの塩基配列を比較したところ、2箇所の塩基の相違が認められた。その他は全て一致した。献血者と患者のHBウイルスはGenotypeCで塩基配列から献血者のSubtypeはayw、患者はadwと推定した。
陽転事例																									
3-1100096	A-11000089	2011/12/28	2012/1/6	照射赤血球濃厚液ーLR(人赤血球濃厚液(放射線照射))	男	80	膀胱癌 右尿管癌	B型肝炎	11/10-11	HBsAg(−) (11/06)	HBsAg(+) (11/11)	-	HBV-DNA(+) HBsAg(+) HBsAb(−) HBcAb(+) (11/11) HBV-DNA(+) HBsAg(+) HBsAb(−) HBcAb(+) (11/11) HBV-DNA(+) HBsAg(+) HBsAb(−) HBcAb(+) (11/12)	陽性(輸血後)	保管検体2本(全部)についてHBV-DNA(−)			4単位	0/2	2本の原料血漿を製造、すべて確保済み	-	非重篤	不明		

日赤番号	識別番号	FAX受付日	報告受領日	販売名(一般名)	患者性別	年代	原疾患	感染症名	投与年月	投与前検査(年月)	投与後検査(年月)	日赤投与前検査	日赤投与後検査	受血者個別NAT	献血者個別NAT	併用血液製剤等	備考	使用単位数	供血者再献血※	同一供血者製剤確保※	同一供血者製剤使用※	感染症等転帰	転帰	供血者発遡及の場合の供血者保管検体(抗原、抗体、NAT)(投与時点)	供血者発遡及の場合の供血者の検査値
3-1200003	A-11000093	2012/1/10	2012/1/24	赤血球濃厚液ーLR(人赤血球濃厚液) 新鮮凍結血漿-LR(新鮮凍結人血漿)	女	80	大動脈弁狭窄症	B型肝炎	2011/06	HBsAg(−)(11/06) HBsAb(−)(−) HBcAb(−)(11/06)	HBsAg(+) (11/12)	HBV-DNA(−) HBsAg(−) HBsAb(−) HBcAb(−) (11/6)	HBV-DNA(+) HBsAg(+) HBsAb(−) HBcAb(+) (12/1)	陰性(輸血前) 陽性(輸血後)	保管検体8本(全部)についてHBV-DNA(−)			9単位 6単位	5/8	4本の原料血漿、1本の新鮮凍結血漿-LR、3本の赤血球濃厚液-LRを製造。原料血漿は全て確保済み。新鮮凍結血漿-LRは確保済み。	赤血球濃厚液-LRは全て医療機関へ供給済み。	重篤	未回復		
3-1200005	A-11000098	2012/1/24	2012/2/8	照射濃厚血小板-LR(人血小板濃厚液(放射線照射)) 照射赤血球濃厚液ー LR(人赤血球濃厚液(放射線照射)) 赤血球濃厚液-LR(人赤血球濃厚液)	男	60	急性リンパ性白血病	B型肝炎	2011/3-9	HBsAg(−)(11/02) HBsAb(−)(−) HBcAb(−)(11/03)	HBV-DNA(−)(11/08) HBV-DNA(+)(11/09) HBV-DNA(+)(12/01)	HBV-DNA(−) HBsAg(−) HBsAb(−) HBcAb(−) (11/03)	HBV-DNA(−) HBsAg(−) HBsAb(−) HBcAb(−) (11/09)	陰性(輸血前) 陰性(輸血中)	保管検体15本(全部)についてHBV-DNA(−)			90単位 2単位 10単位	9/15(HBV関連検査異常なし)	10本の原料血漿、5本の新鮮凍結血漿-LRを製造。原料血漿は8本確保済みあ。新鮮凍結血漿-LRは4本確保済み。	原料血漿は2本使用済み。新鮮凍結血漿-LRは1本医療機関へ供給済み。	重篤	未回復		
3-1200007	A-11000098	2012/1/26	2012/2/8	新鮮凍結血漿-LR(新鮮凍結人血漿) 照射赤血球濃厚液ーLR(人赤血球濃厚液(放射線照射)) 照射濃厚血小板-LR(人血小板濃厚液(放射線照射))	男	60	髄痛性肺襞胞	B型肝炎	2011/7	HBsAg(−)(11/04)	HBV-DNA(+) HBsAg(−) (12/01)	HBV-DNA(−) HBsAg(−) HBsAb(−) HBcAb(−) (11/07)	HBV-DNA(+) HBsAg(−) HBsAb(−) HBcAb(−) (12/01)	陰性(輸血前) 陽性(輸血後)	保管検体44本(全部)についてHBV-DNA(−)			44単位 4単位 20単位	21/44(20人はHBV関連検査陰性、1人はHBs抗体のみ陽性であり、当該献血時においても同様であった)	15本の原料血漿、7本の新鮮凍結血漿-LR、22本の赤血球濃厚液-LR、1本の濃厚血小板-LRを製造。原料血漿は11本確保済み。新鮮凍結血漿-LRは全て確保済み。	原料血漿4本使用済み。赤血球濃厚液-LRは全て医療機関へ供給済み。濃厚血小板-LRは医療機関へ供給済み。	非重篤	未回復		
3-1200012	A-11000104	2012/2/8	2012/2/22	照射赤血球濃厚液ーLR(人赤血球濃厚液(放射線照射)) 新鮮凍結血漿-LR(新鮮凍結人血漿)	男	70	不安定狭心症 冠動脈ステント留置後	B型肝炎	11/09	HBsAg(−)(11/07)	HBsAg(+) HBsAb(−)(11/12) HBsAg(+) HBsAb(−) HBeAg(+) HBeAb(−) IgM-HBcAb(+) (12/02) HBsAg(+) HBsAb(−) (12/02)	-	HBV-DNA(+) HBsAg(+) HBsAb(-) HBcAb(+) (12/02)	陽性(輸血後)	保管検体12本すべてについてHBV-DNA(−)		【献血者陽転化情報】 当該献血11年1月13日 HBV関連検査陰性 保管検体個別NAT陰性 事後検査12年3月13日 HBV-DNA(+)、HBsAg(−)、HBsAb(−)、HBcAb(+) 【製剤情報】当該被疫源(新鮮凍結血漿-LR)の同一採血番号製剤として、1本の赤血球濃厚液-LRが製造され、赤血球製剤を輸血した患者は輸血前検査陰性、輸血後検査は未実施のため、輸血による感染の疑いについては不明	10単位 14単位	11/12(10人はHBV関連検査陰性、1人は事後検査にてHBV-DNA陽性)	5本の原料血漿、7本の赤血球濃厚液-LRを製造。原料血漿はすべて確保済み。	赤血球濃厚液-LRは全て医療機関へ供給済み。	重篤	未回復		患者検体と献血者検体(HBV-NAT陽性)との塩基配列比較は献血者検体のウイルス量が少なくウイルス核酸が増幅検出できなかったため実施できなかった。患者検体のHBV-DNAはGenotyeBで塩基配列からSubtypeはadwと推定された。

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3-1200015	A-11000107	2012/2/14	2012/2/24	照射濃厚血小板－LR(人血小板濃厚液(放射線照射)) 照射赤血球濃厚液－LR(人赤血球濃厚液(放射線照射)) 新鮮凍結血漿-LR(新鮮凍結人血漿)	男	10	急性リンパ性白血病ダウン症	B型肝炎	11/01 11/04	HBsAg(－)(11/01) HBsAg(－)(11/04) HBsAg(＋)(11/06) HBsAg(＋)(11/08) HBsAg(＋)(11/11) HBeAg(＋) HBeAb(－) HBcAb(－) HBeAg(＋) HBeAb(－)(12/02)(この結果を受けて過去に遡って検査実施)	HBsAg(－)(11/01) HBsAg(－)(11/04) HBsAg(＋)(11/06) HBsAg(＋)(11/08) HBsAg(＋)(11/11) HBeAg(＋) HBeAb(－) HBcAb(－) HBeAg(＋) HBeAb(－)(12/02)	－	HBV-DNA(－) HBsAg(－) HBsAb(＋) HBcAb(－)(11/01) HBV-DNA(＋) HBsAg(＋) HBsAb(＋) HBcAb(－)(12/02)	陰性(輸血中) 陽性(輸血後)	保管検体11本すべてについてHBV-DNA(－)		2012年2月8日にうっ血性心不全にて死亡、剖検あり。死亡と本剤との関連性なし(担当医の見解)	50単位 10単位 2単位	10/11(8人はHBV関連検査陰性、二人はHBs抗体の見陽性であり、当該献血時においても同様であった)	5本の原料血漿、1本の新鮮凍結血漿－LR、1本の赤血球濃厚液－LRを製造。	原料血漿は全て使用済み。赤血球濃厚液－LR、新鮮凍結血漿-LRは医療機関へ供給済み。	非重篤	不明		
3-1200024	A-11000117	2012/3/15	2012/3/28	新鮮凍結血漿-LR(新鮮凍結人血漿) 照射赤血球濃厚液－LR(人赤血球濃厚液(放射線照射))	男	60	交通外傷出血性ショック	B型肝炎	11/09-10	HBsAg(－)(08/03) HBsAg(－)(11/01) HBsAg(－)(11/09) HBsAg(－) HBsAb(－) HBcAb(－)(11/09)	HBsAg(＋) HBsAb(－) HBcAb(＋) IgM-HBcAb(＋) HBeAg(＋) HBeAb(－)(12/02)	HBV-DNA(－) HBsAg(－) HBsAb(－) HBcAb(－)(11/09)	HBV-DNA(－) HBsAg(－) HBsAb(－) HBcAb(－) 11/10(輸血期間中) HBV-DNA(＋) HBsAg(＋) HBsAb(－) HBcAb(＋) 12/03(輸血後)	陰性(輸血中) 陽性(輸血後)	保管検体14本すべてについてHBV-DNA(－)			14単位 14単位	5/14(HBV関連検査陰性)	7本の原料血漿、7本の赤血球濃厚液－LRを製造。原料血漿はすべて確保済み。	赤血球濃厚液－LRは全て医療機関へ供給済み。	非重篤	軽快		
輸血後NATで陰性又は輸血前後で陽性																									
3-1200002	A-11000092	2012/1/10	2012/1/17	照射赤血球濃厚液－LR(人赤血球濃厚液(放射線照射))	男	60	多発性骨髄腫	B型肝炎	11/05-06	HBsAg(－) HBcAb(－)(11/5)	HBsAg(＋) HBsAb(－) HBcAb(－)(11/11) HBV-DNA(＋) HBsAg(＋) HBsAb(－) HBeAg(＋) IgM-HBcAb(－)(11/11)	HBV-DNA(－) HBsAg(＋) HBsAb(＋) HBcAb(－)(11/5)	HBsAg(＋) HBsAb(－) HBcAb(－)(11/11)	陰性(輸血前)	保管検体8本(全部)についてHBV-DNA(－)			16単位	5/8(HBV関連検査異常なし)	6本の原料血漿、2本の新鮮凍結血漿－LRを製造。原料血漿・新鮮凍結血漿-LRはすべて確保済み	－	非重篤	軽快		

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				輸血によるHCV感染報告例(疑い例を含む。)																					
供血者陽性事例																									
(該当例なし)																									
陽転事例																									
3-1100095	A-11000088	2011/12/22	2011/12/28	新鮮凍結血漿-LR(新鮮凍結人血漿) 照射濃厚血小板-LR(人血小板濃厚液(放射線照射)) 照射赤血球濃厚液－LR(人赤血球濃厚液(放射線照射))	男	60	弓部大動脈瘤	C型肝炎	2011/10	HCV-Ab(－)(11/10) HCV-Ab(－)(11/10)	HCV-Ab(＋)(11/12)	－	－	－	保管検体15本(全部)についてHCV-RNA(－)	人血清アルブミン 人血由来乾燥血液凝固第13因子 乾燥イオン交換樹脂処理		18単位 20単位 10単位	2/15(HCV関連検査陰性)	3本の原料血漿、3本の新鮮凍結血漿－LR、9本の赤血球濃厚液－LRを製造。原料血漿・新鮮凍結血漿-LRはすべて確保済み	赤血球濃厚液-LRは全て医療機関へ供給済み。	重篤	未回復		
3-1200006	A-11000097	2012/1/26	2012/2/8	照射赤血球濃厚液－LR(人赤血球濃厚液(放射線照射)) 新鮮凍結血漿-LR(新鮮凍結人血漿)	女	70	膀胱癌 糖尿病 閉塞性動脈硬化症	C型肝炎	2011/10	HCV-Ab(－)(11/09)	HCV-Ab(＋)(11/12) HCV-RNA(＋)(11/12) HCVコア抗原(－)(12/01) HCV-Ab(＋)(12/01) 消化管出血、出血性ショックにて死亡。(剖検なし、輸血と死亡との関連性あり)	HCV-RNA(－) HCV-Ab(－)(11/10)	HCV-RNA(＋) HCV-Ab(＋)(12/01)	陰性(輸血前) 陽性(輸血後)	保管検体11本(全部)についてHCV-RNA(－)		消化管出血、出血性ショックにて死亡、剖検なし。輸血と死亡との関連性あり(担当医の見解)	14単位 6単位	7/11(献血または事後検査依頼に再来、HCV関連検査異常なし)	7本の原料血漿、1本の新鮮凍結血漿－LR、3本の赤血球濃厚液－LRを製造。原料血漿は全て確保済み。新鮮凍結血漿-LRは確保済み。	赤血球濃厚液-LRは全て医療機関へ供給済み。	重篤	死亡		
3-1200009	A-11000100	2012/1/31	2012/2/13	照射赤血球濃厚液－LR(人赤血球濃厚液(放射線照射))	女	40	マルファン症候群 大動脈解離 子宮筋腫術後	C型肝炎	11/07-08(他院) 11/10-11	他院にてHCV-Ab(－)(11/10) HCV-RNA(＋) HCV-Ab(＋)(11/12)	－	HCV-RNA(＋) HCV-Ab(－)(11/10(輸血期間中)) HCV-RNA(＋) HCV-Ab(＋)(12/01(輸血後))	陽性(輸血後)	保管検体7本(全部)HCV-RNA(-)			14単位	1/7(HCV関連検査陰性)	7本の原料血漿を製造、全て確保済み	－	非重篤	未回復		

