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２２年１０月２７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２２年７月２６日付け血安第２５６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２２年度第１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２２年１１月２４日（水）に平成２２年度第３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２２年１１月２日（火）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２２年７月２６日付け血安第２５６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 ４ ０ ０ 号
平成２２年１１月２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 22 年 10 月 27 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22年9月30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22年9月30日		
	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			866		
2)個別件数	1,688	69	49	805	43	18
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			961		
2)個別本数	1,877	84	53	899	41	21
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			754		
2)個別本数	1,877	84	53	710	28	16
(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			52		
2)個別件数	144	0	0	52	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	51	0	0
2)医療機関調査中	0	0	0	0	0	0
3)院内で廃棄	6	0	0	4	0	0
4)不明	6	0	0	3	0	0
計	152	0	0	58	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	3	0	0
2)非陽転事例	55	0	0	13	0	0
3)死亡	55	0	0	25	0	0
4)退院・未検査	19	0	0	8	0	0
5)陽性だが輸血前不明	10	0	0	2	0	0
計	140	0	0	51	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	3	0	0

*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２２年６月～平成２２年１１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22年6月28日	平成22年6月25日	赤血球濃厚液-LR「日赤」400mL由来	70-2321-7382	1
平成22年7月27日	平成22年7月26日	赤血球濃厚液-LR「日赤」400mL由来	20-2122-8363	1
平成22年8月16日	平成22年8月14日	照射赤血球濃厚液-LR「日赤」400mL由来	19-0628-4248	1
平成22年8月19日	平成22年8月18日	新鮮凍結血漿-LR「日赤」400mL由来	50-3321-5522	1
平成22年9月7日	平成22年9月6日	新鮮凍結血漿-LR「日赤」400mL由来	70-0627-3590	1
平成22年10月5日	平成22年10月4日	赤血球濃厚液-LR「日赤」400mL由来	72-2527-6035	1
平成22年10月7日	平成22年10月6日	照射赤血球濃厚液-LR「日赤」400mL由来	70-2125-5102	1
平成22年10月22日	平成22年10月20日	赤血球濃厚液-LR「日赤」400mL由来	78-3725-0165	1
平成22年11月1日	平成22年10月29日	新鮮凍結血漿-LR「日赤」400mL由来	37-5120-7484	1
平成22年11月4日	平成22年11月2日	新鮮凍結血漿-LR「日赤」400mL由来	72-1724-7784	1

「血漿分画製剤のウイルス安全対策について」の実施状況について
（平成２２年１１月１１日時点）

○ 経　緯

「血漿分画製剤のウイルス安全対策について」（平成１５年１１月７日付け薬食審査発第 1107001 号、薬食安発第 1107001 号、薬食監発第 1107001 号、薬食血発第 1107001 号。以下「通知」という。）の実施状況について、（社）日本血液製剤協会に所属し、血漿分画製剤を製造又は輸入している会員企業に対し報告を求めたところ、以下の結果が得られた。

① 通知記の３（１）前段に規定するウイルス・プロセスバリデーションの実施の有無

国内製造業者４社及び輸入販売業者５社のいずれにおいても、ウイルス・プロセスバリデーションが行われていた。

② 上記①に関する必要な書類等の整理及び保存の有無

国内製造業者４社及び輸入販売業者５社のいずれにおいても、必要な書類等の整理及び保存が行われていた。

③ 通知記の３（１）後段に規定するウイルスクリアランス指数が９未満の製剤の有無及び該当する製剤がある場合は、ウイルスの除去・不活化の工程の改善の検討状況

ウイルスクリアランス指数が９未満の製剤は、海外血漿を原料とし、日本国内に輸入されている２製剤がある。国内血漿を原料としている製剤及び輸入血漿を原料とし、日本国内で製造されている製剤には、９未満の製剤はない。

該当する製剤がある製造業者又は輸入販売業者の製造元においては、バリデーション結果の見直し、新たな不活化工程の追加等の検討等が行われている。

なお、米国及び欧州で採血された場合は、それぞれの地域における遡及調査ガイドラインに基づいた対応がなされている。

④ 通知記の３（２）に規定する原料のプールにおけるＮＡＴの実施の有無

国内製造業者４社及び輸入販売業者５社の製造元のいずれにおいても、原料のプールにおけるＮＡＴ検査が実施されている。

⑤ 通知記の６に規定する添付文書の改訂の有無

添付文書へ記載する文章及び記載場所について、日本血液製剤協会・添付文書委員会で協議・検討が行われ、平成15年12月17日に厚生労働省医薬食品局安全対策課の了承を得たところであり、平成16年1月から2月にかけて、血漿分画製剤及び人血液を用いる血液製剤代替医薬品の添付文書が改訂された。

薬食審査発第 1107001 号
薬食安発第 1107001 号
薬食監発第 1107001 号
薬食血発第 1107001 号
平成１５年１１月　７日

（社）日本血液製剤協会理事長　殿

厚生労働省医薬食品局審査管理課長

厚生労働省医薬食品局安全対策課長

厚生労働省医薬食品局監視指導・麻薬対策課長

厚生労働省医薬食品局血液対策課長

血漿分画製剤のウイルス安全対策について

標記については、平成１５年１０月２４日に開催された平成１５年度第３回血液事業部会における検討結果を踏まえ、下記のとおりとし、発出日から適用しますので、貴職におかれては、貴会会員に対し当該対策が徹底されるよう周知をお願いします。ただし、平成１５年９月１７日に開催された平成１５年度第３回血液事業部会安全技術調査会において対応を保留することとされた、遡及調査により個別に核酸増幅検査（以下「ＮＡＴ」という。）を実施した結果、陽性血液の混入が判明した原料血漿由来の血漿分画製剤については、本通知の規定を遡って適用することといたします。

また、「血液製剤の当面のウイルス安全対策について」（平成１０年１１月２日付け厚生省医薬安全局安全対策課、監視指導課、血液対策課事務連絡）については、本通知をもって廃止することとします。

記

1　血漿分画製剤（以下「製剤」という。）の製造前には、生物由来原料基準（平成１５年厚生労働省令第２１０号）第２の２の（６）の規定に則り、その原血漿について、ウイルス（ＨＢＶ、ＨＣＶ及びＨＩＶをいう。以下同じ。）のＮＡＴを実施することとし、陽性となった場合は使用しないこと。

2　副作用等の報告（薬事法（昭和３５年法律第１４５号）第７７条の４の２第１項及び第２項に規定する副作用等の報告をいう。以下同じ。）等からの遡及調査に伴い、製剤（ロット）の製造後に個別にＮＡＴを実施することにより、陽性となった血液の原血漿への混入が判明した場合は、混入したウイルスの種類及び量（理論的な上限値を含む。）が特定され、かつ、製造工程において当該ウイルスが十分に除去・不活化されることが確認されれば、個別の分離血漿の段階にある原血漿を除き、当該製剤（ロット）を回収する必要はないものとすること。また、これらの特定及び確認は、厚生労働省医薬食品局血液対策課が、血液事業部会安全技術調査会の意見を聴いて行うものとすること。

なお、この場合において、混入したウイルスの量が、日本赤十字社が現に実施している５０プールのＮＡＴにより陰性が確認されるレベルであって、当該ウイルスに係るウイルスクリアランス指数（ウイルス力価の減少度を対数（$\log_{10}$値）で表したものをいう。以下同じ。）が９以上である製剤（ロット）については、当該ウイルスが十分に除去・不活化されていると平成１５年度第３回血液事業部会において判断されたので、当面は、個別の分離血漿の段階にある原血漿を除き、当該製剤（ロット）を回収する必要はないものとすること。

3　２の前段に規定する確認に資するため、あらかじめ、以下に掲げる措置を講じておくこと。

（１）ウイルスの除去・不活化等に係る書類等の整備及び工程の改善

製剤の製造工程において、ウイルスが十分に除去・不活化されていることを確認できるよう、ウイルス・プロセスバリデーションを実施しておくこと。また、必要な書類等を整理し、保存しておくこと。

さらに、「安全な血液製剤の安定供給の確保等に関する法律」（昭和３１年法律第１６０号）の第７条において、製造業者等の責務として「血液製剤の安全性向上に寄与する技術の開発」に努めることが規定されていることを踏まえ、より安全性の高い製剤の開発に努めること。特に、製造工程におけるウイルスクリアランス指数が９未満である製剤については、早期

にウイルスの除去・不活化の工程について改善を図ること。

（２）原料のプールを製造した際の検査

原料のプールを製造した際、当該プールについてＮＡＴを実施することとし、陽性となった場合は使用しないこと。また、当該ＮＡＴの検出限界が１００ＩＵ／ｍＩの精度となるよう精度管理を行い、必要な書類等を保存しておくこと。

4　以下の場合は、速やかに厚生労働省医薬食品局血液対策課に報告すること。

（１）遡及調査等により原血漿にＮＡＴで陽性となった血液の混入が判明した場合。

（２）３の（２）に規定する原料のプールを製造した際の検査でＮＡＴの陽性が判明した場合。

なお、当該報告があった場合は、「ＮＡＴガイドライン（仮称）」が策定されるまでの間、第三者機関においてＮＡＴの結果を検証することとしているので、血液対策課の指示に基づき当該機関に保管検体を提供すること。

5　副作用等の報告等からの遡及調査に伴い、製剤（ロット）の製造後に個別にＮＡＴを実施することにより、陽性となった血液の原血漿への混入が判明した場合であって、３の（１）及び（２）に掲げる措置が講じられていない等、２の前段に規定する確認ができない場合は、原則として、「医薬品等の回収に関する監視指導要領」（平成１２年３月８日付け医薬発第２３７号別添１）の規定に則り、当該製剤（ロット）を回収すること。