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3-1200013	A-1100-0105	2012/2/9	2012/2/22	照射濃厚血小板(人血小板濃厚液(放射線照射)) 濃厚血小板(人血小板濃厚液) 赤血球M・A・P(人赤血球濃厚液)	女	〜	急性リンパ性白血病	C型肝炎	05/05-10	−	HCV-Ab(−)(05/05) HCV-Ab(−)(05/11) HCV-Ab(−)(06/07) HCV-Ab(−)(07/06) HCV-Ab(+)(12/01) HCV-RNA(+)(12/01)	−	HCV-RNA(+) HCV-Ab(+)(12/02)	陽性(輸血後)	保管検体31本(全部)HCV-RNA(−)			20単位 130単位 16単位	27/31(HCV関連検査陰性)	31本の原料血漿を製造、全て使用済み。	−	重篤	未回復		
3-1200014	A-1100-0106	2012/2/13	2012/2/24	照射赤血球濃厚液−LR(人赤血球濃厚液(放射線照射))	男	70	化膿性脊椎炎MRSA肺炎心房細動陳旧性脳梗塞	C型肝炎	11/11	HCV-Ab(−)(11/08)	HCV-Ab(+)(12/02) HCVコア抗原(+)(12/02)	HCV-RNA(−) HCV-Ab(−)(11/11)	HCV-RNA(+) HCV-Ab(+)(12/02) HCV-RNA(+) HCV-Ab(+)(12/02)	陰性(輸血前) 陽性(輸血後)	保管検体4本(全部)HCV-RNA(−)			8単位	0/4	3本の原料血漿、1本の新鮮凍結血漿−LRを製造。原料血漿は全て確保済み。新鮮凍結血漿−LRは確保済み。	−	重篤	未回復		
3-1200016	A-1100-0108	2012/2/14	2012/2/24	照射赤血球濃厚液−LR(人赤血球濃厚液(放射線照射)) 新鮮凍結血漿−LR(新鮮凍結人血漿)	男	70	感染性心内膜炎	C型肝炎	11/11	HCV-RNA(−) HCV-Ab(−)(11/11)	HCV-RNA(+) HCV-Ab(−) (12/02) HCV-Ab(+)(12/02)	−	HCV-RNA(+) HCV-Ab(+)(12/02)	陽性(輸血)	保管検体19本すべてについてHCV-RNA(−)		本症例は被疑薬4本で情報を入手し、未完了報告を行ったが、その後医療機関からの申し出により被疑薬が5本追加された。	18単位 10単位	3/19(HCV関連検査陰性)	10本の原料血漿、4本の新鮮凍結血漿−LR、5本の赤血球濃厚液−LRを製造。原料血漿はすべて確保済み。新鮮凍結血漿−LRはすべて確保済み。	赤血球濃厚液−LRは全て医療機関へ供給済み。	重篤	未回復		
3-1200018	A-1100-0110	2012/2/22	2012/3/5	赤血球濃厚液−LR(人赤血球濃厚液) 新鮮凍結血漿−LR(新鮮凍結人血漿)	男	60	肺癌	C型肝炎	11/03	HCV-Ab(−)(10/12)	HCV-Ab(+)(12/02) HCV-RNA(+)(12/02)	HCV-RNA(−) HCV-Ab(−)(11/03)	HCV-RNA(+) HCV-Ab(+)(12/02)	陰性(輸血前) 陽性(輸血後)	保管検体6本すべてについてHCV-RNA(−)			8単位 4単位	3/6(HCV関連検査陰性)	4本の原料血漿、2本の赤血球濃厚液−LRを製造。	原料血漿はすべて使用済み。赤血球濃厚液−LRは全て医療機関へ供給済み。	重篤	未回復		
3-1200023	A-1100-0116	2012/3/9	2012/3/22	照射赤血球濃厚液−LR(人赤血球濃厚液(放射線照射))	男	50	骨盤骨折	C型肝炎	11/12	HCV-Ab(−)(11/12)	HCV-Ab(+)(12/02) HCV-RNA(−) HCV-Ab(+)(12/02)	HCV-Ab(−)(11/12)	HCV-RNA(−) HCV-Ab(+)(12/03)	陰性(輸血前) 陰性(輸血後)	保管検体1本HCV-RNA(−)			2単位	0/1	1本の原料血漿を製造、確保済み。	−	非重篤	回復		

日赤番号	識別番号	FAX受付日	報告受領日	販売名(一般名)	患者性別	年代	原疾患	感染症名	投与年月	投与前検査(年月)	投与後検査(年月)	日赤投与前検査	日赤投与後検査	受血者個別NAT	献血者個別NAT	併用血液製剤等	備考	使用単位数	供血者再献血※	同一供血者製剤確保※	同一供血者製剤使用※	感染症等転帰	転帰	供血者発遡及の場合の供血者保管検体(抗原、抗体、NAT)(投与時点)	供血者発遡及の場合の供血者の検査値
輸血後NATで陰性又は輸血前後で陽性																									
3-1200010	A-1100-0102	2012/2/6	2012/2/17	赤血球濃厚液−LR(人赤血球濃厚液) 照射赤血球濃厚液−LR(人赤血球濃厚液(放射線照射)) 新鮮凍結血漿−LR(新鮮凍結人血漿) 照射濃厚血小板−LR(人血小板濃厚液(放射線照射))	男	60	胸腹部大動脈瘤	C型肝炎	11/07 11/09	−	HBcAb(−)(11/08) HCVコア抗原(−)(11/08) HCV-Ab(−)(11/08) 他院にてHCV-Ab(+)(11/10) 他院にてHCV-Ab(+)(11/12)	−	HCV-RNA(−) HCV-Ab(+)(12/02)	陰性(輸血後)	保管検体23本(全部)HCV-RNA(−)			2単位 22単位 18単位 35単位	11/23(HCV関連検査陰性)	10本の原料血漿、4本の新鮮凍結血漿−LR、9本の赤血球濃厚液−LRを製造。原料血漿は全て確保済み。新鮮凍結血漿−LRは全て確保済み。	赤血球濃厚液−LRは全て医療機関へ供給済み。	非重篤	未回復		
				輸血によるHIV感染報告例(疑い例を含む。)																					
(該当例なし)																									
				輸血による細菌等感染報告例(疑い例を含む。)																					
陽性等事例																									
3-1200008	A-1100-0099	2012/1/30	2012/2/8	新鮮凍結血漿−LR(新鮮凍結人血漿)	女	40	血栓性血小板減少性紫斑病	細菌感染	2012/1	−	輸血開始後40〜50分後に痙攣を認めた。患者血液培養は陰性。	投与中止の当該製剤(1本)で細菌培養試験を実施、陰性。非溶血性副作用関連検査実施。抗血漿タンパク質抗体検査:抗C4抗体弱陽性血漿タンパク質欠損検査:欠損無し	−	−	−		採血309日目の新鮮凍結血漿−LR	2単位	−	1本の赤血球濃厚液−LRを製造。	赤血球濃厚液−LRは医療機関へ供給済み。	重篤	軽快		

日赤番号	識別番号	FAX受付日	報告受領日	販売名(一般名)	患者性別	年代	原疾患	感染症名	投与年月	投与前検査(年月)	投与後検査(年月)	日赤投与前検査	日赤投与後検査	受血者個別NAT	献血者個別NAT	併用血液製剤等	備考	使用単位数	供血者再献血※	同一供血者製剤確保※	同一供血者製剤使用※	感染症等転帰	転帰	供血者発遡及の場合の供血者保管検体(抗原、抗体、NAT)(投与時点)	供血者発遡及の場合の供血者の検査値
3-1200022	A-11000114	2012/3/6	2012/3/13	照射赤血球濃厚液－LR(人赤血球濃厚液(放射線照射))	男	80	骨髄異形成症候群	細菌感染	12/03	CRP 0.2mg/dL、BT 37.5℃、BP 158/74mmHg、P 89/min	輸血開始後25分 BT 40℃、BP 147/65mmHg、P 113/min 30分 BT 41℃ 4時間40分後 pH 7.455、PaO2 67%、PaCO2 33.8%、SpO2 92%、カヌラ(3L/min)、CRP 1.0mg/dL 輸血3日後 06:00 CRP 24.6mg/dL 下熱(37℃台)したが、DICを併発。 院内にて実施の患者血液培養よりEscherichia coliを同定した。	投与中止の当該製剤(1本)で細菌培養試験を実施、陰性 非溶血性副作用関連検査実施 抗血漿タンパク質抗体検査:陰性 血漿タンパク質欠損検査:欠損無し	-	-	-		被疑薬:採血10日目の照射赤血球濃厚液-LR(1本) 本症例は、2012年3月2日に副作用名「TRALIの疑い」として情報入手し、30日報告の準備を進めていたが、2012年3月5日に副作用名を「高熱41℃」「細菌感染の疑い」に訂正するとの情報を入手したため、感染症症例として報告するものである。 担当医より「副作用・感染症と輸血血液との因果関係はないと考える」とのコメントが得られた。	2単位	-	1本の原料血漿を製造、確保済み。	-	重篤	未回復 患者は12年3月28日肝炎にて死亡(剖検無し)。 死亡と本剤との関連性無し(担当医の見解)		

平成24年 5月28日開催
薬事・食品衛生審議会
運営委員会提出資料

別紙

日本赤十字社

試行的HEV20プールNAT実施状況について

(輸血後HEV感染の予防対策)

北海道赤十字血液センター管内

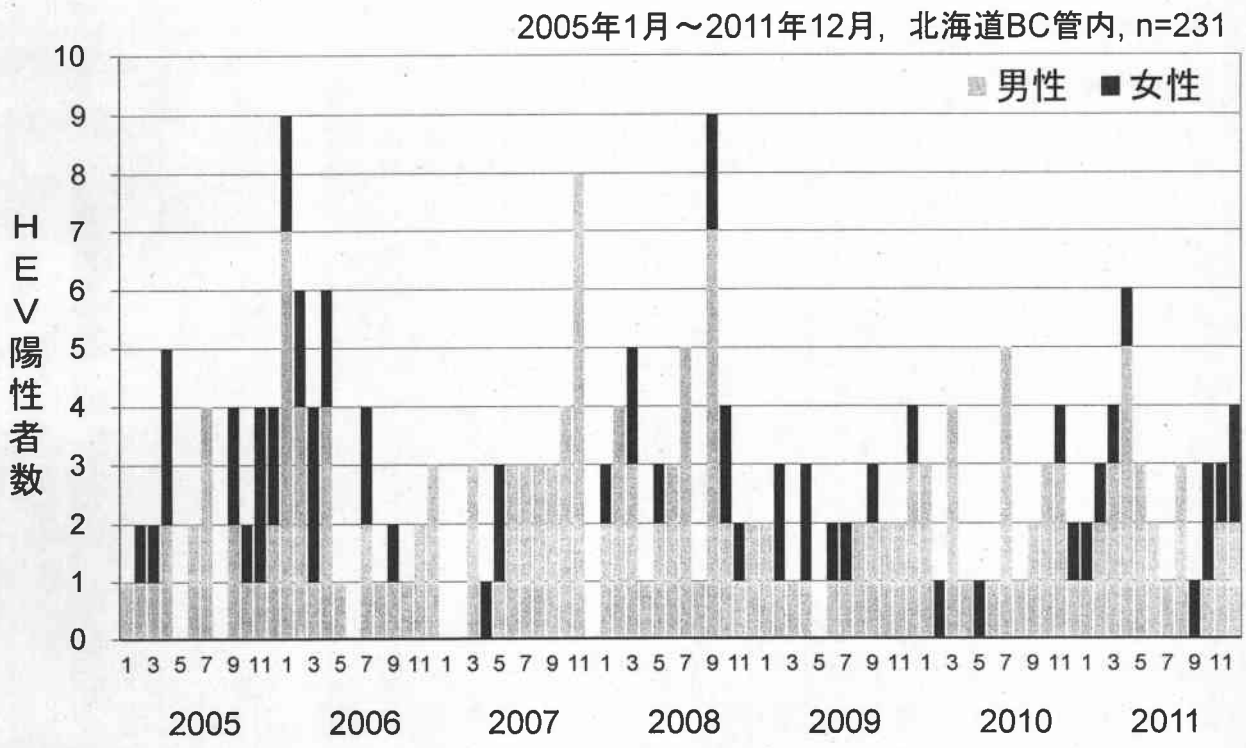
調査期間:平成17年1月1日～平成24年3月31日

	献血者数	HEV-RNA陽性	陽性率
H17. 1～H18. 2*1	341, 174	45	1／7, 582
H18. 3～H24. 3*2	1, 660, 606	195	1／8, 516
合計	2, 001, 780	240	1／8, 341

*1 北海道センターにてNAT実施(ALT高値、検査不合格検体も含む)

*2 血漿分画センターにてNAT実施(ALT高値、検査不合格検体は除く)

HEV NAT陽性献血者の月別発生数



HEV NATスクリーニング

■ 調査期間	2011.1 - 2011.12 (1yr)		2005.1 - 2011.12 (7yr)	
■ 検査総数	279,841		1,931,847	
■ 陽性者数	35		231	
■ 陽性率	0.017%	(1/7,995)	0.012%	(1/8,363)
■ 年齢	39.1+/-10.9	(20-60)	41.1+/-11.9	(17 - 68)
■ 男 : 女	25:10	(71%:29%)	172:59	(74%:26%)
■ Genotype (G3:G4)	30:4	(88%:12%)	212:17	(93%:7%)
■ Anti-HEV抗体				
IgM(-)/IgG(-)	30	(86%)	188	(81%)
IgM(+)/IgG(-)	0	(0%)	3	(1%)
IgM(+)/IgG(+)	5	(14%)	31	(13%)
IgM(-)/IgG(+)	0	(0%)	9	(4%)

事　務　連　絡
平成２４年４月６日

日本赤十字社血液事業本部　御中

薬事・食品衛生審議会血液事業部会事務局
厚生労働省医薬食品局血液対策課

血液製剤に関する報告事項について

血液事業の推進に御努力いただき、厚く御礼申し上げます。

さて、標記につきましては、平成２４年２月７日付け血安第４２号にて貴社から報告を頂いたところですが、平成２４年５月２８日（月）に平成２４年度第１回血液事業部会運営委員会が開催されますので、下記の事項について資料を作成いただき、平成２４年４月２０日（金）までに当事務局あて御提出いただきますようお願いします。記の３については、平成２３年度第４回血液事業部会運営委員会提出資料を更新のうえ、再度御提出ください。

なお、資料の作成に当たっては、供血者、患者及び医療機関の名称並びにこれらの所在地又はこれらの事項が特定できる情報を記載しないよう、個人情報及び法人情報の保護に特段の御配慮をお願いします。

記

１．平成２１年１１月２０日付けで報告された輸血用血液製剤でＨＢＶ（Ｂ型肝炎ウイルス）感染が疑われる事例について、残る２人の供血者のその後の検査結果。来訪がなければ、その旨。