なお、副作用等の報告等からの遡及調査により、製剤（ロット）と感染症の発生との因果関係が否定できない場合には、以上の規定にかかわらず、速やかに厚生労働省医薬食品局安全対策課に報告するとともに、同要領の規定に則り、当該製剤（ロット）を回収すること。

6　既に、「生物由来製品の添付文書に記載すべき事項について」（平成１５年５月１５日医薬発第0515005号）に基づき、製剤のリスクに係る事項が添付文書に記載されているところであるが、なお入念的な措置として、同通知の記の１．（１）⑤に関連して、添付文書の重要な基本的注意に、以下に掲げる趣旨の文言を記載すること。

・　製剤の原材料である血液については、ミニプールでＮＡＴを実施し、ウイルスのＤＮＡ又はＲＮＡが検出されないことが確認されたものを使用しているが、当該ミニプール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こと。

血液製剤に関する報告事項について　（目次）

資料３－２

輸血用血液製剤で感染が疑われる事例について　（平成２２年１１月２日時点）

【ＨＩＶ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
H15.9.5	赤血球製剤	8 人	保管検体個別 NAT 全て陰性 8 人中 6 人が来訪 いずれも感染していないことが確認された。	新鮮凍結血漿：3 本。使用済み。 原料血漿は流通停止。 新鮮凍結血漿を投与された患者 3 名のうち、1 名は原疾患で死亡残り 2 名は輸血後（約 6 ヵ月後）抗体検査で陰性。	平成 17 年 10 月 18 日以降、残る 2 人の来訪なし

【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
H16.3.22	血小板製剤 赤血球製剤	37 人	保管検体個別 NAT 全て陰性 37 人中 32 人が来訪 個別 NAT 陰性：32 人	新鮮凍結血漿：5 本（供血者 4 人分由来） 使用済み。 残りは原料血漿。 新鮮凍結血漿を投与された患者 5 名のうち、陰性 2 名、不明 3 名。	平成 18 年 10 月 17 日以降、残る 5 人の来訪なし
H16.11.26	新鮮凍結血漿 赤血球製剤	48 人	保管検体個別 NAT 全て陰性 48 名中 41 人が来訪 HBV 関連検査陰性：40 人 HBs 抗原、NAT 陰性、HBs 抗体、HBc 抗体陽性：1 人	原料血漿：2 本確保。31 本使用済み。 新鮮凍結血漿：16 本。医療機関へ提供済み。 赤血球製剤：45 本。医療機関へ提供済み。	平成 21 年 4 月 22 日以降、残る 7 人の来訪なし

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
H17.1.12	赤血球製剤 血小板製剤	16人	保管検体個別NAT　陽性2人 16人中12名が来訪 HBV関連検査陰性：12人 （個別NAT陽性の2人は、複数回再献血を行っているが、HBc抗体を含む関連検査が全て陰性であり、感染歴があった可能性は低い。）	原料血漿：3本確保済み。12本使用済み。 新鮮凍結血漿：12本全て医療機関へ提供済み。	平成17年10月18日以降、残る4人の来訪なし。
H17.2.4	赤血球製剤	9人 追跡対象は4人。	保管検体個別NAT：9人全て陰性 （当該患者のHBV-NATが陽性になる前の輸血の供血者は9人中4人。） 4人中3人来訪。 HBV関連検査陰性：2人 HBc抗体陽性：1人	原料血漿：7本確保済み。 新鮮凍結血漿：2本確保済み、2本医療機関へ提供済み。	平成18年4月25日以降、追跡調査対象の残る1人の来訪なし。
H17.6.23	赤血球製剤 新鮮凍結血漿	20人	保管検体個別NAT全て陰性 20人中18人が来訪。 HBV関連検査陰性：18人	原料血漿：17本中10本確保。 新鮮凍結血漿：6本中3本確保。 赤血球製剤：15本全て医療機関へ供給済み。	平成22年7月27日以降、1人が献血に協力いただき、検査は陰性。残る2人の来訪なし。
H18.4.7	血小板製剤 赤血球製剤	53人	保管検体個別NAT全て陰性 53人中46人が来訪。 HBV関連検査陰性：43人 HBc抗体、HBs抗体陽性：2人 HBs抗体陽性：1人	原料血漿：51本中7本確保。44本使用済み。 新鮮凍結血漿：14本全て供給済み。	平成22年7月27日以降、1人が献血に協力いただき、検査は陰性。残る7人の来訪なし。

3

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
H18.6.5	赤血球製剤 新鮮凍結血漿	29人	保管検体個別NAT全て陰性 29人中28人来訪 HBV関連検査陰性：25人 HBs抗体、HBc抗体陽性：1人 HBs抗体陽性：1人	原料血漿：27本中11本確保。16本使用済み。 新鮮凍結血漿：8本中6本確保。2本供給済み。 赤血球製剤：18本全て使用済み。	平成21年4月22日以降、残る1人の来訪なし。
H19.2.20	赤血球濃厚液	3人	保管検体個別NAT全て陰性 3人中2人来訪 HBV関連検査陰性：2人	原料血漿：3本全て確保。	平成19年10月19日以降、残る1人の来訪なし。
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 23人中20人来訪 HBV関連検査陰性：20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22年2月13日以降、残る3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
H18.2.15	赤血球製剤 血小板製剤 新鮮凍結血漿	81人	保管検体個別NAT全て陰性 81人中78人来訪 HCV関連検査陰性：78人	原料血漿：70本中67本確保。3本使用済み。 新鮮凍結血漿：14本中11本確保。3本供給済み。 赤血球製剤：6本全て供給済み。	平成19年10月19日以降、残る3人の来訪なし

平成２２年度感染症報告事例のまとめ（前回報告分以降）について

１　平成２２年７月２０日報告分から２２年１０月２５日までに報告（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２８件である。輸血用血液製剤の内訳は、
（１）Ｂ型肝炎報告事例：　９
（２）Ｃ型肝炎報告事例：　７
（３）ＨＩＶ感染報告例：　０
（４）その他の感染症報告例：　１２

２　Ｂ型肝炎報告事例
（１）輸血前後に感染症検査でHBs抗原（又はHBV-DNA）等が陽転した事例は９例（輸血後NATで陰性又は輸血前後で陽性は２例）。
（２）　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２例。
（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例（劇症化例含む。）である。

３　Ｃ型肝炎報告事例
（１）輸血前後に抗体検査（又はHCV-RNA）等が陽転した事例は４例（輸血後NATで陰性又は輸血前後で陽性は１例）。
（２）使用した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例。
（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例。

４　ＨＩＶ報告事例
（１）輸血前後に抗体検査等が陽転した事例は０例。
（２）使用した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例。
（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例。

５　その他感染症報告事例
（１）Ｂ型肝炎及びＣ型肝炎以外の肝障害報告事例は０例。
（２）細菌等感染症報告事例において、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例。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例。

【国内輸血例】

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	供用血液製剤等	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																									
3-1000039	A-10000021	2010/6/8	2010/6/21	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))	男	60	血液腫瘍	B型肝炎	09/12 09/12	HBsAb(－) HBcAb(－) (09/12) HBsAg(－) (09/12)	HBsAg(＋) HBsAb(－) HBcAb(＋) (10/05) HBV-DNA (＋)HBsAg (＋)HBcAb (－) (10/06)	HBV-DNA(－) (09/12)	HBV-DNA (－) HBsAg(－) HBsAb(－) HBcAb(－) (09/12) HBV-DNA (＋)HBsAg (＋)HBsAb (－)HBcAb (－) (10/05)	陰性(輸血前) 陽性(輸血後)	保管検体8本全部についてHBV-DNA(－)1本+HBV-DNA(＋)		被疑薬3本で第1報を入手し、未完了報告を行ったが、医療機関からの申し出により被疑薬0本が追加された。 ＊HBV-DNA陽性輸血用血液(献血者)についての情報 ①同一採血番号製剤：1本の原料血漿を製造し、確保済み ②供血者の再来献血：当該献血者にその後の献血は確認されていない ③当該以前の献血：なし	2単位 16単位	3/9(HBV関連検査陰性)	9本の原料血漿を製造、全て確保済み	-	重篤	未回復		献血者保管検体はHBV-DNAに対して1検体が個別NAT陽性であった。患者検体と献血者(HBV陽性保管検体)とでPreS/S領域を含むP領域の前半部の1550bpの塩基配列を比較したところ、両者の塩基配列は全て一致した。 献血者と患者のHBウイルスは共にGenotype Cで塩基配列からSubtype adrと推定した。
3-1000057	A-10000041	2010/9/2	2010/9/15	新鮮凍結血漿－LR(新鮮凍結人血漿)	男	70	胆・肝・膵腫瘍	B型肝炎	10/04	HBsAg(－) (10/04) HBsAb(－) HBcAb(－) HBeAg(－) HBeAb(－) (10/04)	HBsAg(＋) (10/08) HBV-DNA (＋) (10/08)		HBV-DNA (＋) (10/09)	陽性(輸血後)	保管検体8本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 ・同一採血番号製剤：1本の赤血球濃厚液－LRを製造し、医療機関へ供給済み。 ・再来献血：1回献血に再来。(HBV関連検査陰性)(保管検体についてHBV-DNA(－)) ・1本の原料血漿、1本の赤血球濃厚液－LRを製造。原料血漿は確保済み。赤血球濃厚液－LRは医療機関へ供給済み。 ・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。 医療機関報告10102034-001	18単位	8/9(HBV関連検査陰性)	9本の赤血球濃厚液－LR製造。赤血球濃厚液－LR12本廃棄済み。	赤血球濃厚液－LR7本は医療機関へ供給済み。	非重篤	回復		患者検体と献血者(HBV陽性保管検体)とでS領域193bp＊の塩基配列を比較したところ、全て一致した。献血者と患者のHBウイルスは共にGenotypeAで、塩基配列からSubtypeはadwと推定した。 ＊献血者検体のPreS/S領域を含む前半部の1556bpの塩基配列はウイルス量が少なくPCRで増幅できなかった。
陽転事例																									
3-1000049	A-10000030	2010/8/12	2010/8/26	照射赤血球濃厚液－LR(人赤血球濃厚液(放射線照射))	男	60	循環器疾患	B型肝炎	10/04	HBsAg(－) (10/04)	HBsAg(－) (10/08) IgM-HBcAb (＋) (10/08) HBV-DNA (＋) (10/08) HBsAb(－) HBeAg(－) HBcAg(＋) (10/08)	HBV-DNA(－) (10/04)	HBV-DNA(＋) HBsAg(－) HBsAb(－) HBcAb(＋) (10/08)	陰性(輸血前) 陽性(輸血後)	保管検体1本(全部)HBV-DNA(－)			2単位	0/1	1本の新鮮凍結血漿－LRを製造、確保済み	-	重篤	未回復		

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
3-1000051	A-1000-0034	2010/8/20	2010/9/3	照射赤血球濃厚液-LR(人赤血球濃厚液(放射線照射))	女	80	血液腫瘍	B型肝炎	10/03	HBsAg(-)(08/12)	HBsAg(+)(10/07) HBcAb(+)(10/07) HBsAg(+)(10/07) HBeAg(-)(10/07) HBV-DNA(+)(10/07) HBsAg(+)(10/07)	-	-	調査なし	保管検体3本(全部)HBV-DNA(-)			4単位	1/3(HBV関連検査陰性)	3本の原料血漿を製造、すべて確保済み	-	非重篤	未回復		
3-1000053	A-1000-0036	2010/8/25	2010/9/7	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	脳疾患	B型肝炎	10/02 10/02	HBsAg(-)(10/02)	HBsAg(+)(10/07) HBeAg(+)(10/08) IgM-HBcAb(+)(10/08) HBsAg(+)(10/07) HBcAb(+)(10/07) HBeAg(-)(10/07) HBeAb(+)(10/08)	HBV-DNA(-)(10/02) HBsAg(-)(10/02) HBeAb(-)(10/02) HBcAb(-)(10/02)	HBV-DNA(+)(10/08) HBsAg(+)(10/08) HBeAb(-)(10/08) HBcAb(+)(10/08)	陰性(輸血前) 陽性(輸血後)	保管検体7本(全部)HBV-DNA(-)		被疑薬4本で第1報を入手し、その後医療機関からの申し出により被疑薬3本追加	8単位 6単位	2/7(HBV関連検査陽性)	2本の原料血漿、2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	軽快		
3-1000067	A-1000-0053	2010/9/29	2010/10/12	赤血球M・A・P(人赤血球濃厚液) 濃厚血小板(人血小板濃厚液) 新鮮凍結血漿(新鮮凍結人血漿)	女	60	血液腫瘍	B型肝炎	04/03-05 04/03-04 04/03-04	HBsAg(-)(04/03)	HBsAg(+)(10/08) HBeAg(+)(10/08) HBcAb(+)(10/08) IgM-HBcAb(-) HBeAg(+)(10/08) HBeAb(-)(10/08)	-	HBV-DNA(+)(10/08)	陽性(輸血後)	保管検体20本(全部)HBV-DNA(-)	ヴェノグロブリン-IH		14単位 50単位 14単位	13/20	19本の原料血漿、7本の新鮮凍結血漿、7本の赤血球MAPを製造。	原料血漿は全て使用済み。新鮮凍結血漿はすべて医療機関へ供給済み。赤血球MAPは医療機関へ供給済み。	非重篤	未回復		
3-1000069	A-1000-0055	2010/10/4	2010/10/14	濃厚血小板(人血小板濃厚液) 濃厚血小板-LR(人血小板濃厚液) 赤血球濃厚液-LR(人赤血球濃厚液)	男	50	血液腫瘍	B型肝炎	09/12-10/02 10/02-03 09/12-10/03	HBsAg(-)(09/10) HBsAb(-)(09/10) HBcAb(-)(09/10) HBsAg(-)(09/11) HBsAb(-)(09/11) HBcAb(-)(09/11)	HBV-DNA(-)(10/01) HBsAg(-)(10/04) HBeAg(+)(10/04) HBsAb(-)(10/04) HBeAb(-)(10/04) HBcAb(+)(10/09)	HBV-DNA(-)(09/11) HBsAg(-)(09/11) HBeAb(-)(09/11) HBcAb(-)(09/11)	HBV-DNA(+)(10/09) HBsAg(+)(10/09) HBeAb(-)(10/09) HBcAb(+)(10/09)	陰性(輸血前) 陽性(輸血後)	保管検体58本(全部)HBV-DNA(-)			190単位 220単位 34単位	38/58(35人はHBV関連検査陰性。二人はHBsおよびHBc抗体陽性。一人は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。	56本の原料血漿、2本の新鮮凍結血漿-LRを製造。原料血漿は52本確保済み。	原料血漿は4本使用済み。新鮮凍結血漿-LRはすべて医療機関へ供給済み。	重篤	未回復		

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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																									
3-1000044	A-1000-0025	2010/7/29	2010/8/12	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) 新鮮凍結血漿(新鮮凍結人血漿)	男	20	先天性疾患	B型肝炎	09/9-12 09/09-10 09/09	HBsAg(-)(09/07)	HBsAg(-)(10/05) HBeAg(+)(10/07) HBeAg(+)(10/07) HBV-DNA(-)(10/07)	HBV-DNA(-)(09/09) HBsAg(-)(09/09) HBsAb(-)(09/09) HBcAb(-)(09/09)	HBV-DNA(-)(10/07) HBsAg(-)(10/07) HBsAb(+)(10/07) HBcAb(-)(10/07) HBeAg(+)(10/07) HBeAb(+)(10/07)	陰性(輸血前) 陰性(輸血後)	保管検体37本に全てについてHBV-DNA(-)			18単位 8単位 120単位	28/36(23人はHBV関連検査陰性。3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。＊被疑薬は37本	8本の原料血漿、1本の新鮮凍結血漿-LR、4本の赤血球濃厚液-LRを製造。原料血漿は4本確保済み。	新鮮凍結血漿-LR、赤血球濃厚液-LRは全て医療機関に供給済み。原料血漿は4本使用済み。	非重篤	未回復		
3-1000050	A-1000-0033	2010/8/17	2010/8/31	照射赤血球濃厚液-LR(人赤血球濃厚液(放射線照射))	女	80	その他の疾患	B型肝炎	10/04-06	-	HBsAg(-)(10/05) HBsAb(-)(10/08) HBcAb(-)(10/08) HBeAg(+)(10/08) HBsAb(-)(10/08) HBeAg(+)(10/08) HBcAb(-)(10/08)	-	HBV-DNA(-)(10/06) HBsAg(-)(10/06) HBsAb(-)(10/06) HBcAb(-)(10/06) HBV-DNA(-)(10/08) HBsAg(+)(10/08) HBsAb(-)(10/08) HBcAb(-)(10/08)	陰性(輸血後)	保管検体6本(全部)HBV-DNA(-)			12単位	0/6	5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	-	非重篤	不明		
陽転未確認事例																									
(該当例なし)																									
				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																									
3-1000047	A-1000-0029	2010/8/10	2010/8/24	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(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	循環器疾患 消化器疾患	C型肝炎	10/01-03 10/03 10/03	HCV-Ab(-)(10/01) HCV-RNA(-)(10/01) HCV-Ab(-)(10/01)	HCV-RNA(-)(10/03) HCV-Ab(+)(10/08) HCV-RNA(+)(10/08) HCV-Ab(+)(10/08)	HCV-RNA(-)(10/01) HCV-Ab(-)(10/01)	HCV-RNA(-)(10/03) HCV-Ab(-)(10/03) HCV-RNA(+)(10/08) HCV-Ab(+)(10/08)	陰性(輸血前) 陽性(輸血後)	保管検体13本(全部)HCV-RNA(-)	プミネート		16単位 15単位 20単位	5/13(HCV関連検査陰性)	9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は全て確保済み	-	重篤	未回復		