２．試行的ＨＥＶ２０プールＮＡＴについて、その後の調査実施状況。

血安第１７７号
平成２４年４月２０日

厚生労働省医薬食品局血液対策課長 様

日本赤十字社
血液事業本部長

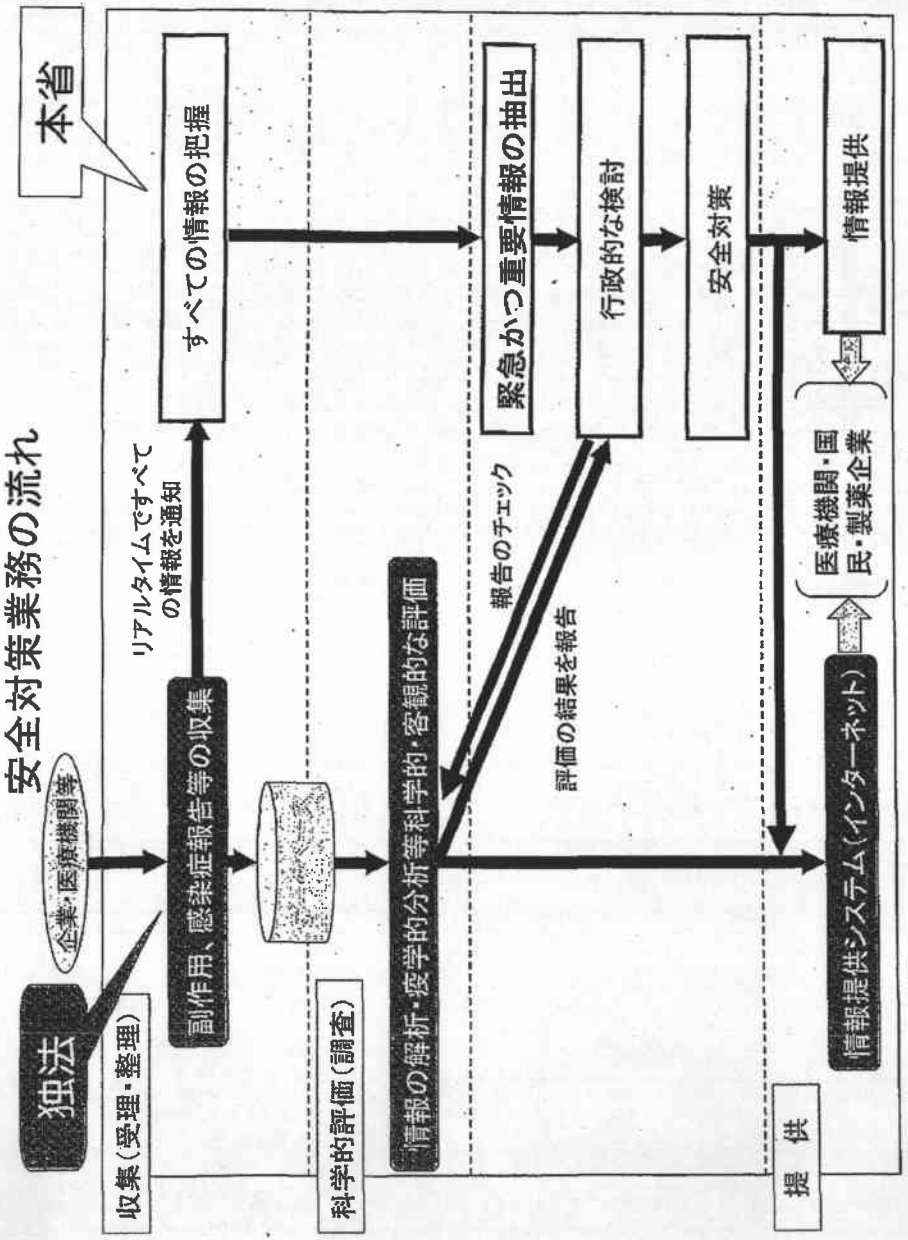
血液製剤に関する報告事項について（回答）

平成 24 年 4 月 6 日付事務連絡によりご依頼のありました標記の件について、下記のとおり資料を作成しましたので報告いたします。

記

１．平成 21 年 11 月 20 日付けで報告された輸血用血液製剤でＨＢＶ（Ｂ型肝炎ウイルス）感染が疑われる事例について、残る２人のその後の来訪なし。（23 名中 21 名が来所、検査は全て陰性）

２．試行的ＨＥＶ２０プールＮＡＴについて、その後の調査実施状況については別紙のとおり。



献血件数及びＨＩＶ抗体・核酸増幅検査陽性件数

年	献血件数（検査実施数）	陽性件数 （）内女性 ［］内核酸増幅検査のみ陽性	10万件当たり
	件	件	件
１９８７年　（昭和62年）	8,217,340	１１（１）	0.134
１９８８年　（昭和63年）	7,974,147	９（１）	0.113
１９８９年　（平成 元年）	7,876,682	１３（１）	0.165
１９９０年　（平成 2年）	7,743,475	２６（６）	0.336
１９９１年　（平成 3年）	8,071,937	２９（４）	0.359
１９９２年　（平成 4年）	7,710,693	３４（７）	0.441
１９９３年　（平成 5年）	7,205,514	３５（５）	0.486
１９９４年　（平成 6年）	6,610,484	３６（５）	0.545
１９９５年　（平成 7年）	6,298,706	４６（９）	0.730
１９９６年　（平成 8年）	6,039,394	４６（５）	0.762
１９９７年　（平成 9年）	5,998,760	５４（５）	0.900
１９９８年　（平成10年）	6,137,378	５６（４）	0.912
１９９９年　（平成11年）	6,139,205	６４（６）	1.042
２０００年　（平成12年）	5,877,971	６７（４）［３］	1.140
２００１年　（平成13年）	5,774,269	７９（１）［１］	1.368
２００２年　（平成14年）	5,784,101	８２（５）［２］	1.418
２００３年　（平成15年）	5,621,096	８７（８）［２］	1.548
２００４年　（平成16年）	5,473,140	９２（４）［２］	1.681
２００５年　（平成17年）	5,320,602	７８（３）［２］	1.466
２００６年　（平成18年）	4,987,857	８７（５）［１］	1.744
２００７年　（平成19年）	4,939,550	１０２（３）［６］	2.065
２００８年　（平成20年）	5,077,238	１０７（３）［０］	2.107
２００９年　（平成21年）	5,287,101	１０２（６）［２］	1.929
２０１０年　（平成22年）	5,318,586	８６（３）［１］	1.617
２０１１年　（平成23年）	5,252,182	８９（８）［３］	1.695
２０１２年　（平成24年）（１～３月）	1,325,793（速報値）	１４（１）［１］	1.056

（注１）・ 昭和６１年は、年中途から実施したことなどから、3,146,940件、うち、陽性件数１１件（女性０）となっている。
（注２）・ 抗体検査及び核酸増幅検査陽性の血液は廃棄され、製剤には使用されない。
・ 核酸増幅検査については、平成１１年１０月より全国的に実施している。
（注３）・ 平成２４年は、１月～３月の速報値で集計している。

HIV抗体・核酸増幅検査陽性献血者数内訳

1. 性別・年齢区分・国別

	男性			女性			合計		
	日本人	外国人	計	日本人	外国人	計	日本人	外国人	計
	人	人	人	人	人	人	人	人	人
16～19歳	36	1	37	11	0	11	47	1	48
20～29歳	548	30	578	49	4	53	597	34	631
30～39歳	527	13	540	26	2	28	553	15	568
40～49歳	189	1	190	12	1	13	201	2	203
50～69歳	84	0	84	8	0	8	92	0	92
合　計	1384	45	1429	106	7	113	1490	52	1542

※ 昭和61年～平成24年3月（昭和61年については年途中から集計）

2. 都道府県別（献血地別）

県別	61年	62年	63年	元年	2年	3年	4年	5年	6年	7年	8年	9年	10年	11年	12年	13年	14年	15年	16年	17年	18年	19年	20年	21年	22年	23年	24年	合計	構成割合	ブロック室	
	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(件)	(%)	陽性献血件数 (件)	構成割合 (%)
1.北海道			1			1	2	1	1	1		1	1	3	2	2	3	2	2	3	2	3	2	2	2		40	2.6		
2.青森			2									1					1	1	2	1	1	1		1	2		13	0.8		
3.岩手										1			1											3		1		6	0.4		
4.宮城							1	1				1	1	1	1	1	1	1	1	2				2				13	0.8	北海道	
5.秋田															1				1				1		1			4	0.3	・東北	
6.山形													1			1								1	1			4	0.3		
7.福島							1					2		1	1			1					1		1	2		10	0.6	90	5.8
8.茨城					1	1	4	2			1	2		1	2	1	1				1	1	1	5	1	1	1	27	1.8		
9.栃木					3	1				2	1	1		1		3		1			1	4	2	1	1	3		26	1.7		
10.群馬					1	1		1				1		1	3	1			2		3		2	1	3	1		21	1.4		
11.埼玉		1				1	1	2	1	2	3	3	3	3	3	3	3	3	3	5	2	1	3	2	8	3	1	57	3.7		
12.千葉							1	6	2	2	3	7	2	4	5	4	5	3	3	2	2	2	6	9	5	6	8	88	5.7	関東	
13.東京	10	6	4	10	10	11	12	11	14	21	18	18	19	27	26	29	23	25	24	22	24	17	21	19	25	18	3	467	30.3		
14.神奈川		1		1	1	4	1	3	4	2	5	3	4	3	5	3	5	5	8	4	5	5	5	1	2	4	1	85	5.5	771	50.0
15.新潟				1		1		1						1		2			1		2			2			1	12	0.8		
16.富山					2						1				1	1					1							6	0.4		
17.石川																2				1		3		1				7	0.5	北陸・	
18.福井			1							2						1	1											5	0.3	甲信越	
19.山梨					1	1					1					1												4	0.3		
20.長野							1	1			2						1					1	1					7	0.5	41	2.7
21.岐阜							1									1			1					1	2	1	2	9	0.6		
22.静岡						1	3		1						1	1		1	1			4		2	1	2		18	1.2	東海	
23.愛知		1			3	2		3	1	1			4	3	2	3	2	2	4	4	5	4	10	4	2	3		63	4.1		
24.三重											1	1	1		1			1		2				1				8	0.5	98	6.4
25.滋賀																1	3						1	2				7	0.5		
26.京都								2		2	1	1			2	5	2			4	5	1			2	5		32	2.1		
27.大阪	1	1	1	1	3		1		4	2	1	8	14	6	8	10	10	15	17	19	17	26	26	13	18	16	2	240	15.6		
28.兵庫						2			1	2				2	1	1	4	5	3		3	3	4	3	2		1	37	2.4	近畿	
29.奈良										1	2	3	1		1		1	1	1				1	1				13	0.8		
30.和歌山																		2					1	1		2		6	0.4	335	21.7
31.鳥取									1							1				1			1		1			5	0.3		
32.島根						1															1			3	1			6	0.4		
33.岡山									2									2	1	2	2	3	1		1			14	0.9	中国	
34.広島							2	1	1				1			1	2	6			2	2	1	4		1		24	1.6		
35.山口					1						2												1		1			5	0.3	54	3.5
36.徳島									1					1						1		1	1	1		1		7	0.5		
37.香川							1												1		1	2	2	1				8	0.5	四国	
38.愛媛													1	1	2	3	2	2		1	1	2		1		2		18	1.2		
39.高知																		1	1		1		1	1	1			6	0.4	39	2.5
40.福岡							1		2	2	2	1	1	1	1	2	4	2	2		3	1	3	2	4	4		38	2.5		
41.佐賀																											1	1	0.1		
42.長崎																2						1	1	2		2		8	0.5		
43.熊本						1				2	1			1	2		1	2		1	1	2	2	2	1	3		22	1.4		
44.大分									1									2	1									4	0.3	九州	
45.宮崎													2							2		1	1	1		1		8	0.5	・沖縄	
46.鹿児島							1					2			1	1		1			1	1	2		2	1		13	0.8		
47.沖縄		1									1							2	3		1	5	1	3	1	1	1	20	1.3	114	7.4
合計	11	11	9	13	26	29	34	35	36	46	46	54	56	64	67	79	82	87	92	78	87	102	107	102	86	89	14	1542	100	1542	100

※ 「構成割合」は端数処理しているため、合計が必ずしも１００％にはならない

※ 平成24年については、1月～3月の速報値で集計

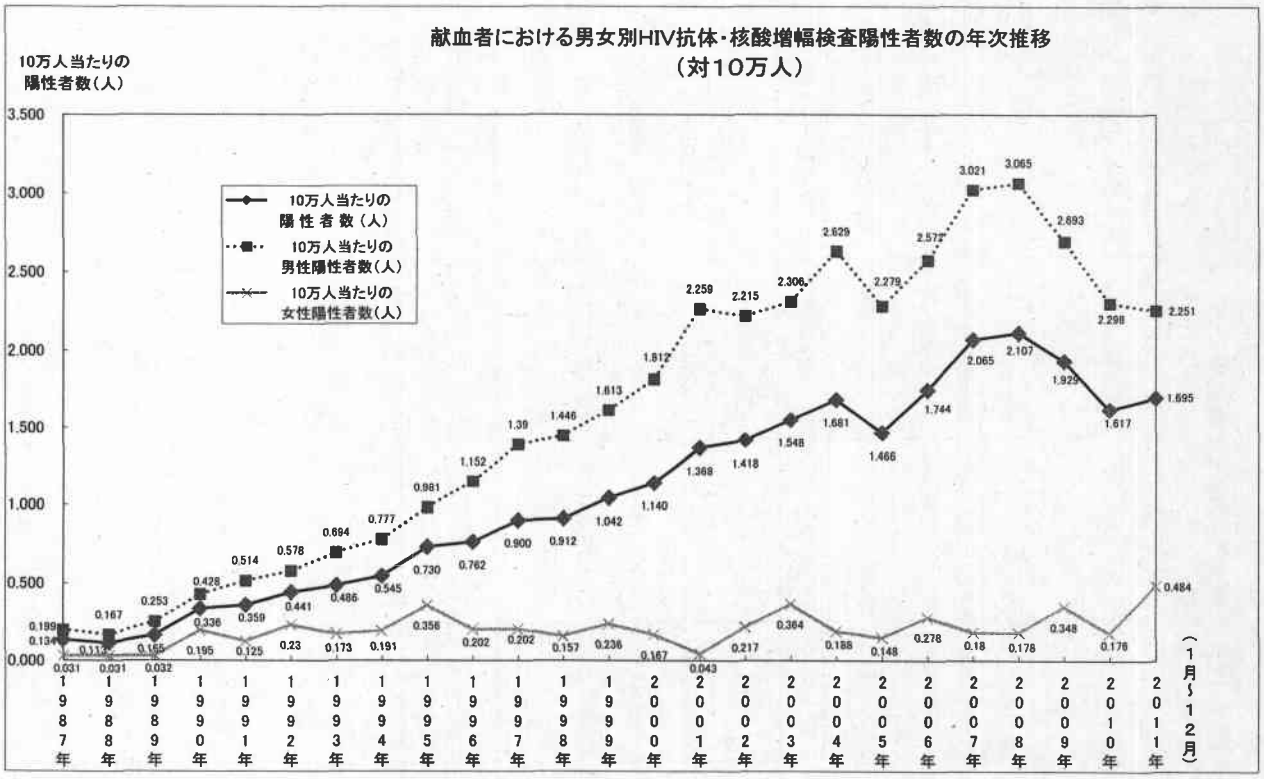
ブロック別ＨＩＶ抗体･核酸増幅検査陽性献血者

	平成20年			平成21年			平成22年			平成23年			平成24年（1月～3月）（速報値）		
	献血者	陽性	10万人当たり	献血者	陽性	10万人当たり	献血者	陽性	10万人当たり	献血者	陽性	10万人当たり	献血者	陽性	10万人当たり
	人	件	件	人	件	件	人	件	件	人	件	件	人	件	件
北海道・東北	651,215	5	0.768	677,073	9	1.329	690,050	7	1.014	645,896	5	0.774	168,456	0	0.000
			件			件			件			件			件
関東	1,621,408	40	2.467	1,705,070	42	2.463	1,698,561	38	2.237	1,649,186	36	2.183	421,067	8	1.900
			件			件			件			件			件
北陸・甲信越	335,848	0	0.000	340,901	3	0.880	340,203	0	0.000	349,241	0	0.000	86,751	1	1.153
			件			件			件			件			件
東海	562,610	11	1.955	584,495	9	1.540	589,557	4	0.678	586,872	7	1.193	145,463	0	0.000
			件			件			件			件			件
近畿	833,556	33	3.959	863,744	20	2.316	876,750	22	2.509	873,048	23	2.634	219,539	3	1.366
			件			件			件			件			件
中国	316,509	4	1.264	329,443	4	1.214	330,284	5	1.514	324,416	3	0.925	80,356	0	0.000
			件			件			件			件			件
四国	166,332	4	2.405	173,914	5	2.875	176,923	2	1.130	176,841	3	1.696	42,647	0	0.000
			件			件			件			件			件
九州・沖縄	589,760	10	1.696	612,461	10	1.633	616,258	8	1.298	646,682	12	1.856	161,514	2	1.238
			件			件			件			件			件
合計	5,077,238	107	2.107	5,287,101	102	1.929	5,318,586	86	1.617	5,252,182	89	1.695	1,325,793	14	1.056

年齢別HIV抗体･核酸増幅検査陽性献血者

	平成19年			平成20年			平成21年			平成22年			平成23年		
	献血者	陽性	10万人当たり	献血者	陽性	10万人当たり	献血者	陽性	10万人当たり	献血者	陽性	10万人当たり	献血者	陽性	10万人当たり
	人	件		人	件		人	件		人	件		人	件	
16才～19才	324,414	5	1.541	308,019	2	0.649	295,811	3	1.014	292,853	5	1.707	286,534	2	0.698
	人			人			人			人			人		
20才～29才	1,135,102	38 (2)	3.348	1,141,746	41	3.591	1,139,991	37 (1)	3.246	1,080,385	21 (1)	1.944	1,037,257	41 (4)	3.953
	人			人			人			人			人		
30才～39才	1,369,241	35 (1)	2.556	1,391,141	50 (1)	3.594	1,414,747	42 (3)	2.969	1,376,596	43 (1)	3.124	1,317,138	31 (2)	2.354
	人			人			人			人			人		
40才～49才	1,088,410	17	1.562	1,171,449	11 (1)	0.939	1,272,397	17 (2)	1.336	1,350,490	10	0.740	1,379,078	8 (1)	0.580
	人			人			人			人			人		
50才～59才	770,663	5	0.649	785,280	3 (1)	0.382	841,168	3	0.357	872,113	6 (1)	0.688	878,562	6 (1)	0.683
	人			人			人			人			人		
60才～	251,720	2	0.795	279,603	0	0.000	322,987	0	0.000	346,149	1	0.289	353,613	1	0.283
	人			人			人			人			人		
合計	4,939,550	102 (3)	2.065	5,077,238	107 (3)	2.107	5,287,101	102 (6)	1.929	5,318,586	86 (3)	1.617	5,252,182	89 (8)	1.695

(注)陽性件数の()内女性



平成24年5月28日開催
血液事業部会運営委員会

日本赤十字社　血液事業本部

HBV感染既往献血者への対応スケジュール予定

資料４

	年	2012
	月	1 2 3 4 5 6 7 8 9 10 11 12
	週	1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4
事前通知の試行的実施 問合せ対応		○対象となる献血者の抽出 ○試行的通知の開始（一部の血液センターで試行） ○試行的通知の問題点等にかかる検討
職員教育訓練		○問合せ担当者、献血推進担当者及び採血担当者への教育訓練の実施
事前通知 問合せ対応		○対象となるHBV感染既往者のうち、複数回献血者及び登録者を優先的に順次通知
判定基準の変更 （HBc抗体検査C.O.Iの変更）		○新判定基準により検査された血液の供給 基準変更
保管中のFFPへの対応		○対象製剤の抽出 ○対象製剤の抽出 ○対象製剤の抽出

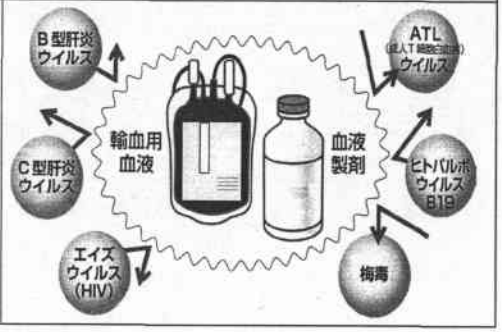
「お知らせとお願い」の連絡を受けた皆様へ

HBc 抗体陽性、HBs 抗原陰性とは？

1.献血時に血液検査（ウイルス検査）を行うのはなぜ？

→献血した血液にウイルスが入っていないかを調べ、輸血用血液にウイルスが混入するのを防ぐためです。

ウイルスに感染している人の血液を輸血すると患者さんに感染が起こることがあります。そのため、献血されたすべての血液に対し、さまざまなウイルス検査を行い、輸血用血液へのウイルスの混入を防いでいます。

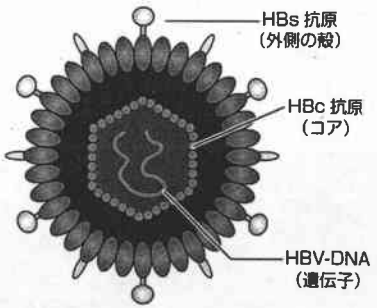


2.B 型肝炎ウイルスに感染しているかどうかは何を調べているの？

→B型肝炎ウイルスがつくる蛋白（HBs抗原）やウイルス感染に対する免疫反応によってつくられる抗体（HBc抗体）を調べています。

B 型肝炎ウイルスは球形のウイルスで、外側を被う殻（HBs 抗原と呼ばれる蛋白が埋め込まれている）と、中心にあるコア（HBc 抗原と呼ばれる蛋白）、とDNA（遺伝子）から構成されています（右図）。

下表に B 型肝炎ウイルス関連検査の種類とその検査が陽性の場合の状態を記載しています。



B型肝炎ウイルス

※B 型肝炎ウイルス関連検査

HBs 抗原	ウイルスの外側を被う蛋白 陽性：現在感染している。
HBc 抗体	HBc 抗原に対する抗体 陽性：現在感染しているか、過去に感染したことがある。
HBs 抗体	HBs 抗原に対する抗体（HBV の感染を防御する働き） 陽性：過去に感染したことがある。
HBV-DNA	B 型肝炎ウイルスの遺伝子 陽性：現在感染している（検査の精度が高い）

3.HBc 抗体陽性、HBs 抗原陰性の検査結果は何を意味しているの？

→HBc 抗体が陽性、HBs 抗原が陰性ということは、「過去にB型肝炎ウイルスに感染したことがあるが、現在は治っている状態」であると考えられます。

B型肝炎ウイルス感染者の70～80%は肝炎症状が起こらず自然に治ってしまいます。したがいまして、これまで献血いただいた血液は輸血に使用されてまいりました。しかし近年、非常に低い頻度ではありますが、治った後でも血液中に微量のB型肝炎ウイルスが見つかることが明らかとなってまいりました。

4.すぐにしないといけないことは？

→特にありません。**普段の生活に問題はありません。**

5.医療機関に受診する必要は？

→今回の検査結果をもって**医療機関（病院）を受診する必要はありません。**

ただし、今後 入院治療するような大きな病気（癌や移植など）に罹った場合は、念のため担当医師に今回の内容をお伝えください。

また、今回のお知らせに限らず、すべての方々が年に１回程度、健診などで肝機能検査を受けられることをお勧めします。

6.今後の献血は？

→非常に低い頻度ではありますが、治った後でも血液中に微量のＢ型肝炎ウイルスが見つかることが明らかとなってまいりました。（上記３．を参照ください。）

そこで患者さんへの万が一の感染を防ぐために、

これからの献血は控えていただきたくお願い申し上げます。

より安全な輸血のため、どうかご理解をお願いいたします。

＊血液センターでの検査は、安全な輸血のため少しでも疑わしいものも含めて「陽性」と判定しています。そのため、本来は陰性であるにもかかわらず、あたかも陽性と反応してしまうこともあります。その場合、医療機関で同様の検査を受けた結果、陰性となることもあります。

日本赤十字社
Japanese Red Cross Society

資料５

欧州調査報告　　運営委員会委員　岡田　義昭

出張者

三宅　智（厚生労働省血液対策課課長）、伯野　春彦（同課長補佐）

日野　学（日本赤十字社血液事業本部）、岡田　義昭（国立感染症研究所）

１．Sanquin研究所（オランダ）

オランダでは多数の血液バンクが存在したが、12年前にSanquin研究所が血液供給の唯一の事業体となった。

（FFP）

現在、不活化法は導入されていないがquarantineによって対応している。プリオン除去フィルターが開発され、vCJDリスクが軽減したためS/Dプラズマに変更する予定。

（血小板）

90％が全血由来で有効期間は7日間、全例に無菌検査が実施されている。不活化は 臨床治験は実施しているが導入されていない。理由として、有効期間が5日に短縮され、コストが高くなるため。

２．Federal Agency for Medicines and Health Products(ベルギー)

赤十字が全体の94％を採血している。他に病院と軍の血液バンクがある。

（FFP）

vCJDのリスクから個別のバックで処理できるメチレンブル（MB）法が2004年から導入され、現在95％以上がMB処理されている。特に副作用は多くない。S/Dプラズマは医薬品として登録されているが、使用量は少ない。

（血小板）

2010 年までに全ての血小板に不活化法を導入することが決定されたが、南半分でアモトサレン法が導入しているのみ。北部ではリボフラビン法を予定しているが現在のところ導入されていない。血小板の有効期間は７日間であるが、不活化処理された血小板は５日間である。また、不活化処理されていない血小板は全例、無菌検査を実施しているが、偽陰性のため感染例があった。

３．ワルシャワ血液センター（ポーランド）

ポーランド全体で21の地域にそれぞれ血液バンクがあり、他に警察と軍の血液バンクが存在する。訪問したバンクは、最も大きなバンクである。

（FFP）

2010年1月からリボフラビン法による不活化を導入した。当該施設が製造するFFPの60％が不活化処理されている。これまでに約6万単位のFFPを製造したが、有害事象の報告はない。

（血小板）

2009年7月からリボフラビン法による不活化処理を開始し、2011年12月までに15,700検体を不活化処理した。血小板の有効期間は5日間、導入前後で変えていない。有害事象の報告はない。

（リボフラビン法を導入した理由）

各種の不活化法を検討した結果、FFPやクリオにも使用できること、処理時間が10分（アモトサレン法は不活化処理時間に加えて、アモトサレンの除去に少なくとも4時間かかる）であること、処理しても凝固活性やフィブリノゲンの減少は認められなかったこと、等から選択した。

４．French Agency for the Safety of Health Products(Afssaps:フランス)

Etablissement Francais du Sang(EFS)が採血･供給事業を行い、Afssapsが監視･評価を行っている。

（FFP）

2008 年から導入され、S/D 処理、MB 処理、アモトサレン法の 3 つの方法が地域によってそれぞれ選択されている。もっとも多く MB が使用されていたが、アレルギーの頻度が高く、1 名が死亡（MB 処理が原因とは確定していない）したため供給を停止している。安全性の確保のために quarantine（２ヶ月後に再来した時に感染症マーカーが陰性であった場合に出庫）で対応しているが、将来的にはS/D プラズマとアモトサレン法の併用、又はS/D プラズマになる予定である。

（血小板）

フランス本土では、アルザス地方の血液センターでのみアモトサレン法による不活化を実施している。3つの海外県ではチクングニアウイルス対策等で導入しているが、今のところ全土に拡大する計画はない。なお、血小板の有効期間は5日間。

抗HBs人免疫グロブリン製剤の国内自給に係る原料血漿収集のための B型肝炎ワクチン接種プログラムに関するガイドライン案

1. 目的

平成 15 年 7 月に施行された「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）では、国内で使用される血液製剤が原則として国内の献血により得られた血液を原料として製造される体制の構築を目指すとされている。これに基づいて出された「血液製剤の安全性の向上及び安定供給の確保を図るための基本的な方針」（平成 20 年厚生労働省告示第 326 号）では、「アルブミン製剤及び免疫グロブリン製剤については、平成 25 年を目途に国内自給の達成を目指す。」こととし、特に、特殊免疫グロブリン製剤については、「特殊免疫グロブリン製剤等多くを輸入に依存している製剤については、国内自給の方策を具体的に検討していく必要がある。」並びに「国内での原料血漿確保の実現可能性を考慮しながら、国内製造の方策を引き続き検討していく。」ことが示されている。

現在、特殊免疫グロブリン製剤の一つである抗 HBs 人免疫グロブリン製剤（以下、HBIG）のごく一部は国内献血血漿を原料に製造されているが、これらの血漿は日本赤十字社が全献血者を対象に高力価の抗体を有する血漿を選別したものである。この受動的収集方法では限界があり、HBIG の自給に必要な原料血漿量を国内献血により確保するのは困難で、より積極的かつ能動的な収集法を考慮する必要がある。その一つとして、諸外国の例に倣いB型肝炎ワクチン（以下、HBワクチン）接種により抗体産生を促した献血者から血漿を集めることが考えられる。

これまで、厚生労働科学研究結果（医薬品・医療機器レギュラトリーサイエンス総合研究事業「抗HBs人免疫グロブリンの国内製造用原料血漿収集におけるB型肝炎ワクチン接種の有効性に係わる基礎的検討」（八橋班）、平成 22～23 年度実施）から、HB ワクチン接種により現在 HBs 抗体を保有している者（以下、ワクチン接種既往者）を対象に HB ワクチンを追加接種した場合、効率よく高力価抗 HBs 抗体産生者を見出し得ることが明らかにされている。