日赤番号	識別番号	FAX受付日	報告受領日	販売名(一般名)	感染性別	年代	原疾患	感染症名	投与月	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	供血者発遣及の場合の供血者保管検体(抗原、抗体、NAT)(投与時点)	供血者発遣及の場合の供血者の検査値
3-1000055	A-1000038	2010/8/26	2010/9/9	赤血球濃厚液-LR(人赤血球濃厚液)	女	70	循環器疾患	C型肝炎	10/04	-	HCVコアAg(-)(10/04) HCVコアAg(+)(10/05) HCVコアAg(+)(10/08)	HCV-RNA(-) HCV-Ab(-)(10/4)	HCV-RNA(+) HCV-Ab(-)(10/05)	陰性(輸血前) 陽性(輸血後)	保管検体2本(全部)HCV-RNA(-)			4単位	1/2(HCV関連検査陰性)	2本の原料血漿を製造、すべて確保済み	-	重篤	未回復		
3-1000058	A-1000042	2010/9/15	2010/9/16	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	90	泌尿器腫瘍	C型肝炎	10/04 10/04	HCV-Ab(-)(10/03) HCVコア抗原(-)(10/04)	HCVコア抗原(+)(10/07)	HCV-RNA(-)(10/04)	HCV-RNA(+)(10/09)	陰性(輸血前) 陽性(輸血後)	保管検体18本(全て)HCV-RNA(-)			20単位 16単位	8/18(HCV関連検査陰性)	8本の原料血漿、10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		
輸血後NATで陰性又は輸血前後で陽性																									
3-1000061	A-1000044	2010/9/9	2010/9/17	照射赤血球濃厚液-LR(人赤血球濃厚液(放射線照射))	男	60	肝・胆・膵疾患消化器疾患その他の疾患	C型肝炎	10/04	HCVコア抗原(-)HCV-Ab(-)(10/04)	HCVコア抗原(+) HCV-Ab(-)(10/08)	HCV-RNA(-) HCV-Ab(-)(10/04)	HCV-RNA(-) HCV-Ab(-)(10/09)	陰性(輸血前) 陰性(輸血後)	保管検体2本(全て)HCV-RNA(-)			4単位	0/2	2本の新鮮凍結血漿-LRを製造、全て確保済み。	-	非重篤	不明		
陽転未確認事例																									
3-1000065	A-1000050	2010/9/16	2010/10/1	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	消化器腫瘍	C型肝炎	10/05-06 10/05	HCVコア抗原(-)(10/05) HCV-Ab(+)(10/05)	HCVコア抗原(+) HCV-Ab(+)(10/09)	調査中	調査中	HCV関連検査実施予定	保管検体11本についてHCV-RNA(-)		医療機関報告(10101662-001	9単位 10単位	3/11(HCV関連検査陰性)	2本の原料血漿、3本の新鮮凍結血漿-LR、8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	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)	感染性別	年代	原疾患	感染症名	投与月	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	供血者発遣及の場合の供血者保管検体(抗原、抗体、NAT)(投与時点)	供血者発遣及の場合の供血者の検査値
3-1000066	A-1000054	2010/10/1	2010/10/14	照射赤血球濃厚液-LR(人赤血球濃厚液(放射線照射))	男	90	消化器疾患	C型肝炎	09/10	HCV-Ab(-)(09/10)	HCV-RNA(+) HCV-Ab(+)(10/09)	調査中	調査中	HCV関連検査実施予定	保管検体3本(全部)HCV-RNA(-)			4単位	2/3(HCV関連検査陰性)	調査中	調査中	非重篤	未回復		
3-1000071	A-1000057	2010/10/5	2010/10/18	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(新鮮凍結人血漿)	女	70	腎臓疾患	C型肝炎	09/12 09/12	HCV-Ab(-)(09/11)	HCV-Ab(+)(10/09) HCV-RNA(+)(10/10)	HCV-RNA(-) HCV-Ab(-)(09/12)	HCV-RNA(+) HCV-Ab(+)(10/10)	陰性(輸血前) 陽性(輸血後)	保管検体4本(全部)HCV-RNA(-)			6単位 5単位	2/4(HCV感染検査陰性)	6本の原料血漿を製造、すべて確保済み	-	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																									
3-1000043	A-1000024	2010/7/26	2010/8/6	照射赤血球濃厚液-LR(人赤血球濃厚液(放射線照射))	女	90	血液疾患	細菌感染	10/07	18:00 BT36.9℃ BP90/34 (10/07)	初日輸血後 21:00 BT36.6℃ BP112/77 初日輸血翌日 11:00 BT36.6℃ BP96/52 一、二本目輸血 15:00 BT36.8℃ BP126/64 輸血終了後翌日 10:00BT37.8℃ BP136/87 輸血終了後翌々日 19:00BT37.7 輸血終了8日後病院にて死亡。患者の血液培養は未実施。胸水の検査結果にて、細胞診クラスII、培養陰性。感染症は口腔内常在菌のみ検出。	同一採血番号の血液(3本)で無菌試験を実施、適合。非溶血性副作用関連検査実施 抗血小板タンパク質抗体検査:陰性 血漿タンパク質欠損検査:欠損なし	-	-	-	人血清アルブミン	担当医より「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。	4単位	-	1本の原料血漿、2本の新鮮凍結血漿-LRを製造。原料血漿は確保済み。新鮮凍結血漿-LRはすべて確保済み。	-	重篤(死亡)	死亡		

日赤管理番号	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	供血者発遣及の場合の供血者保管検体(抗原、抗体、NAT)(投与時点)	供血者発遣及の場合の供血者の検査値
3-1000048	A-1000-0027	2010/7/30	2010/8/16	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	血液腫瘍	細菌感染	10/07 10/07	-	血漿製剤2単位輸血施行後、前出血あり(経皮)。赤血球製剤2単位輸血施行3時間後、心肺停止、肺出血著明。同日、患者肺出血にて死亡確認。剖検なし。	当該製剤のセグメントチューブで細菌培養試験を実施、陰性。	-	-	-		担当医の見解:死亡と本剤との関連性なし。	2単位 2単位	-	1本の新鮮凍結血漿-LR、確保済み	-	非重篤	不明(部出血にて死亡。剖検なし。死亡と本剤の関連性なし(担当医の意見)		
3-1000048	A-1000-0028	2010/8/11	2010/8/24	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))	男	70	外傷・整形外科的疾患	細菌感染	10/08 10/08	BT38.6℃	13:45 輸血中止 13:45 BT40℃、BP232/98、P140 13:50 BP231/99 14:10 BP157/62 14:20 BP133/56 院内にて実施の患者血液培養により MRS Staphylococcus epidermidisを同定。	当該製剤のセグメントチューブ(2本)および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:欠損なし	-	-	-		被疑薬:採血3日目の照射濃厚血小板-LR(2本) 採血15日目の照射赤血球濃厚液-LR(1本) 担当医の見解 敗血症性ショックと輸血血液との因果関係はないと考える。	20単位 2単位	-	3本の原料血漿を製造、すべて確保済み	-	重篤	回復		
3-1000050052	A-1000-0035	2010/8/23	2010/9/3	照射赤血球濃厚液-LR(人赤血球濃厚液(放射線照射))	男	40	血液腫瘍	細菌感染	10/08	BT 37.3℃、BP 83/50	14:00 輸血施行。 14:05 悪寒・戦慄出現。輸血中止。BP 125/84、pulse 111、SpO2 98%、意識清明 15:00頃 BT 39.1℃に上昇。解熱剤、抗生剤開始。解熱。夜間 BP 99/50 院内にて実施の患者血液培養により Staphylococcus epidermidis MRSE同定	投与中止の当該製剤(1本)で細菌培養試験を実施、陰性。 当該製剤でエンドトキシン濃度 0.8pg/mL以下(基準値1.0pg/mL) 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:欠損なし	-	-	-		被疑薬:採血17日目の照射赤血球濃厚液-LR(1本) 担当医より、細菌感染起因輸血血液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、すべて確保済み	-	重篤	回復		

日赤管理番号	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	供血者発遣及の場合の供血者保管検体(抗原、抗体、NAT)(投与時点)	供血者発遣及の場合の供血者の検査値
3-1000050054	A-1000-0037	2010/8/26	2010/9/7	照射赤血球濃厚液-LR(人赤血球濃厚液(放射線照射))	女	50	婦理器疾患消化器疾患	細菌感染	10/08	BT 36.8℃	11:20 輸血開始。 12:20頃 悪寒・シバリング発熱あり、39.0℃。直ちに輸血中止した。血液培養提出(1回目)。 翌日解熱。血液培養2回目提出。 10/08/24 血液培養(2回目の検体)よりグラム陰性桿菌検出。後日、Escherichia coliと同定。	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:欠損なし 抗HLA抗体検査:クラスI抗体陽性 クラスII抗体陽性	-	-	-		被疑薬:採血12日目の照射赤血球濃厚液-LR(1本) 担当医の見解 細菌感染と輸血血液との因果関係は不明である。	2単位	-	1本の原料血漿を製造、すべて確保済み	-	非重篤	回復		
3-1000050059	A-1000-0045	2010/9/8	2010/9/21	照射濃厚血小板-LR(人血小板濃厚液(放射線照射))	女	60	血液疾患	細菌感染	10/09	BT 36.2℃、BP 111/64、P 64	患者血液培養は陽性。当該製剤の血液培養にて菌:陰性 Staphylococcus hominisを同定。	同一採血番号の血漿(1本)で無菌試験を実施、適合。 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:欠損なし	-	-	-		被疑薬:採血3日目の照射濃厚血小板-LR(1本)	10単位	-	1本の原料血漿を製造、確保済み	-	非重篤	軽快		
3-1000060060	A-1000-0043	2010/9/8	2010/9/17	照射濃厚血小板-LR(人血小板濃厚液(放射線照射))	男	60	血液腫瘍	敗血症	10/09	-	16:21血小板製剤投与開始。 17:15BT 40℃輸血中止。その後解熱。 輸血翌日 5:35BT 36.9℃ 院内にて実施の患者血液培養より Acinetobacter baumanniを同定した。	投与中止の当該製剤(1本で)細菌培養試験実施、陰性。 当該輸血用血液のセグメントチューブでエンドトキシン濃度 14.2pg/mL(基準値 1.0pg/mL) 当該輸血用血液と同時製造品の血漿のセグメントチューブでエンドトキシン濃度 0.8pg/mL以下(基準値 1.0pg/mL)	-	-	-		被疑薬:採血2日目の照射濃厚血小板-LR(1本) 検査結果を受けて担当医より「副作用・感染症と輸血用血液の因果関係ありと考える。とのコメントが得られた。	10単位	-	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
3-1000070	A-1000-0056	2010/10/5	2010/10/18	照射濃厚血小板-LR(人血小板濃厚液(放射線照射))	男	80～	血液疾患	細菌感染	10/09	-	9:25 血小板製剤輸血施行。 10:40 悪寒あり。BT 39.4℃、SpO2 94% 11:20 SpO2 89%、O2はマスクで3L/min。 12:40 BT 39.9℃、SpO2 91-92%(3L/min) 16:00 BT 38.6℃、SpO2 95-97%(3L/min) 19:00 BT 40.5℃ 院内にて実施の患者血液培養にてEscherichia coliを同定した。 感染検査にてPseudomonas seruginosaとα-Streptococcusを同定した。	-	-	-	被疑薬:採血4日目の照射濃厚血小板-LR(1本)	10単位	-	調査中	調査中	重篤	軽快		
3-1000072	A-1000-0058	2010/10/5	2010/10/18	赤血球濃厚液-LR(人赤血球濃厚液)	女	0～	先天性疾患	敗血症	10/09	-	赤血球製剤投与翌日 BT 40.1℃、P 150/min 院内にて実施の患者血液培養よりEnterobacter aerogenesを同定した。	同一採血番号の血漿(1本)で無菌試験を実施予定。	-	-	-		被疑薬:採血4日目の赤血球濃厚液-LR(1本)	1単位	-	調査中	調査中	重篤	調査中		
3-1000063	A-1000-0047	2010/9/14	2010/9/28	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))	女	1～	その他の疾患	サイトメガロウイルス感染	10/07 10/07	-	IgM-CMV-Ab(+)(10/08) 髄液IgM-CMV-Ab(-)(10/06) 血液IgM-CMV-Ab(+)(10/08) 血液CMV-DNA(+)、髄液CMV-DNA(-) 尿CMV-DNA(+)	-	CMV-DNA(+)(10/09)	陽性(輸血後)	保管検体(2本) (1)血清学的検査 1本IgM-CMV抗体陽性、IgG-CMV抗体陽性、 1本IgM-CMV抗体陽性、IgG-CMV抗体判定保留 (2)遺伝子検査全てCMV-DNA(-).	ベニロン		1単位 2単位	-	1本の原料血漿、1本の赤血球濃厚液-LRを製造。原料血漿は確保済み。	赤血球濃厚液-LRは医療機関へ供給済み。	重篤	後遺症有		