本ガイドライン案は、HBIG の原料血漿収集の目的と意義を理解し、無償かつ自発的に血漿提供者となることに同意したワクチン接種既往者へ HB ワクチンを追加接種し、抗体価の高い血漿を提供（以下、本プログラム）いただくための、倫理的かつ医療技術的に適切なストラテジー及び手順概要を定めたものである。

2. 本プログラムの基本ストラテジー

① 本プログラムは、「血液製剤は、国内自給が確保されることを基本とする」血液法の基本理念にのっとり、「基本的かつ総合的な施策を策定し、実施しなければならない。」責務を負う国の国庫補助事業として、日本赤十字社が実施主体となり行う。

② 日本赤十字社は国と共に、予め、医療機関等の関係機関（以下、医療関係施設）へ本プログラム事業の啓発と献血への協力の依頼を行う。（下記項目3参照）

③ 日本赤十字社血液センターは、献血時のスクリーニング検査結果を活用し、HBs 抗体保有献血者【HBs抗体 100mIU/mL 以上でかつ HBc抗体陰性】を抽出し、40歳以下の者を HB ワクチン追加接種候補者とする。（下記項目4参照）

④ 日本赤十字社および協力医療関係施設は、上記③の HB ワクチン追加接種候補者への具体的な説明により協力依頼を行い、本プログラムを実施する。（下記項目5、6参照）

⑤ 本プログラムによる高力価抗体保有該当者に対して、年2回以上の血漿成分献血の協力を依頼する。（下記項目7参照）

⑥ 現在の HBIG の自給率は 3%以下であることから、暫次委託する施設数を増やし、段階的に自給率の向上を図ることとする。

⑦ 平成27年度を目途に「母子感染による新生児のB型肝炎予防、およびHBs抗原陽性血液の汚染事故後のB型肝炎発症予防」に使われる筋注用製剤を、平成 30 年度を目途に「HBs抗原陽性のレシピエント、あるいはHBc抗体陽性ドナーからの肝移植後のレシピエントにおけるB型肝炎発症抑制」に使われる静注用製剤の国内自給達成を目指す。

3. 医療関係施設等への啓発及び協力依頼

医療関係施設の従事者または学生の多くは、就労または臨床実習開始時に HB ワクチンを接種している。これらの人は HBIG の必要性についても理解が深い。日本赤十字社は国と共に、医療関係施設に、HBIG の国内自給の現状と HBIG 用原料血漿収集の必要性について、文書等により説明し本プログラムへの理解と協力を求める。

本プログラムへの協力に賛同していただいた施設の長もしくはその権能を委譲された者は、自らが管轄する医療関係施設従事者等で、既に B 型肝炎ウイルスの針刺し事故等による感染予防のために B 型肝炎ワクチンを接種している40歳以下の者（接種回数は問わず）に対して、血液法の趣旨、HBIG 国内自給の現状とHBIG 原料血漿収集の必要性を説明し（参考：別紙 3、別紙 5）、成分献血（血漿・血小板の何れか）を促す（別紙 1）。

4. HB ワクチン追加接種者の要件と必要人数

これまでの厚生労働科学研究（八橋班）の結果から、抗 HBs 抗体価が 100mIU/mL 以上で、且つ HBc抗体陰性の者に HB ワクチンを追加接種すると HBIG 原料血漿基準を満たす高力価の HBs抗体が効率的に産生されることが分かっている。とりわけ 40 歳

以下では免疫応答性が優れている。したがって、HB ワクチン追加接種候補者は、次の条件を全て満たす者とする。

①40歳以下、②抗 HBs 抗体 100mIU/mL 以上、③HBc抗体陰性

なお、2010 年度の献血データでは、HBs抗体 200mIU/mL 以上かつ HBc抗体陰性（C.O.I＜1）の献血者は約 7.5 万人で、その多くは HB ワクチン接種既往のある医療従事者であると予想される。

また、平成 22 年度の HBIG 使用実績を基に HBIG 国内自給のための原料血漿量を確保するために必要な HB ワクチン接種を受ける対象者数は、数万人と試算される。但し、将来需要が変化する、あるいは新たな HBIG が開発された場合には血漿提供者数を適宜変更することもありうる。

5. 献血者の中からのワクチン追加接種候補者の選別等

① 日本赤十字社血液センター（以下、血液センター）は献血者の中から HB ワクチン追加接種候補者【（40歳以下、かつ、HBs抗体 100mIU/mL 以上、かつ、HBc抗体陰性（C.O.I＜1）】を抽出し、文書にて HBIG の国内自給の状況、原料血漿収集のための HB ワクチン追加接種の目的と意義を説明し、血液センターへの連絡を依頼する（別紙 2）。

② 連絡のあった献血者に血液センター等への来所を依頼し、医師が HBIG の国内自給の状況、原料血漿収集のための HB ワクチン再接種の必要性と意義、プログラムの具体的な内容を説明し（インフォームドコンセント、（別紙 3））、本プログラムへの参加協力を求める。

③ 協力する意向の献血者について書面にて同意を確認し、同意が得られた献血者をワクチン追加接種対象者とする。なお、同意書には下記の項目が記されており（別紙4）、3通綴りにして、本人、献血する血液センターが各1通保管し、残る1通はHB ワクチン接種施設への提出用とする。

1） HBIG の国内自給に必要な原料血漿収集のための B 型肝炎ワクチン接種に関して十分な説明を受け、その意義目的を理解したこと

2） HB ワクチンの追加接種を承諾すること（B 型肝炎ワクチン接種による副作用が生じうることを含む）

3） 追加接種後は4週間から2カ月以内に日本赤十字社血液センターにて血漿成分献血をすること

4） 十分な抗 HBs 抗体の上昇が確認された場合には血液センターに HBIG ドナーとして登録し、献血に協力すること

5） HBs 抗体価が下がった際には前回接種より 1 年以降に再度 HB ワクチン接種を受け、継続的に抗 HBs 人免疫グロブリン製剤の国内自給へ協力すること

6） HB ワクチン接種にかかる経費（当該ワクチン、注射・診療諸費用）は全て無料であること

7） HB ワクチン接種および採血に起因する健康被害が発生した場合には、補償が受けられること

8） 献血は自発的、無償で行われ、謝礼はないこと

④ 同意が得られた献血者について、健康保持の観点から、前回 HB ワクチン接種から 1 年以上経ち、HBs 抗体価が HBIG 原料血漿基準（10,000mIU/mL）未満であることを確認し、下記項目6に従い追加接種の手続きを進める。

6. HB ワクチンの追加接種

HB ワクチンの追加接種は、血液センターもしくは血液センターの依頼の下で協力する契約医療関係施設にて行う。

① 血液センターで HB ワクチン追加接種を行う場合は、前記項目5④の HB ワクチン追加接種対象者と調整し、指定場所（献血ルーム等）および指定日時を設定する。

② 契約医療関係施設で HB ワクチン追加接種を行う場合は、血液センターは前記項目5④の対象者と施設と調整し、追加接種する施設および日時を設定する。（要 施設宛送付；連絡書、費用請求様式等）

③ 追加接種に用いる HB ワクチンは、日本国において認可されている製品のうち、接種後の抗体価の上昇が高い製品を使用する。接種は添付文書の記載に従って行う。なお、HB ワクチン追加接種に起因すると考えられる健康被害等が発生した場合には、日本赤十字社が整備した民間保険による救済制度を適用する。また、同接種に起因すると考えられる副反応報告は、日本赤十字社にて情報を集積し、分析を行う。

④ 契約医療関係施設は、追加接種実施後、HB ワクチン代金を含めた経費を血液センターあてに請求する。なお、HB ワクチン追加接種にかかる費用として1人 5,000～5,500 円（ワクチン代金含む）相当が算定されるため、血液センターは国庫補助事業費から契約医療関係施設に必要な経費を支払う。

⑤ 血液センターは、ワクチン追加接種対象者が追加接種した事を確認し、接種後4週間から2カ月以内に血漿成分献血の予約を行う。

7. HB ワクチン追加接種者の血漿成分献血とドナー登録

① 血液センターは、前記項目6の対象者がワクチン追加接種後4週間から2カ月以内であることを確認し、日本赤十字社が定める採血手順にしたがって血漿成分採血を行う。なお、献血時の健康被害については、献血者健康被害救済制度が適応され

る。

② 献血時スクリーニング検査によって候補者が HBIG 原料血漿基準(10,000mIU/mL 以上)に該当することが確認された場合には、抗 HBIG 原料血漿提供者としてドナー登録(以下、登録者)する。また2週から1カ月以内での再献血を依頼し、承諾が得られたなら次回献血日を予約する。

③ さらに、血液センターは、献血6カ月以降1年以内に登録者に連絡して血漿成分献血を依頼し、献血の予約を確保する。

④ 血液センターは、登録者が上記②または③の予約にしたがって献血に訪れた時のスクリーニング検査で HBs 抗体価が HBIG 原料血漿基準を下回った際(10,000mIU/mL 未満)には、登録者に連絡し、前回ワクチン接種1年以降に再度ワクチン接種を依頼する(接種予定1カ月前に再度連絡)。

8. HB ワクチンの再接種

① 前記項目7の④の該当登録者は、上記項目6の手続きに従いワクチン再接種を受ける。

② 再接種は1年以上の間隔を空ける。

9. その他

① 血液センターは、HB ワクチン接種者の氏名、HB ワクチン接種後の抗体価、接種時の副作用情報について、また、登録者の経年的抗体価、献血時の副作用情報の情報について、各々データベース化し、さらに、献血者情報と統合して管理する。

② 今後、本プログラムに協力いただける方がより多くなるよう、日本赤十字社はワクチン接種場所や時間の配慮等、利便性の向上に努める必要がある。

以上

別紙 1

B 型肝炎ワクチン接種により HBs 抗体を保有されている方へ 成分献血を勧めていただく施設長へのお願い

－抗 HBIG の国内自給にかかる原料血漿収集のための B 型肝炎ワクチン接種プログラムに係る事前のお願い－

ご依頼する趣旨

血液製剤である抗 HBs 人免疫グロブリン(HBIG)は、わが国においては、①B 型肝炎母子感染予防、②B 型肝炎ウイルス汚染針による針刺し事故後の感染予防、③B 型肝炎患者の移植後の B 型肝炎ウイルス再活性化予防、などの目的をもった医療用医薬品として広く用いられています。

一方、「安全な血液製剤の安定供給の確保等に関する法律」では、国内で使用される血液製剤が原則として国内の献血により得られた血液を原料として製造される体制の構築を目指すとされています。しかし現在の献血者から選別する受動的収集法では、HBIG の国内自給率は 3%にも満たず、その多くは外国からの血液に依存しています。

したがって HBIG 自給に必要な量の原料血漿を国内献血により確保するためには、より積極的、能動的収集法を考慮する必要があると考えています。

その方法の一つとして、一定の抗体価を持つ方で同意が得られた献血者に B 型肝炎ワクチン(HB ワクチン)を接種して HBs抗体価を上昇させた後に献血いただく方法が考えられます。

今回お願いする内容

貴施設において、B 型肝炎ウイルスの針刺し事故等による感染予防のため、B 型肝炎ワクチンを接種した40歳以下の方＜接種回数は問わず＞)に対して、血液法の趣旨、HBIG 国内自給の現状と HBIG 原料血漿収集の必要性を説明(別紙 5(参考資料))し、成分献血(血漿・血小板の何れか)を促していただくようお願いいたします。

本プログラムの具体的内容について

別添えの参考資料(別紙 3 が相当)をご覧ください。

なお、HBワクチン追加接種は、貴施設で同ワクチンを接種後1年以上経過した後にお願いする日程を予定させていただきます。

別紙 2

平成 24 年　月　日

様

○○○○赤十字血液センター所長

抗 HBs人免疫グロブリン製剤製造のための血漿成分献血のお願い

日頃から献血にご理解ご協力を頂き、誠にありがとうございます。頂きました血液は輸血用血液として有効に使用させて頂いております。

さて、平成　年　月　日に献血して頂いた血液について検査を行いましたところ、あなたの血液にはB型肝炎ウイルスに対する免疫力があり、B 型肝炎ワクチンを接種された方と思われました。この免疫力は抗体と呼ばれる血液中のタンパク質で、体内に入ってきたB型肝炎ウイルスの感染を防ぐことができます。また、あなたの血液からB型肝炎の発症予防に有効な医薬品（抗 HBs人免疫グロブリン製剤）を製造出来る可能性があります。この製品を作るには特に強い抗体を持つ方の血液が必要ですが、そのような方は献血者 1 万人中 1～2 人と非常に少ないのです。

「安全な血液製剤の安定供給の確保等に関する法律」では、国内で使用される血液製剤は原則として国内の献血により得られた血液を原料として製造されると決められていますが、強い抗体を持つ方が少ないため、抗 HBs人免疫グロブリン製剤の原料となる血液の殆どは外国から輸入しています。外国ではB型肝炎ウイルスに対してある程度免疫力を持っている人に再度 B 型肝炎ワクチンを接種して、免疫力の高い血液を集めています。もし既に免疫力を持つあなたにワクチン接種のご協力が頂ければ、あなたの血液から貴重な抗 HBs人免疫グロブリン製剤を製造することが出来ます。是非「ワクチン接種とその後の血漿成分献血」にご協力頂きたくお願い申し上げます。

このお手紙を受け取られた際には、お手数ですが、下記血液センターまでご連絡下さい。より詳しくご説明を差し上げたいと存じます。

記

問い合わせ先　　○○○○赤十字血液センター　○○課
TEL：

別紙 3

抗 HBs 人免疫グロブリン製剤の国内自給にかかる原料血漿収集のための B 型肝炎ワクチン接種プログラムに参加いただく方への説明文書

B 型肝炎ワクチン接種の意義と目的

血液製剤である抗 HBs 人免疫グロブリン（HBIG）は、わが国においては、①B 型肝炎母子感染予防、②B 型肝炎ウイルス汚染針による針刺し事故後の感染予防、③B 型肝炎患者の移植後の B 型肝炎ウイルス再活性化予防、などの目的をもった医療用医薬品として広く用いられています。

一方、「安全な血液製剤の安定供給の確保等に関する法律」では、国内で使用される血液製剤が原則として国内の献血により得られた血液を原料として製造される体制の構築を目指すとされていますが、現在の献血者から選別する受動的収集法では、HBIG の国内自給率は 3%にも満たず、その多くは外国からの血液に依存しています。したがって HBIG 自給に必要な量の原料血漿を国内献血により確保するためには、より積極的、能動的収集法を考慮する必要があると考えています。その方法の一つとして同意が得られた献血者に B 型肝炎ワクチン（HB ワクチン）を接種して HBs抗体価が上昇した後に献血いただく方法が考えられます。特に HB ワクチン接種により現在 HBs 抗体を保有している方に再度ワクチンを接種した場合には、非常に効率よく高力価 HBs抗体保有者が見つかる事が明らかにされています。HBIG の国内自給には数万人の方の協力が必要です。