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
3-1000062	A-1000-0046	2010/9/9	2010/9/24	照射濃厚血小板-LR(人血小板濃厚液(放射線照射))	女	10～	血液機構	敗血症	10/09	輸血当日 5:00 BT38.7℃、P 84 9:30 BT38.5℃、BP119/69、P 111	10:20 血小板製剤投与開始。BP 105/73 11:20 BT 38.6℃、BP 140/80、P 138 シバリングあり。血小板製剤投与中止。 11:50 BT 39.7℃ 16:30 BT 39.6℃ 院内にて実施の患者血液培養よりグラム陰性桿菌を検出。	投与中止の当該製剤(1本で)細菌培養試験実施、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:欠損なし 後日、Klebsiella pneumoniaeと同定	-	-	-		被疑薬:採血4日目の照射濃厚血小板-LR(1本) 調査結果を受けて。担当医より「細菌感染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。	10単位	-	1本の原料血漿を製造、確保済み。	-	重篤	軽快		
3-1000056	A-1000-0040	2010/8/30	2010/9/13	照射濃厚血小板-LR(人血小板濃厚液(放射線照射))	男	50～	血液腫瘍	敗血症	10/08	BT 36.4℃、BP 115/83、P 73	12:36 血小板製剤投与開始。 14:10 悪寒、戦慄、呼吸困難感あり。 14:15 BT36.5℃、BP 147/82、P 104、SpO2 83% 輸血中止。 15:15 BT36.8℃、BP 148/77、P 126 17:05 BT38.4℃、BP 112/64、P 90、SpO2 99% 輸血翌日 13:53 BT36.5℃、BP 107/60、P 70 輸血2日後 19:30 BT36.8℃ 血液培養から、グラム陽性球菌検出。その後、Enterococcus faecium と同定された。また患者の尿、便のほか、IVH患者接触部からもEnterococcus faecium が確認された。	投与中止の当該製剤(1本)で細菌培養試験実施、陰性。 非溶血性副作用関連検査、異常なし。	-	-	-		被疑薬:採血4日目の照射濃厚血小板-LR(1本)	10単位	-	1本の原料血漿を製造、すべて確保済み	-	重篤	不明		

日赤管理番号	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	供血者発遣及の場合の供血者保管検体(抗原、抗体、NAT)(投与時点)	供血者発遣及の場合の供血者の検査値
3-1000073		2010/10/14		照射赤血球濃厚液-LR(人赤血球濃厚液(放射線照射))	女	50	消化器疾患	細菌感染	10/10	-	輸血後BT38.5℃、BP170/90院内にて実施の患者血液培養よりグラム陰性桿菌を検出した。	投与中止の当該製剤(1本)で細菌培養試験を実施予定。	-	-	-	-	被疑薬:採血12日目の照射赤血球濃厚液-LR(1本)	2	-	調査中	調査中	非重篤	調査中		
3-1000074		2010/10/22		濃厚血小板HLA-LR(人血小板濃厚液HLA)	男	50	血液疾患	細菌感染	10/10	BP 103/60	16.45輸血開始17時過ぎ38.0℃、悪寒(+)、BP 118/78血培、喀痰培養実施。	投与中止の当該製剤(1本)で細菌培養試験を実施予定。	-	-	-	-	被疑薬:採血3日目の濃厚血小板HLA-LR(1本)	10	-	1本の原料血漿を製造。原料血漿は使用の有無を調査中。	調査中	重篤	軽快		

【国内血漿分画製剤例】

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(簡略名)	感染症名	投与年月	投与前検査(年月)	投与後検査(年月)	患者検体確保状況	受血者個別NAT	原料血漿・製品NAT検査(再検査・製造時検査の別)	併用血液製剤等	備考	使用単位数	ロット番号	同一製剤ロット使用状況等	感染症転帰	転帰
A-10000031	2010/8/12 2010/8/20	2010/8/27	タココンブ組織接着用シート(フィブリノゲン配合剤)	女	60	呼吸器腫瘍	B型肝炎表面抗原陽性トレポネーマ検査用性	2010/6	B型肝炎について2010年4月26日:HBs抗原陰性。梅毒について2010年4月26日:RPR定性陰性。TPEIA定性陰性。	B型肝炎について2010年8月2日:HBs抗原陰性。 2010年8月9日:HBs抗原陰性。HBs抗体(CLIA)陰性。HBe抗原(CLIA)陰性。HBe抗体(CLIA)陽性。 梅毒について2010年8月2日:RPR定性陰性。TPEIA定性陽性。TPHA定量陽性。 2010年8月9日:RPR定性陰性。TPEIA定性陽性。TPHA定量陽性。FTA-ABS(IgG)陰性。FTA-ABS定量(IgG)陰性。					(RPR:カルジオリピン抗体検査/TPEIA,TPHA,FTA-ABS:梅毒TP抗体検査)		10509203	本ロットは2010年3月29日から2010年6月18日にかけて、4,113枚が弊社から出荷され、2010年8月16日時点、528施設へ3,688枚納入された。 当該ロット製品における感染症の報告は国内からも海外安全管理部門からもない。		軽快

平成22年11月24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）

1. 試行的HEV20プールNAT実施状況

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22年9月30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～H22. 9*2	1, 241, 293	142	1／8, 742
合計	1, 582, 467	187	1／8, 462

*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）

2. HEV-RNA陽性献血者の内訳

別添

別添

2. HEV-RNA陽性者の内訳

調査期間：2005年1月1日～2010年9月30日

No.	採血日	年齢	性別	ALT (IU/L)	HEV抗体 IgM	HEV抗体 IgG	HEV RNA	問診該当※1	喫食歴調査 肉の種類	喫食歴調査 食べ方	濃厚対象献血製剤	安血者情報
1	2005/01/04	32	M	57	−	−	+	無	不明レバー	生	無	
2	2005/02/07	38	F	11	−	−	+	無	ブタレバー	生	無	
3	2005/02/13	41	M	103	−	−	+	無	回答なし		無	
4	2005/03/25	65	F	17	−	−	+	無	回答なし		無	
5	2005/03/27	28	M	38	−	−	+	有	不明レバー（問診時）	生	有	赤血球製剤破損のため院内廃棄
6	2005/04/10	54	F	20	−	−	+	無	ウシ精肉	半生	無	
7	2005/04/15	59	F	16	−	−	+	無	ブタホルモン、シカ精肉	十分加熱	無	
8	2005/04/15	35	F	16	−	−	+	無	シカ精肉、ウシ精肉 ウシレバー、ヒツジ精肉 ウシホルモン、ヒツジ精肉	半生 十分加熱 半生	無	
9	2005/04/20	25	M	24	+	+	+	無	ウシレバー、ウシ精肉 ウシホルモン、ヒツジ精肉	十分加熱 半生 十分加熱	有	感染なし
10	2005/04/28	22	M	44	−	−	+	無	回答なし		無	
11	2005/06/07	42	M	24	+	+	+	無	ウシ精肉 ウシホルモン、ブタ精肉、ヒツジ精肉	半生 十分加熱	有	原疾患により死亡
12	2005/06/22	51	M	52	−	−	+	無	回答なし		無	
13	2005/07/03	58	M	219	+	+	+	無	不明レバー、ブタ精肉	十分加熱	無	
14	2005/07/05	22	M	23	+	−	+	無	回答なし		無	
15	2005/07/05	38	M	15	−	−	+	無	ブタホルモン、ウシ精肉、ブタ精肉	半生	無	
16	2005/07/13	24	M	19	−	−	+	無	ウシレバー	生	有	原疾患により死亡
17	2005/09/02	33	M	49	−	−	+	無	ウシ精肉 ヒツジ精肉	生 半生	無	
18	2005/09/01	29	F	100	+	+	+	無	ウシホルモン、ヒツジ精肉 ウシレバー、ウシ精肉、ブタ精肉	半生 十分加熱	無	
19	2005/09/20	42	M	31	−	−	+	無	ブタホルモン、不明レバー、ヒツジ精肉	十分加熱	有	HEV感染（H17.11.1 運営委員会報告済み）
20	2005/09/27	20	F	10	−	−	+	無	ウシ精肉、ブタホルモン、ヒツジ精肉	十分加熱	無	
21	2005/10/21	41	M	12	−	−	+	無	回答なし		無	
22	2005/10/25	44	F	38	+	+	+	無	ウシ精肉、ブタ精肉	十分加熱	無	
23	2005/11/07	30	F	21	−	−	+	無	ブタホルモン、ウシ精肉、ヒツジ精肉	半生	無	
24	2005/11/07	31	F	12	+	+	+	有	ブタホルモン、ウシ精肉、ブタ精肉、ヒツジ精肉	十分加熱	無	
25	2005/11/20	28	M	47	+	+	+	有	ブタレバー、ブタホルモン、ウシ精肉	十分加熱	無	
26	2005/11/29	35	F	333	+	+	+	有	ウシレバー、ウマ精肉 ブタホルモン、ウシ精肉、ブタ精肉	生 十分加熱	無	
27	2005/12/13	42	M	30	−	−	+	有	回答なし		無	
28	2005/12/13	30	M	11	−	−	+	有	ウシ精肉、ヒツジ精肉 不明レバー、ブタ精肉	半生 十分加熱	有	原疾患により死亡
29	2005/12/22	62	F	14	−	−	+	無	不明レバー	十分加熱	有	HEV感染（H18.01.26 運営委員会報告済み）
30	2005/12/27	42	F	14	−	−	+	無	回答なし		無	
									回答なし		無	

No.	採血日	年齢	性別	ALT (IU/L)	HEV抗体		HEV RNA	問診該当 ※1	喫食歴調査		遡及対象供給製剤	受血者情報
					IgM	IgG			肉の種類	食べ方		
31	2006/01/02	22	F	12	−	−	+	有	ウシレバー、ウシ精肉	十分加熱	無	
32	2006/01/06	68	M	23	−	−	+	無	ウシレバー、ブタホルモン、ヒツジ精肉	半生	無	
33	2006/01/13	36	M	42	−	−	+	無	ウマ精肉、不明レバー ウシ精肉、ヒツジ精肉	生 半生	無	
34	2006/01/18	53	M	238	+	+	+	有	ウシレバー、ウシホルモン	十分加熱	無	
35	2006/01/13	31	M	43	−	−	+	有	不明レバー ブタ精肉、ヒツジ精肉	半生 十分加熱	無	
36	2006/01/17	48	M	25	−	−	+	無	回答なし		無	
37	2006/01/25	52	M	25	−	−	+	無	不明レバー、ヒツジ精肉	十分加熱	有	輸血後89日現在、HEVマーカーの陽転は見られず追跡調査終了
38	2006/01/30	39	F	22	−	−	+	無	回答なし		無	
39	2006/01/30	25	M	32	−	−	+	有	ウシ精肉、ウシホルモン、ブタ精肉	十分加熱	無	
40	2006/02/02	39	F	35	−	+	+	有	ウシレバー ウシレバー	生 半生	無	
41	2006/02/07	57	M	13	−	−	+	無	不明	不明	無	
42	2006/02/07	40	F	172	+	+	+	無	ウシ精肉	十分加熱	無	
43	2006/02/17	39	M	28	−	−	+	無	ブタホルモン、ブタレバー、ブタガツ、ヒツジ精肉 イノシシ精肉、ブタ精肉	半生 十分加熱	無	
44	2006/02/20	58	M	22	−	−	+	無	ヒツジ精肉	十分加熱	無	
45	2006/02/21	45	M	30	−	−	+	無	ウシ精肉 ブタ精肉、ブタレバー、ヒツジ精肉	半生 十分加熱	無	
46	2006/03/01	46	F	15	−	−	+	無	回答なし		無	
47	2006/03/01	50	F	29	−	−	+	無	回答なし		無	
48	2006/03/02	54	M	47	+	+	+	無	ウシ・ブタ(精肉、レバー、ホルモン)、ヒツジ精肉	十分加熱	無	
49	2006/03/27	40	F	12	−	−	+	無	回答なし		無	
50	2006/04/01	31	F	16	−	−	+		ヒツジ精肉	半生	無	
51	2006/04/04	30	F	14	−	−	+		ブタ精肉、不明レバー	十分加熱	無	
52	2006/04/12	38	M	45	+	+	+		ブタレバー、ウシ精肉、ブタ精肉、ヒツジ精肉	十分加熱	無	
53	2006/04/18	21	M	26	−	−	+		ウシ精肉、ウシホルモン ウシ精肉、ウシホルモン	半生 十分加熱	無	
54	2006/04/22	28	M	14	+	+	+		回答なし		無	
55	2006/04/26	46	M	19	−	−	+		ブタレバー	半生	無	
56	2006/05/18	62	M	27	−	−	+		ヒツジレバー	十分加熱	無	
57	2006/07/07	17	M	33	−	−	+		回答なし		無	
58	2006/07/11	34	F	10	−	−	+		回答なし		無	
59	2006/07/12	21	F	27	−	−	+		回答なし		無	
60	2006/07/22	49	M	46	+	−	+		ウシ精肉、ブタ精肉、ブタホルモン、ブタレバー	十分加熱	無	

No.	採血日	年齢	性別	ALT (IU/L)	HEV抗体		HEV RNA	問診該当 ※1	喫食歴調査		遡及対象供給製剤	受血者情報
					IgM	IgG			肉の種類	食べ方		
61	2006/08/01	62	M	18	−	−	+		ブタホルモン、ウシ精肉、ブタ精肉、ヒツジ精肉	十分加熱	無	
62	2006/09/06	44	F	14	−	−	+		喫食歴なし		無	
63	2006/09/29	68	M	15	−	−	+		ブタ精肉、ヒツジ精肉	十分加熱	無	
64	2006/10/21	29	M	22	−	−	+		不明		無	
65	2006/11/19	48	M	58	−	−	+		ウシ精肉、ブタ精肉	十分加熱	無	
66	2006/11/23	54	M	18	−	−	+		回答なし		無	
67	2006/12/01	43	M	55	−	+	+		ブタ精肉	十分加熱	無	
68	2006/12/04	60	M	46	+	+	+		ウシ精肉	十分加熱	無	
69	2006/12/04	47	M	40	+	+	+		ウシ精肉、ウシホルモン	十分加熱	無	
70	2007/03/01	33	M	41	−	−	+		ウシレバー	生	無	
71	2007/03/15	42	M	32	−	+	+		ブタレバー、ブタホルモン	半生	無	
72	2007/03/27	55	M	30	−	−	+		不明レバー	十分加熱	無	
73	2007/04/07	22	F	9	−	−	+		ユッケ ウシホルモン、ヒツジホルモン	生 十分加熱	無	
74	2007/05/16	47	F	15	−	−	+		ヒツジ精肉、ブタホルモン	十分加熱	無	
75	2007/05/18	40	F	27	+	+	+		ブタ生ハム(自家製)	半生	無	
76	2007/05/30	33	M	26	−	+	+		ヒツジ精肉、ブタホルモン	十分加熱	無	
77	2007/06/22	38	M	20	−	−	+		ウシ精肉、ヒツジ精肉	十分加熱	無	
78	2007/06/25	45	M	37	+	+	+		ブタ精肉 ヒツジ精肉	十分加熱 半生	無	
79	2007/06/27	37	M	18	−	−	+		ブタ精肉	十分加熱	無	
80	2007/07/24	57	M	24	−	−	+		喫食歴なし		無	
81	2007/07/29	37	M	48	−	−	+		不明レバー、ブタホルモン 不明レバー、ブタホルモン	十分加熱 半生	無	
82	2007/07/31	48	M	30	−	−	+		ブタ精肉、ブタホルモン、ブタレバー	十分加熱	無	
83	2007/08/01	48	M	33	−	−	+		ブタ精肉 ウシ精肉、ヒツジ精肉	十分加熱 半生	無	
84	2007/08/04	53	M	28	−	−	+		ヒツジ精肉 ヒツジ精肉	十分加熱 半生	無	
85	2007/08/26	50	M	60	−	−	+		ヒツジ精肉 ウシ精肉	十分加熱 生	無	
86	2007/09/05	41	M	29	−	−	+		喫食歴なし		無	
87	2007/09/18	41	M	23	−	−	+		ウシ精肉、ブタ精肉、ウシホルモン、ブタホルモン	半生	無	
88	2007/09/21	57	M	19	−	−	+		ブタホルモン	十分加熱	無	
89	2007/10/03	59	M	39	−	−	+		ブタレバー、ブタ精肉	十分加熱	無	
90	2007/10/03	19	M	40	−	−	+		喫食歴なし		無	