なお、HB ワクチン投与後の HBs抗体価は、自然感染例とは異なり時間とともに徐々に低下し、多くの例において数年以内に HBs抗体は消失します。しかし、今回、追加ワクチン投与をおこなった場合、HBs抗体価が上昇することから、追加ワクチンを投与しない場合に比較して HBs抗体の陽性時期が長期に持続することが期待されます。

本プログラム協力者に協力いただくこと

HBs 抗体を保有する事が確認されるも HBs抗体価が基準に満たない方で、プログラムへの参加に理解と同意が得られた方に対して、前回接種から 1 年以上経過していることを確認した後に HB ワクチンの接種を行ないます。そして、ワクチン接種4週間後から2カ月以内に血液センターにて血漿成分献血に協力していただきます。献血時のスクリーニング検査で十分な抗 HBs 抗体があることが確認された方には、日本赤十字社血液センターに献血者登録させていただきます。

本プログラムへのご参加の任意性と同意撤回の自由について

本プログラムへ参加するかどうかは、あなたの自由意思で決めていただくことになります。このプログラム参加されなくても、今後の献血に関係することで不利益を受けることは一切ありません。また一旦同意された後でもその同意をいつでも撤回することができます。この場合においても、今後の献血に関係することであなたが不利益を受けるは一切ありません。

ワクチン接種および採血によって健康被害が発生した場合の対処法

本プログラムで接種される HB ワクチンは、日本で市販され、あなたが過去に接種を受けたものと同じ、ないし類似のものです。またHBワクチンは20年以上にわたってわが国で数百万人に接種され、その安全性は証明されています。

しかし、本プログラムに起因すると考えられる健康被害が発生した場合には、日本赤十字社が整備した民間保険による救済制度、また成分献血時の健康被害については献血者健康被害救済制度が適応されます。また、同接種に起因すると考えられる副反応報告は、日本赤十字社にて情報を集積し、分析を行ないます。

個人情報保護について

本プログラム参加後の献血時の検体を含む個人情報は、日本赤十字社の管轄の下で、第三者から特定できない状態で厳重に管理いたします。個人情報の漏洩が起こることがないよう万全の体制で実施しておりますが、万一漏洩が起きた場合、参加された方々へのご迷惑が最少限となるよう日本赤十字社の責任の下で直ちに原因を特定し、再発防止を徹底いたします。

検体と記録の保管期間について

本プログラムにご参加された方の検体や記録は、通常の献血に準じて保管いたします。保管期間中は、それらを本プログラムの目的外に使用することはございません。保管期間終了後は匿名化を確認した後に廃棄いたします。

費用について

本プログラムに参加する際の HB ワクチン接種にかかる経費(当該ワクチン、注射・診療諸費用)は全て無料です。なお、献血に対する謝礼はございません。

以上の説明文書の中で、何か疑問点や不明な点がありましたら、ご遠慮なく担当医師にお尋ねください。

担当医師:　　所属　　職名　　氏名

別紙 4

同 意 書

○○○○赤十字血液センター所長 殿

私は、抗 HBs 人免疫グロブリン製剤の国内自給に必要な原料血漿収集のためのB型肝炎ワクチン接種プログラムに関して十分な説明を受け、その意義と目的を理解いたしました。

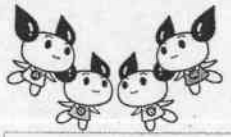
私はこの目的の為に下記の協力を行うことに同意します。

① B 型肝炎ワクチンの接種を受けること（ただし、前回接種から 1 年以上経過後）
② B 型肝炎ワクチンの接種４週間後から２カ月以内に、日本赤十字社血液センターにて血漿成分献血に参加すること
③ スクリーニング検査で十分な抗 HB s 抗体価が確認された場合には、抗 HBs 人免疫グロブリン製剤用原料血漿として使用されること
④ スクリーニング検査で十分な抗 HB s 抗体価が確認されなかった場合には、一般の分画原料血漿として使用されること
⑤ 献血血漿が抗 HBs 人免疫グロブリン製剤用原料血漿に使用された際には、HBIG ドナーとして日本赤十字社に登録されること
⑥ HBs 抗体価が下がった際には前回接種より 1 年以降に再度 HB ワクチン接種を受け、継続的に抗 HBs 人免疫グロブリン製剤の国内自給へ協力すること
⑦ B 型肝炎ワクチン接種にかかる経費(当該ワクチン、注射・診療諸費用)は全て無料であること
⑧ B 型肝炎ワクチン接種および採血に起因する健康被害が発生した場合には、無償で救済が受けられること
⑨ 献血は自発的、無償で行われること

本人署名欄＿＿＿＿＿＿＿＿＿＿＿＿

平成　　年　　月　　日

別紙5（参考資料）



－ お願い －

HBIG用原料血漿確保における B型肝炎ワクチン接種プログラムの必要性について

背景現状

・「抗HBs免疫グロブリン(HBIG)」製剤の用途は、①垂直母子感染の予防、②HBV血液針刺事故の発症予防、③肝移植時B型肝炎の再発抑制、です。

・しかし、残念ながら一般の献血から自然に確保できる率はごく僅かで、現在、その原料となる血液は、97%以上が輸入に頼っている状況です。

方針

・H15年に施行された「血液法」において、血液製剤が国内で安定的に供給されるために「国内自給化の推進」が定められました。

・また、H20年に改正された同法の基本方針には「特殊免疫グロブリン(HBIGを含みます。)の国内自給化の方策を具体的に検討して行く必要がある。」旨が明記されました。

今回のお願い

・そこで、欧米諸国においては既に実績のある方法があり、ワクチンを積極的に付与して、その方々から原料となる血液を確保して行こうというものです。

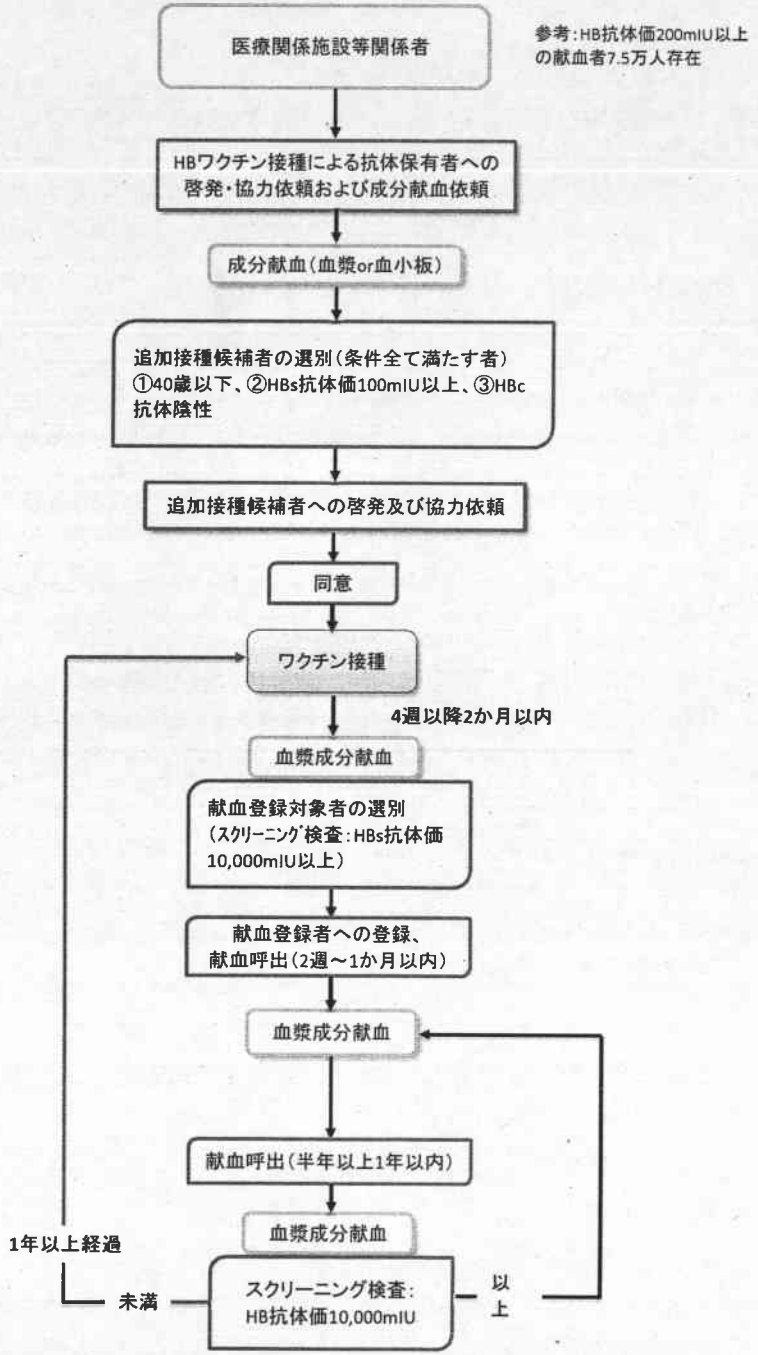
・この方法では、ワクチン接種によって抗体をお持ちの方が、より高い効果(抗体を産生する)があることが研究的にわかっています。

・従いまして、今回、貴施設でB型肝炎ウイルスの針刺し事故感染予防用のB型肝炎ワクチンを接種した４０歳以下の方に、成分献血（血漿・血小板）をお願いいたします。

・なお、献血時検査結果で一定以上の高い抗体価をお持ちの方については、改めて本プログラムのご参加のお願いさせていただくことを予定しています。

日本赤十字社
Japanese Red Cross Society

厚労科学研究（八橋班）：HBワクチンプロジェクト・ガイドライン案のフロー概要



資料7

「血液製剤の安全性の向上及び安定供給の確保に関する基本的な方針」の改訂スケジュール（案）

	作業内容
平成24年6月	↕ 改訂（案）作成
7月	
8月	
9月	第2回運営委員会 ●改訂（案）協議
10月	↕ 改訂（案）修正
11月	
12月	第3回運営委員会 ●改訂（案）協議 第1回血液事業部会 ●改訂（案）審議
平成25年1月	↕ 改訂（案）修正
2月	↕ 改訂（案）について意見募集（30日間）
3月	第2回血液事業部会 ●改訂（案）審議 薬事分科会 ●報告
4月以降	告示、施行

参考

○血液製剤の安全性の向上及び安定供給の確保を図るための基本的な方針

（平成二十年厚生労働省告示第三百二十六号）

安全な血液製剤の安定供給の確保等に関する法律（昭和三十一年法律第百六十号）第九条第三項の規定に基づき、血液製剤の安全性の向上及び安定供給の確保を図るための基本的な方針（平成十五年厚生労働省告示第二百七号）の全部を次のように改正し、平成二十年七月三十日から適用する。

血液製剤の安全性の向上及び安定供給の確保を図るための基本的な方針

我が国の血液事業は、昭和三十九年の閣議決定を契機として、関係者による多大の努力が積み重ねられてきた結果、輸血用血液製剤については昭和四十九年以降、国内自給が達成されている。しかしながら、血漿分画製剤に関しては、一部の製剤について、国内自給率は上昇してきたものの、その他の製剤についてはいまだ相当量を輸入に依存している状況にある。このような現状を踏まえ、血液製剤（安全な血液製剤の安定供給の確保等に関する法律（昭和三十一年法律第百六十号。以下「法」という。）第二条第一項に規定する血液製剤をいう。以下同じ。）の安定的な供給が確保され、かつ、国内自給が推進されるよう一層の取組を進めることが必要である。

我が国は、過去において、血液凝固因子製剤によるＨＩＶ（ヒト免疫不全ウイルス）感染問題という、深甚な苦難を経験しており、これを教訓として、今後、重大な健康被害が生じないよう、血液製剤の安全性を向上するための施策を進めることが必要である。

本方針は、これらの経緯等を踏まえ、法の基本理念である血液製剤の安全性の向上、献血によって得られた血液による国内自給の確保、血液製剤の安定供給、適正使用の推進及び公正かつ透明な血液事業の実施体制の確保を図るため、法第九条第一項に基づき策定する基本的な方針であり、今後の血液事業の方向性を示すものである。血液事業は、本方針、本方針に基づき定める献血推進計画及び需給計画、都道府県が定める都道府県献血推進計画並びに採血事業者が定める献血受入計画に基づいて一体的に進められることが必要である。

本方針は、血液事業を取り巻く状況の変化等に的確に対応する必要があること等から、法第九条第三項に基づき、少なくとも五年ごとに再検討を加え、必要があると認めるときは、これを変更するものとする。

第一　血液製剤の安全性の向上及び安定供給の確保に関する基本的な方向

一　基本的な考え方

血液製剤は人体から採取された血液を原料とする有限で貴重なものであることを、まず十分認識することが必要である。

国並びに地方公共団体（都道府県及び市町村をいう。以下同じ。）、採血事業者、製造販売業者等（製造販売業者、製造業者及び販売業者をいう。以下同じ。）、医療関係者など血液事業に関わる者は、法に基づき課せられた責務を確実に果たすとともに、法に掲げられた以下の四つの基本理念の実現に向け、各般の取組を進めることが必要である。

1　安全性の向上

血液製剤は医療の領域に多くの成果をもたらしてきており、また、科学技術の進歩により、病原体の発見、その検査法や不活化技術の開発・導入等を通じ、血液製剤を介して感染症等が発生するリスクは著しく低減してきている。しかし、人の血液を原料として製造されていることから、当該リスクを完全には否定できない可能性があること、製造過程における病原体の不活化処理等には限界がある場合があることなどの特徴を有する。このため、常に最新の科学的知見に基づき、血液の採取から製造、供給、使用に至るまで、一貫した遡及調査体制を構築するなど、安全性の確保及びその向上に向けた不断の努力が必要である。

これまで、血液製剤については、薬事法（昭和三十五年法律第百四十五号）に基づき、その安全性の確保を図ってきたところであるが、我が国は、過去において、血液凝固因子製剤によるＨＩＶ感染問題という、深甚な苦難を経験しており、より一層の安全確保対策の充実が求められている。国は、平成十四年七月に公布された薬事法及び採血及び供血あつせん業取締法の一部を改正する法律（平成十四年法律第九十六号）を踏まえ、安全性情報の収集・評価等の安全対策が迅速かつ的確に行われ、常にその実効性が検証されるような体制によって血液事業を運営していくこととする。

2　国内自給の原則と安定供給の確保

法第三条第二項において血液製剤の国内自給が確保されることを基本とすることが規定されているとおり、倫理性、国際的公平性等の観点に立脚し、国内で使用される血液製剤が、原則として国内で行われる献血により得られた血液を原料として製造され、海外の血液に依存しなくても済む体制を構築すべきである。このため、中期的な需給見通しに基づき、有限で貴重な血液製剤を献血により確保し、医療需要に応じて過不足なく安定的に供給するとともに、血液製剤の適正使用を推進する必要がある。