No.	採血日	年齢	性別	ALT (IU/L)	HEV抗体		HEV RNA	問診該当 ※1	喫食歴調査		遡及対象供給製剤	受血者情報
					IgM	IgG			肉の種類	食べ方		
91	2007/10/09	35	M	19	−	−	+		ブタ精肉	十分加熱	無	
92	2007/10/18	30	M	31	−	−	+		ウシ精肉、ブタ精肉、ヒツジ精肉	十分加熱	無	
93	2007/11/16	24	M	5	−	−	+		不明		無	
94	2007/11/16	54	M	22	−	+	+		ブタホルモン、ブタレバー	十分加熱	無	
95	2007/11/16	45	M	47	−	−	+		ブタ精肉 ブタレバー	十分加熱 半生	無	
96	2007/11/19	58	M	13	−	−	+		レバー、ホルモン	不明	無	
97	2007/11/19	24	M	46	−	−	+		不明		無	
98	2007/11/24	36	M	25	−	−	+		不明		無	
99	2007/11/29	42	M	21	−	+	+		不明		無	
100	2007/11/30	31	M	42	+	+	+		レバー	不明	無	
101	2008/01/08	35	M	36	−	−	+		ウシ精肉、ブタ精肉	十分加熱	無	
102	2008/01/17	48	F	13	+	+	+		ブタホルモン、シカ精肉 ウシ精肉	十分加熱 半生	無	
103	2008/01/29	57	M	22	−	−	+		ブタレバー、ブタホルモン	十分加熱	無	
104	2008/02/04	31	M	47	+	+	+		不明		無	
105	2008/02/06	57	M	20	−	−	+		ブタホルモン	十分加熱	無	
106	2008/02/13	42	M	35	−	−	+		不明レバー	十分加熱	無	
107	2008/02/13	60	M	37	+	+	+		不明		無	
108	2008/03/11	30	M	21	−	−	+		不明		無	
109	2008/03/25	34	F	26	−	−	+		喫食歴なし		無	
110	2008/03/26	32	M	41	+	+	+		ブタ精肉、ウシ精肉	十分加熱	無	
111	2008/03/29	54	M	26	−	−	+		ブタ精肉	十分加熱	無	
112	2008/03/30	19	F	9	−	−	+		不明レバー	十分加熱	無	
113	2008/04/16	48	M	13	−	−	+		不明		無	
114	2008/05/12	33	M	12	−	−	+		ブタ精肉、ブタホルモン	半生	無	
115	2008/05/28	39	F	29	−	−	+		不明		無	
116	2008/05/28	47	M	46	−	−	+		ブタホルモン	十分加熱	無	
117	2008/06/04	43	M	38	+	+	+		ウシレバー ウシホルモン、ウシ、ブタ、ヒツジ精肉	生 十分加熱	無	
118	2008/06/07	42	M	11	−	−	+		ウシレバー ブタ精肉	生 十分加熱	無	
119	2008/06/23	48	M	17	−	−	+		ウシ、ブタ、ヒツジ精肉	半生	無	
120	2008/07/10	39	M	32	−	−	+		ウシ、ブタ、ヒツジ精肉 ウシ、ブタ、ヒツジ精肉	半生 十分加熱	無	

No.	採血日	年齢	性別	ALT (IU/L)	HEV抗体		HEV RNA	問診該当 ※1	喫食歴調査		遡及対象供給製剤	受血者情報
					IgM	IgG			肉の種類	食べ方		
121	2008/07/11	39	M	28	−	−	+		不明		無	
122	2008/07/26	34	M	35	−	−	+		ウシ精肉、ブタ精肉	十分加熱	無	
123	2008/07/27	36	M	45	−	−	+		不明		無	
124	2008/07/30	24	M	10	−	−	+		不明		無	
125	2008/08/20	19	M	17	+	−	+		不明		無	
126	2008/09/03	30	M	28	−	−	+		不明		無	
127	2008/09/08	35	M	16	−	−	+		不明		無	
128	2008/09/09	23	F	24	−	−	+		ブタ、ヒツジ精肉	十分加熱	無	
129	2008/09/16	33	F	18	+	+	+		不明		無	
130	2008/09/16	58	M	21	−	−	+		不明		無	
131	2008/09/17	62	M	37	−	−	+		ウシレバー、ブタレバー	十分加熱	無	
132	2008/09/23	42	M	36	−	−	+		ブタ精肉、ブタレバー	十分加熱	無	
133	2008/09/25	35	M	16	−	−	+		不明		無	
134	2008/09/27	30	M	22	−	−	+		不明		無	
135	2008/10/10	50	M	31	−	−	+		ウシ、ブタ、ヒツジ精肉	不明	無	
136	2008/10/11	39	F	15	−	−	+		ウマ精肉	生	無	
137	2008/10/14	56	M	13	−	−	+		不明レバー	生	無	
138	2008/10/18	38	F	23	−	−	+		不明		無	
139	2008/11/03	37	M	22	−	−	+		ウシホルモン、ブタ精肉	半生	無	
140	2008/11/11	41	F	11	−	−	+		不明		無	
141	2008/12/05	52	M	18	−	−	+		ブタレバー	十分加熱	無	
142	2008/12/20	47	M	22	−	−	+		ウシ、ブタ、ヒツジ精肉	十分加熱	無	
143	2009/01/13	50	M	27	−	−	+		ウシ・ブタ(精肉、レバー、ホルモン)	十分加熱	無	
144	2009/01/27	55	M	17	−	−	+		不明		無	
145	2009/02/11	37	M	28	−	−	+		不明ホルモン	十分加熱	無	
146	2009/02/18	59	F	23	−	−	+		ブタレバー	不明	無	
147	2009/02/23	20	F	42	−	+	+		ウシ、ブタ精肉	半生	無	
148	2009/03/11	29	M	49	−	−	+		ブタレバー、ホルモン	十分加熱	無	
149	2009/04/16	35	F	29	−	−	+		ウシレバー ウシ・ブタホルモン	生 半生	無	
150	2009/04/24	36	F	42	−	−	+		不明ホルモン	不明	無	

No.	採血日	年齢	性別	ALT (IU/L)	HEV抗体		HEV RNA	問診該当 ※1	喫食歴調査		遡及対象供給製剤	受血者情報
					IgM	IgG			肉の種類	食べ方		
151	2009/04/27	45	M	50	−	−	+		不明		無	
152	2009/06/04	65	F	24	−	−	+		不明ホルモン	不明	無	
153	2009/06/09	63	M	26	−	−	+		ブタ肉 シカ精肉	十分加熱 生	無	
154	2009/07/01	47	M	40	+	+	+		ウシ精肉、ブタホルモン	十分加熱	無	
155	2009/07/23	26	F	11	−	−	+		ブタホルモン ウシ精肉	十分加熱 生	無	
156	2009/08/01	40	M	26	−	−	+		ウシ精肉、ブタホルモン	十分加熱	無	
157	2009/08/14	41	M	14	−	−	+		不明		無	
158	2009/09/04	43	M	45	−	−	+		ウマ精肉	生	無	
159	2009/09/09	54	F	14	−	−	+		ウシレバー	半生	無	
160	2009/09/09	51	M	19	−	−	+		ブタ精肉	十分加熱	無	
161	2009/10/12	27	M	41	−	−	+		不明		無	
162	2009/10/27	52	M	29	−	−	+		ブタ精肉、ブタレバー	十分加熱	無	
163	2009/11/17	53	M	19	−	−	+		ウシ・ブタ精肉、ウシレバー	半生	無	
164	2009/11/28	28	M	29	−	−	+		不明		無	
165	2009/12/13	37	M	35	−	−	+		ウシレバー	生	無	
166	2009/12/17	37	M	15	−	−	+		ウシ精肉、ブタレバー、ウシ・ブタホルモン	十分加熱	無	
167	2009/12/24	64	M	40	−	−	+		ヒツジ精肉	不明	無	
168	2009/12/28	34	F	16	−	−	+		不明		無	
169	2010/01/17	41	M	25	−	−	+		ウシレバー	生	無	
170	2010/01/19	34	M	36	−	−	+		ブタホルモン	十分加熱	無	
171	2010/01/21	39	M	24	−	−	+		ブタレバー、ウシ精肉、ブタ精肉	十分加熱	無	
172	2010/02/26	26	F	15	−	−	+		ウシ精肉	十分加熱	無	
173	2010/03/04	50	M	21	−	−	+		シカ精肉	半生	無	
174	2010/03/17	47	M	18	−	−	+		ブタ精肉	十分加熱	無	
175	2010/03/17	26	M	11	−	−	+		不明		無	
176	2010/03/26	60	M	38	−	−	+		ブタ精肉	十分加熱	無	
177	2010/04/17	54	M	37	−	−	+		ウシレバー、ブタホルモン	十分加熱	無	
178	2010/05/19	36	F	14	−	−	+		不明ホルモン		無	
179	2010/06/19	35	M	31	−	−	+		不明ホルモン		無	
180	2010/07/13	25	M	17	−	−	+		回答なし		無	

No.	採血日	年齢	性別	ALT (IU/L)	HEV抗体		HEV RNA	問診該当 ※1	喫食歴調査		遡及対象供給製剤	受血者情報
					IgM	IgG			肉の種類	食べ方		
181	2010/07/14	49	M	26	−	−	+		不明		無	
182	2010/07/19	49	M	21	−	−	+		なし		無	
183	2010/07/20	58	M	30	−	+	+		不明ホルモン、レバー	不明	無	
184	2010/07/29	44	M	17	−	−	+		ブタ精肉・レバー、ウシ・ヒツジ精肉	半生	無	
185	2010/08/01	50	M	14	−	−	+		ウシ精肉・ホルモン、ヒツジ精肉	十分加熱	無	
186	2010/09/19	60	M	23	−	−	+		不明レバー・ホルモン	半生	無	
187	2010/09/19	42	M	30	+	+	+		ウシ・ブタホルモン・レバー	十分加熱	無	