特に、血漿分画製剤については、供給の見通しを踏まえた検討を行った上で、毎年度、需給計画を定めることにより、安定的な供給を確保するものとする。

3　適正使用の推進

医療関係者は、血液製剤が人の血液に由来する有限で貴重なものであること及び原料に由来する感染のリスク等について特段の注意を払う必要があることを十分認識し、患者に真に必要な場合に限って血液製剤を使用するなど、適切かつ適正な使用を一層推進する必要がある。これは国内自給及び安定供給の確保の観点からも重要である。

このため、医療機関において、血液製剤の管理体制を整備し、血液製剤の使用状況を正確に把握するなど、血液製剤の適正な使用を推進する必要がある。

また、国は、血液製剤の適切かつ適正な使用を推進するため、血液製剤の適正使用や輸血療法の実施等に関する指針を状況の変化に応じて改定し、その普及を図るとともに、医療機関における血液製剤の使用状況について定期的に評価を行うなど、適正使用を更に促進するための方策を講ずることとする。

4　公正の確保及び透明性の向上

国、地方公共団体、採血事業者、製造販売業者等、医療関係者など血液事業に関わる者は、献血者の善意にこたえ、国民の理解と協力を得ることができるよう、献血の推進、適正使用の推進等血液事業に係る施策の策定及び実施に当たり、血液製剤の安全性や供給の状況等につき、十分な情報を公開する必要がある。

また、国、地方公共団体その他の血液事業に関わる者は、血液事業の公正かつ透明な運営を確保するものとする。

二　血液製剤代替医薬品の取扱い

用法、効能及び効果について血液製剤と代替性がある医薬品（以下「血液製剤代替医薬品」という。）についても、その安全性の確保及び向上が必要である。

また、血液製剤代替医薬品は、安定供給を確保するため、計画的に製造及び供給が行われる必要があるとともに、それぞれの患者への必要に応じて、適切かつ適正に使用されることが求められる。

血液製剤代替医薬品の安全性や供給の状況等についても、血液製剤と同様に十分な情報を公開する必要がある。

三　国民の理解と参加

国民一人一人が、献血に由来する血液製剤を用いた医療が提供されることによって生命と健康が守られているということを理解し、積極的に献血に協力すること等を通じ、国民が今後の血液事業の健全な展開に参加することが期待される。

こうした国民の血液事業への参加を促すため、血液事業に関わる者は、国民に対し、血液事業や血液製剤を用いた医療に関する分かりやすい情報の積極的な提供に努めることが必要である。

第二　血液製剤についての中期的な需給の見通し

血液製剤及び血液製剤代替医薬品の需給動向を勘案しつつ、それらの中期的な需給の見通しとして、平成二十五年度までの今後五年間の状況について考察する。

一　輸血用血液製剤

輸血用血液製剤は、昭和四十九年以降、すべて国内献血で賄われている。平成十九年においては、全血製剤、赤血球製剤、血小板製剤及び血漿製剤について、血液量に換算して合計八十五万リットルが供給されており、血漿分画製剤の原料血漿を含め、約四百九十四万人の献血者からの血液によって供給された。

輸血用血液製剤は、引き続き医療需要に応じた供給が確保される必要がある。また、献血者の確保のための努力が続けられる一方で、血液製剤の適正使用の推進がさらに図られることにより、医療に必要な輸血用血液製剤は今後とも国内献血で賄われると見込まれる。

二　血漿分画製剤

1　原料血漿

原料血漿については、毎年度、需給計画において翌年度に確保されるべき原料血漿の量の目標を定めた上で、計画的に原料血漿を確保し、供給している。平成十八年度の原料血漿確保目標量は九十三万リットル、平成十九年度は九十七万リットルと定め、原料血漿の確保を行ったことにより、これまで需要に見合う供給が行われてきている。過去の供給状況等を勘案すると、平成二十五年度において百二十万リットル程度までの量が供給可能と予測され、血液製剤代替医薬品の供給状況にもよるが、今後とも、需要に見合う供給が可能であると見込まれる。

2　免疫グロブリン製剤及びアルブミン製剤

血漿分画製剤のうち、免疫グロブリン製剤及びアルブミン製剤の供給量は、製造に要する原料血漿量に換算して、平成十九年においてそれぞれ九十六万リットル及び百五十七万リットルであり、うち国内献血に由来するものの供給量は、それぞれ九十一万リットル及び九十八万リットルである。

これらの製剤の今後の需要予測は、過去の使用状況等を勘案すると、製造に要する原料血漿量に換算して、平成二十五年度においてそれぞれ九十四万リットル～九十八万リットル程度及び百二十五万リットル～百二十八万リットル程度であると見込まれ、これらは国内の製造業者の現在の製造能力約百三十万リットルを超えないものである。

原料血漿の供給量及び血漿分画製剤の国内製造業者の製造能力等を勘案すると、今後は、遺伝子組換え製剤の開発も重要な課題である。

3　血液凝固因子製剤等

血液凝固第Ⅷ因子製剤（遺伝子組換え製剤を除く。）及び血液凝固第Ⅸ因子製剤（複合体を除く。）は、すべて国内献血で賄われている。

これらの製剤については、今後とも国内自給が確保されることが見込まれるが血漿由来製剤及び遺伝子組換え製剤の特性及び副作用の発現状況並びに危機管理的な対応を考慮した製造体制及び製造能力の確保が必要であり、国内献血由来製剤を一定量確保する必要がある。

なお、特殊免疫グロブリン製剤等多くを輸入に依存している製剤については、国内自給の方策を具体的に検討していく必要がある。

三　血液製剤代替医薬品

血液凝固第Ⅷ因子製剤については、血液製剤代替医薬品として、血液に由来する製剤の外に遺伝子組換え製剤が輸入により供給されている状況にある。なお、将来的には遺伝子組換え第Ⅷ因子製剤の国内での製造の可能性も検討する必要がある。

また、新たに開発された遺伝子組換えアルブミン製剤について承認がなされたところであり、今後、徐々に供給されていくと見込まれるが、当該製剤の製造及び供給状況を確認していくことが必要である。

第三　血液製剤に関し国内自給が確保されるための方策に関する事項

一　基本的な考え方

血液製剤は安全性の向上に常に配慮しつつ安定的に供給されなければならず、かつ、国内の献血に基づく国内自給が確保されることを基本とするものである。このことから、平成十九年現在、国内自給を達成している輸血用血液製剤、血液凝固第Ⅷ因子製剤（遺伝子組換え製剤を除く。）及び血液凝固第Ⅸ因子製剤（複合体を除く。）に加え、アルブミン製剤（遺伝子組換え製剤を除く。）及び免疫グロブリン製剤等の血液製剤についても、平成二十五年を目途に国内自給の達成を目指すものとする。なお、アルブミン製剤（遺伝子組換え製剤を除く。）の国内自給については、今後の遺伝子組換えアルブミン製剤の供給状況も影響することに留意する必要がある。

また、アルブミン製剤（遺伝子組換え製剤を除く。）及び免疫グロブリン製剤等の血漿分画製剤については、国内自給を推進するために、国内の需要を満たすために必要な献血量の確保、原料血漿の有効利用、献血由来原料血漿を使用した生産の拡大、医療関係者に対する献血由来製剤の意義の啓発、患者への情報提供、血液製剤の適正使用の推進等の方策を各関係者が実践して取り組むことが必要である。

なお、特殊免疫グロブリン製剤については、国内での原料血漿確保の実現可能性を考慮しながら、国内製造の方策を引き続き検討していく。

二　国内自給が確保されるための具体的な方策

1　献血量の確保

国、地方公共団体及び採血事業者は、第二に示した血液製剤についての中期的な需給の見通しを踏まえ、第四に示すとおり、計画的な献血の推進に努め、血液製剤の国内自給のために必要な献血量を確保することが求められる。

2　国内における献血由来製剤及び血液製剤代替医薬品の製造と供給

国、採血事業者、製造販売業者及び製造業者は、第五に示すとおり、国内の献血により得られた血液及び原料血漿がすべて有効に利用され、医療需要に応じて、血液製剤として国内に過不足なく供給されるよう、血液製剤の国内自給に向けた製造及び供給のための体制を整備し、血液事業の安定的な運営を通じて、血液製剤の安定供給を確保する必要がある。

このため、採血事業者、製造販売業者及び製造業者は、採血から製造及び供給に至るすべての段階において、事業の最大限の効率化及び合理化を図ることが必要である。

また、国は、国内自給を推進するに当たって、採血事業者、製造販売業者等、患者又はその家族、医療関係者、献血者等血液事業に関わる者の意見を十分踏まえるとともに、遺伝子組換えアルブミン製剤の開発状況及び承認後の供給状況、国内の献血に由来する血液製剤及び輸入される血液製剤の供給をめぐる動向等も十分に考慮するものとする。

3　医療関係者等に対する啓発等

国、地方公共団体、採血事業者及び製造販売業者等は、医療関係者及び患者に対し、国内の献血により得られた血液に由来する製剤の意義についての啓発に取り組

む必要がある。

医療関係者においては、献血により確保されている血液製剤は貴重なものであることを含め、そのような血液製剤に関して、患者への分かりやすい情報提供に努めることが重要である。

4　適正使用の推進

免疫グロブリン製剤の使用量は近年やや増加傾向にあり、今後とも適切かつ適正な使用の推進が求められる。アルブミン製剤の使用量は、適正使用の推進の結果として、減少傾向にあるものの、引き続き適正使用が図られる必要がある。

医療機関においては、血液製剤の適正使用の一層の推進に努めることが、アルブミン製剤等の国内自給を推進する方策としても重要である。

第四　献血の推進に関する事項

一　基本的な考え方

国、地方公共団体、採血事業者、献血推進協議会、民間の献血推進組織等は、本方針及び献血推進計画を踏まえ、協力して、相互扶助及び博愛の精神に基づき、献血推進運動を展開する必要がある。また、その際には、献血について国民に正確な情報を伝え、その理解と協力を得る必要がある。

中長期的な課題として、今後の人口動態を考慮すると、献血可能人口が減少すると推定されていることから、血液製剤の安定供給には国民一人一人の一層の協力が不可欠であると考えられる。こうした状況にかんがみ、献血についての理解を広め、献血者を増やすため、幼少期も含めた若年層を中心に普及啓発を一層推進する必要がある。

また、四〇〇ミリリットル全血採血及び成分採血は、献血量を確保しやすくなるとともに、感染症等のリスクを低減させるなどの利点があるため、今後も、一層の普及が必要である。

なお、献血者の理解を深めるためには、血液製剤の使用状況に関する情報提供や医療機関における患者等への説明等を通じ、血液製剤の適正使用に関する理解を得ることも重要である。

また、血液製剤、特に赤血球製剤の安定供給を確保するためには、国、都道府県及び採血事業者は、在庫水準を常時把握し、在庫が不足する場合には供給に支障を及ぼす危険性を回避するよう早急な対策を講ずることが必要である。

さらに、国及び地方公共団体は、あらかじめ災害時の対応を検討するとともに、災害時における献血が確保され、血液が適切に供給されるよう所要の措置を講ずるものとする。採血事業者は、あらかじめ災害時における献血受入体制を構築し、各採血所間における需給調整が迅速にできるよう備えることにより、災害時における献血量の確保に協力する必要がある。

二　献血推進計画及び都道府県献血推進計画

国は、献血により確保すべき血液の目標量、その目標量確保のための基本的な施策、献血の推進に関する事項について、毎年度、薬事・食品衛生審議会（以下

「審議会」という。）の意見を聴いて献血推進計画を策定し、公表する。また、献血推進計画に基づき、国民の献血への理解と協力を得るための教育及び啓発、採血事業者による献血の受入れや献血者の保護に対する協力等を行う。

都道府県は、本方針及び国の定める献血推進計画に基づき、毎年度、血液製剤の需給の状況、適正使用の推進状況、人口動態等を考慮して、効果的な都道府県献血推進計画を策定し、公表する。また、住民の献血への理解を深めるための広報、献血推進組織の育成、献血の受入れの円滑な実施等の措置を講ずることが重要である。

市町村は、国及び都道府県とともに献血推進のための所要の措置を講ずることが重要である。

三　献血受入計画

採血事業者は、本方針及び国の定める献血推進計画に基づき、毎年度、献血受入計画を作成し、国の認可を受けなければならない。事業の実施に当たっては、献血受入体制を着実に整備し、献血の受入れに関する目標を達成するための措置を講ずることが必要である。例えば、採血時の安全性の確保、事故への対応、献血者の個人情報の保護、採血による献血者等の健康被害の補償等献血者が安心して献血できる環境の整備、採血に際しての血液検査による健康管理サービスの充実及び献血者登録制度による献血者との連携の確保を図ることが重要である。また、希少血液の確保に引き続き取り組むことが求められる。

四　献血推進施策の進捗状況等に関する確認及び評価

国及び地方公共団体は、献血推進施策の進捗状況について確認及び評価を行うとともに、採血事業者による献血の受入れの実績についての情報を収集する体制を構築し、必要に応じ、献血推進施策の見直しを行うことが必要である。

第五　血液製剤の製造及び供給に関する事項

一　基本的な考え方

血液製剤は安定的に供給されなければならないことから、血液製剤の供給に当たっては、緊急時の輸入、国内で製造が困難な血液製剤の輸入等やむを得ない場合を除き、海外の血液に依存しなくても済むよう、原則として国内の献血に基づく国内自給を推進することが求められる。また、国内の献血によって得られた血液が有効に利用され、血液製剤として安定的に供給される必要がある。さらに、一部の製剤で供給に支障が生じるような緊急事態に対応できるよう、製造販売業者等は所要の在庫を保有しておくことが重要である。

このため、保健衛生上の観点から、医療に必要な血液製剤を確保して安定的に供給するために、厚生労働大臣が製造、供給等の需給動向を適時適切に把握する必要のある血漿分画製剤については、血液製剤代替医薬品を含め、法第二十五条に基づき、第二に示した中期的な需給の見通しを踏まえ、需給計画を策定し、公表するものとする。なお、輸血用血液製剤については、災害時等の緊急的な対応を常に考慮しつつ、その需給が季節的に変動すること等も踏まえ、献血推進計画

等により、安定的な供給を確保する必要がある。

二　血液製剤の安定供給の確保のための需給計画

需給計画を策定する際には、当該血漿分画製剤の需給動向のみならず、その製造に使用する原料血漿の量の動向、当該製剤に代替する医薬品、治療法等を考慮し、審議会の意見を聴いて策定する。

血漿分画製剤の製造販売業者等は、需給計画に沿って、計画的に血漿分画製剤の製造及び供給に取り組む必要があるとともに、その製造実績等を厚生労働大臣に報告することが必要である。厚生労働大臣は、当該報告を受け、安定供給の確保の観点から、需給計画を尊重して適正に製造及び供給が行われるよう、必要に応じ勧告等の措置を講ずるものとする。

また、国内の献血に由来する血液製剤を取り扱う製造販売業者等は、その供給の確保に努めることが重要である。

なお、国内の献血に由来する原料血漿を一旦海外へ輸出して外国の工場において製剤化して日本へ輸入する血液製剤を取り扱うことが特に必要であるとされた場合には国内での安定供給及び国内自給の推進と両立する範囲内において実施することについて、課題毎に具体的な検討が必要である。

三　原料血漿の配分

国は、製造販売業者及び製造業者の製造能力及び製造効率を勘案し、安定供給に必要な血漿分画製剤の適正な水準の生産が確保されるよう、審議会における公正かつ透明な審議を踏まえ、需給計画において採血事業者から製造販売業者及び製造業者への血漿の配分量及び配分する際の標準価格を規定するものとする。

採血事業者、製造販売業者及び製造業者は、需給計画を尊重して原料血漿を配分することが必要であり、厚生労働大臣は、計画が尊重されているかを把握するため、原料血漿の配分結果の報告を求めるものとする。

四　血液製剤の製造及び供給の在り方

血液製剤の今後の製造及び供給の在り方については、「血漿分画製剤の製造体制の在り方に関する検討会」での議論を踏まえ、安定供給の確保の観点から血液事業が安定的に運営できるよう、各関係者が取り組むことが必要とされる。また、その際には、輸血用血液製剤及び血漿分画製剤がともに人の血液に由来する有限で貴重なものであり、安全性の向上、安定供給の確保、適正使用の推進等の点で他の医薬品とは異なる性格を有するものであることを十分踏まえた取扱いが必要である。

なお、国は、災害等の場合にあっても、血液製剤の供給に支障を来すことがないよう、製造販売業者及び製造業者による安定供給に必要な量の備蓄の状況等に関し、適宜、確認を行うなど、その安定供給を確保することとする。

第六　血液製剤の安全性の向上に関する事項

一　安全性の向上のための取組

薬事法に基づき、生物由来製品について、その感染のリスク等を踏まえ、原材

料の採取及び製造から市販後に至る各段階において、一般の医薬品等における各種基準に加え、以下に掲げる基準等が定められた。これらを柱として、血液製剤の一層の安全性の確保を図ることとする。

1　原材料採取の方法等について保健衛生上の観点から定める品質等の付加的な基準

2　構造設備、製造管理及び品質管理の方法について、その特性に応じた付加的な基準

3　直接の容器又は直接の被包等において、感染のリスク等を有することから適正に使用すべき医薬品等であることを明らかにするため、安全性の確保に関し必要な付加的な表示を行うこと。

4　病原体の混入が判明した場合に遡及調査を速やかに講ずることを可能とするため、製造販売業者等及び医療関係者は必要な事項について記録を作成し、保存すること。

製造販売業者及び外国特例承認取得者は、薬事法第六十八条の八に定める感染症定期報告を行うことが必要であり、製造業者は、特定生物由来製品について、遡及調査のために必要な量を適切に保存することが必要である。

医療関係者は、特定生物由来製品を使用する際には、原材料に由来する感染のリスク等について、特段の注意を払う必要があることを十分認識する必要がある。また、薬事法第六十八条の七に基づき、その有効性及び安全性その他当該製品の適正な使用のために必要な事項について、患者又はその家族に対し、適切かつ十分な説明を行い、その理解と同意を得るよう努めるものとする。

都道府県及び保健所を設置する市（特別区を含む。以下「都道府県等」という。）は、必要に応じ、医療関係者が安全対策を適切に実施するよう、指導に努めることが重要である。

採血事業者は、血液製剤を介して感染症等が発生するリスクをできる限り排除するために、献血時における問診の充実を図ることが必要である。また、国、地方公共団体及び採血事業者は、あらかじめ献血者に対し、検査を目的とした献血を行わないよう周知徹底する必要がある。

医療関係者は、血液製剤の免疫学的副作用の発生にも留意する必要がある。

なお、血液製剤代替医薬品についても、安全性の確保を図ることが重要である。

二　迅速かつ適切に安全対策を実施するための体制整備

国、採血事業者、製造販売業者等及び医療関係者は、感染症等、血液製剤に係る安全性に関する情報を把握し、その情報を評価し、安全対策の実施を迅速かつ適切に行うとともに、遡及調査を速やかに実施できる体制を整えることが必要である。

血液製剤の安全性に関する情報については、審議会において、専門家、患者等と遅滞なく情報を共有するとともに、国民に対し適時適切かつ迅速に情報を公開し、提供するものとする。

三　血液製剤の使用により感染症の発生等が判明した場合の対応

国は、血液製剤の使用により、感染症等の保健衛生上の危害の発生又は拡大を防止するため必要があると認めるときは、遡及調査を速やかに実施し、ほかの患者等への健康被害が拡大しないよう、薬事法第六十九条の三に基づく製品の販売等の一時停止や同法第七十条第一項及び第二項に基づく回収等の措置を講ずることとする。また、患者又はその家族に対する不利益や偏見、差別に配慮しつつ、患者又はその家族を始めとする国民や医療機関等へ各種の手法により迅速に情報を提供するとともに、原因の究明、改善の指示等を行うものとする。

四　安全性の向上のための技術の開発促進及び早期導入

製造販売業者等は、病原体の不活化・除去技術の向上、より高感度かつ高精度の検査方法の開発等を通じ、より安全性の高い血液製剤の開発等に努めることが必要である。

また、国は、血液製剤の安全性の向上に係る技術に関する情報を収集し、技術開発を支援し、採血事業者及び製造業者がそれらの技術を早期導入するように指導するものとする。

五　自己血輸血等の取扱い

輸血により、感染症、免疫学的副作用等が発生するリスクは完全には否定できない可能性があることから、自己血輸血は推奨される手法である。自己血輸血を行う際は、法第二十四条第二項に基づき定める基準及びその実施に関する指針に沿って適切に行う必要がある。

また、自己血輸血を除き、院内血輸血は、安全性の問題及び患者又はその家族に対する負担の問題があることから、原則として行うべきではない。

第七　血液製剤の適正な使用に関する事項

一　血液製剤の適正使用の推進

医療関係者は、血液製剤の特徴を十分に理解し、その適正な使用を一層推進する必要がある。また、医療関係者に対する教育、研修等、様々な機会を通じて働きかけていくことが重要である。

国は、血液製剤の適正使用、輸血療法の実施等に関する指針を医療機関に示してきたところであるが、医療機関における血液製剤の使用状況等について報告を求め、定期的に評価し、必要に応じて当該指針を見直す等、適正使用の推進のためのより効果的な方法を検討するものとする。

二　院内体制の整備

医療機関においては、血液製剤を用いた医療が適正になされるよう、院内の血液製剤を管理し、使用するための体制を整備することが重要である。このため、国及び都道府県等は、そのような医療機関に対し、様々な機会を通じて、院内における輸血療法委員会及び輸血部門の設置並びに責任医師の任命を働きかけるものとする。

三　患者等に対する説明

医療関係者は、それぞれの患者に応じて血液製剤の適切な使用に努めることが

重要であり、患者又はその家族に対し、血液製剤に関して適切かつ十分な説明を行い、その理解と同意を得るよう努めるものとする。

第八　その他献血及び血液製剤に関する重要事項

一　血液製剤代替医薬品に関する事項

遺伝子組換え血液凝固第Ⅷ因子製剤をはじめとする血液製剤代替医薬品は、血液製剤の需給動向に重要な影響を与えるため、第五に示したとおり、その計画的な製造及び供給が行われる必要がある。

また、血液製剤代替医薬品の安全対策については、第六に示した薬事法に基づく規制を適用することとする。なお、患者又はその家族への説明及び同意あるいは記録の保存等についても、必要に応じ、特定生物由来製品と同様に行うことが求められる。

血液製剤代替医薬品は、血液に由来する製剤と同様に、それぞれの患者に応じ適切に、また適正に使用することが求められる。

二　採血基準の見直し

四〇〇ミリリットル全血採血等の対象年齢等を規定している採血基準に関しては、献血により得られる血液量の確保とともに、献血者の安全確保を図るために、体重、採血間隔、血中ヘモグロビン値、比重等のデータや新たな感染症の発生状況等の最新の科学的知見に基づき、諸外国の状況も勘案し、専門家の意見を聴きながら、採血基準の見直しを行うことが必要である。

三　血液製剤の表示

血液製剤については、患者又はその家族の選択の機会を確保するため、製造販売業者等は、直接の容器又は直接の被包に、採血国及び献血又は非献血の区別を表示することが必要である。

また、血液製剤代替医薬品のうち、特定生物由来製品についても、採血国及び献血又は非献血の区別を表示することが必要である。

四　血液製剤等の研究開発の推進

血液製剤の安全性の向上の観点から、国は、血液製剤の安全性の向上に係る技術開発の支援等を行い、製造販売業者等は、より安全性の高い血液製剤の開発等に努めることが必要である。

また、血液製剤の安定供給及び国内の献血に基づく国内自給等の観点から、原料血漿の供給量、血漿分画製剤の国内製造業者の製造能力等を勘案すると、今後とも、遺伝子組換え製剤等の血液製剤代替医薬品の開発は重要な課題である。

いわゆる人工血液等、新たに開発される血液製剤代替医薬品については、血液製剤との比較において優れた安全性及び有効性を有するものの製品化が促進されるよう、研究開発を推進する必要がある。

五　研究開発等における血液製剤の使用に関する基準の策定

国民の善意の献血によって得られる血液を主たる原料とする血液製剤は有限で貴重なものであり、研究開発等の使用に当たっても、倫理的な観点からの慎重

な配慮が必要である。血液製剤の適用外使用により、本来の効能及び効果を目的として供給される血液製剤が不足したり、医療に支障を生じることがあってはならない。

しかしながら、研究開発等に当たり、人の血液を使用せざるを得ない場合もあるため、本来の効能及び効果を目的とした血液製剤の供給に支障を生じないよう、国は、研究開発等における血液製剤の使用に関する基準を策定し、これを様々な機会を通じて医療関係者等に徹底させるものとする。

資料８

Press Release

日本赤十字社
Japanese Red Cross Society

平成 24 年 5 月 8 日

報道関係　各位

日 本 赤 十 字 社
田辺三菱製薬株式会社

血漿分画事業に係る統合新法人「一般社団法人 日本血液製剤機構」の設立について

日本赤十字社（本社：東京都港区、社長：近衞 忠煇）と田辺三菱製薬株式会社（本社：大阪市中央区、代表取締役社長：土屋 裕弘）は、平成 23 年 6 月 17 日の基本合意に基づき、日本赤十字社の血漿分画事業部門と田辺三菱製薬株式会社の完全子会社で、血漿分画製剤の製造販売会社である株式会社ベネシスの血漿分画事業を統合するため、鋭意検討を進めてまいりました。

今般、新法人「一般社団法人 日本血液製剤機構」を設立し、両社が持つ血漿分画事業を各々が拠出または事業譲渡することにより、平成 24 年 10 月 1 日から事業を開始することに合意しましたので、お知らせいたします。

日本血液製剤機構は、献血者の善意に基づき無償で得られた血液を原料とした血漿分画製剤による国内自給達成という、公益性の高い目標のために取り組みます。また、事業統合で得られるスケール・メリットを生かし、生産段階および供給段階でのコストを低減させ、事業の健全性を確保してまいります。

日本赤十字社および田辺三菱製薬株式会社は、日本血液製剤機構が、安全な血液製剤の安定供給の確保等に関する法律の基本理念に則り、血液製剤の国内自給の達成に貢献し、将来に亘って国民の保健衛生の向上に広く寄与していくものと考えております。

なお、新法人の概要は、次頁に記載のとおりです。

以　上

【本件に関するお問い合わせ先】
日本赤十字社　血液事業本部　経営企画課　TEL：03-3437-7708
田辺三菱製薬株式会社　広報部　TEL：06-6205-5211

Press Release

日本赤十字社
Japanese Red Cross Society

＜新法人の概要＞

法　人　名：一般社団法人 日本血液製剤機構（Japan Blood Products Organization）
本社所在地：東京都港区浜松町二丁目4番1号 世界貿易センタービルディング7階
工場所在地：北海道千歳市泉沢1007番31
　　京都府福知山市長田野町二丁目11番
研究所所在地：兵庫県神戸市（予定）
事業開始日：平成24年10月1日（予定）
代表理事：理事長　上田 英彦　※現 日本赤十字社血液事業本部血液事業経営会議委員
　　副理事長　秋山 裕治　※現 株式会社ベネシス顧問
従業員数：約900名
売　上　高：約370億円　※現在の両社血漿分画製剤売上高合算（薬価ベース）
主な製品名：①人免疫グロブリン製剤
・日赤ポリグロビン®N5%静注 5g/100mL（現 日本赤十字社品）
・献血ヴェノグロブリン®IH5%静注 5g/100mL（現 ベネシス社品）
②人血清アルブミン製剤
・赤十字アルブミン 25%静注 12.5g/50mL（現 日本赤十字社品）
・献血アルブミン 25%静注 12.5g/50mL「ベネシス」（現 ベネシス社品）
・赤十字アルブミン 20%静注 10g/50mL（現 日本赤十字社品）
・献血アルブミン 5%静注 12.5g/250mL「ベネシス」（現 ベネシス社品）
・赤十字アルブミン 5%静注 12.5g/250mL（現 日本赤十字社品）
③血液凝固第Ⅷ因子製剤
・クロスエイト M®静注用 1000単位（現 日本赤十字社品）
④抗HBs人免疫グロブリン製剤
・抗HBs人免疫グロブリン筋注200単位/1mL「日赤」（現 日本赤十字社品）
・ヘブスブリン®IH静注1000単位（現 ベネシス社品）
⑤乾燥濃縮人アンチトロンビンⅢ製剤
・ノイアート®静注用1500単位（現 ベネシス社品）
⑥人ハプトグロビン製剤
・ハプトグロビン静注2000単位「ベネシス」（現 ベネシス社品）

（参考）新法人ロゴ

日本血液製剤機構
Japan Blood Products Organization

資料9

ひと、くらし、みらいのために
厚生労働省
Ministry of Health, Labour and Welfare

Press Release

平成24年5月25日
医薬食品局血液対策課
（担当・内線）課長補佐　伯野（2905）
血液安全係長　松本（2908）
（代表電話）03(5253)1111
（直通電話）03(3595)2395

報道関係者　各位

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16年12月9日に公表したフィブリノゲン製剤納入先医療機関を対象として、平成19年11月7日付で実施した追加調査の結果について、前回の報告から平成24年5月11日までに、医療機関から新たに届いた回答はありませんでしたので、平成24年4月27日に公表した調査結果からの変更はありません。

（参考）C型肝炎ウイルス検査受診の呼びかけ（下記の厚生労働省ホームページにリンク）
http://www.mhlw.go.jp/houdou/2008/01/h0117-2/index.html