※1：問診喫食歴調査内容
05年 1月1日～05年10月31日：「過去3ヶ月以内にブタ、シカ、イノシシあるいは動物種不明の生肉、生レバーの喫食歴」
05年11月1日～06年03月31日：「過去3ヶ月以内に生肉（半生も含む）、レバー、ホルモン（動物種、焼き方を問わず）の喫食歴」、なお本調査は06年03月31日をもって終了

事　務　連　絡
平成２２年１０月２７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２２年７月２６日付け血安第２５７号にて貴社から報告を頂いたところですが、平成２２年１１月２４日（水）に平成２２年度第３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２２年１１月２日（火）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１２については、平成２２年８月１１日開催平成２２年度第２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１５年９月５日付けで報告された輸血用血液製剤でＨＩＶの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．平成１６年３月２２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５人の供血者のその後の検査結果。来訪がなければ、その旨。

３．平成１６年１１月２６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７人の供血者のその後の検査結果。来訪がなければ、その旨。

４．平成１７年１月１２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４人の供血者のその後の検

査結果。来訪がなければ、その旨。

５．平成１７年２月４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１人の供血者のその後の検査結果。来訪がなければ、その旨。

６．平成１７年６月２３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３人の供血者のその後の検査結果。来訪がなければ、その旨。

７．平成１８年４月７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８人の供血者のその後の検査結果。来訪がなければ、その旨。

８．平成１８年６月５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１人の供血者のその後の検査結果。来訪がなければ、その旨。

９．平成１９年２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１人の供血者のその後の検査結果。来訪がなければ、その旨。

１０．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３人の供血者のその後の検査結果。来訪がなければ、その旨。

１１．平成１８年２月１５日報告、３月８日付けで追加報告された輸血用血液製剤でＨＣＶ（Ｃ型肝炎ウイルス）感染が疑われる事例について、残る３人の供血者のその後の検査結果。来訪がなければ、その旨。

１２．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 3 9 9 号
平成２２年１１月２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22年10月27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は、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15年9月5日付けで報告された輸血用血液製剤でＨＩＶの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。(8名中6名が来所、検査は全て陰性)

２．平成16年3月22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5人のその後の来訪なし。(37名中32名が来所、検査は全て陰性)

３．平成16年11月26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7人のその後の来訪なし。(48名中41名が来所、検査は全て陰性)

４．平成17年1月12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4人のその後の来訪なし。(16名中12名が来所、検査は全て陰性)

５．平成17年2月4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１人のその後の来訪なし。(追跡調査対象の4名中3名が来所、HBV-DNAは全て陰性。1名はHBc抗体がEIA法のみ陽性HI法陰性、その他の者は全て陰性)

６．平成17年6月23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、1人がその後献血に協力頂き、検査は陰性。残る２人の来訪なし。(20名中18名が来所、検査は全て陰性)

７．平成18年4月7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、１人がその後献血に協力頂き、検査は陰性。残る７人のその後の来訪なし。(53名中46名が来所、HBV-DNAは全て陰性。2名はHBc抗体及びHBs抗体陽性、1名はHBs抗体のみ陽性、その他の者は全て陰性)

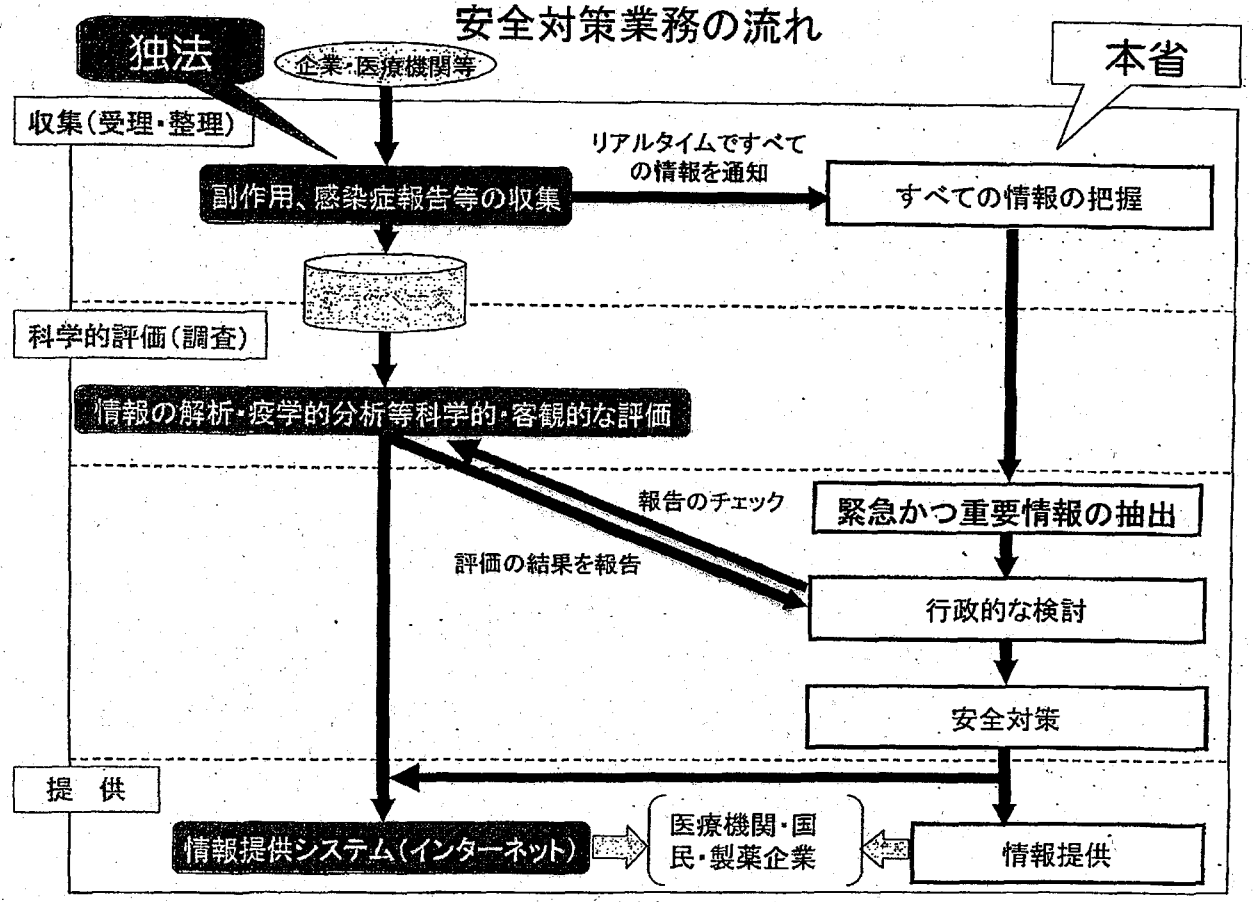
８．平成18年6月5日付けで報告された輸血用血液製剤でＨＢＶ（Ｂ型肝炎ウイルス）感染が疑われる事例について、残る１人のその後の来訪なし。(29名中28名が来所、HBV-DNAは全て陰性。2名はHBc抗体及びHBs抗体陽性、1名はHBs抗体のみ陽性、その他の者は全て陰性)

９．平成19年2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１人のその後の来訪なし。(3名中2名が来所、検査は全て陰性)

１０．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3人のその後の来訪なし。(23名中20名が来所、検査は全て陰性)

１１．平成18年2月15日報告、3月8日付けで追加報告された輸血用血液製剤でＨＣＶ（Ｃ型肝炎ウイルス）感染が疑われる事例について、残る 3 人のその後の来訪なし。(81名中78名が来所、検査は全て陰性)

１２．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。


安全対策業務の流れ
独法
企業・医療機関等
本省
収集(受理・整理)
副作用、感染症報告等の収集
リアルタイムですべての情報を通知
すべての情報の把握
科学的評価(調査)
情報の解析・疫学的分析等科学的・客観的な評価
報告のチェック
緊急かつ重要情報の抽出
評価の結果を報告
行政的な検討
安全対策
提　供
情報提供システム(インターネット)
医療機関・国民・製薬企業
情報提供

29

資料３－３

献血件数及びＨＩＶ抗体・核酸増幅検査陽性件数

	（検査実施数）	陽性件数 内女性 核酸増幅検査のみ陽性	10万件当たり
１９８８年（昭和63年）	7,974,147	11（1）	0.134
１９８９年（平成元年）	7,876,682	9（1）	0.113
１９９０年（平成２年）	7,743,475	13（1）	0.165
１９９１年（平成３年）	8,071,937	26（6）	0.336
１９９２年（平成４年）	7,710,693	29（4）	0.359
１９９３年（平成５年）	7,205,514	34（7）	0.441
１９９４年（平成６年）	6,610,484	35（5）	0.486
１９９５年（平成７年）	6,298,706	36（5）	0.545
１９９６年（平成８年）	6,039,394	46（9）	0.730
１９９７年（平成９年）	5,998,760	46（5）	0.762
１９９８年（平成10年）	6,137,378	54（5）	0.900
１９９９年（平成11年）	6,139,205	56（4）	0.912
２０００年（平成12年）	5,877,971	64（6）	1.042
２００１年（平成13年）	5,774,269	67（4）[3]	1.140
２００２年（平成14年）	5,784,101	79（1）[1]	1.368
２００３年（平成15年）	5,621,096	82（5）[2]	1.418
２００４年（平成16年）	5,473,140	87（8）[2]	1.548
２００５年（平成17年）	5,320,602	92（4）[2]	1.681
２００６年（平成18年）	4,987,857	78（3）[2]	1.466
２００７年（平成19年）	4,939,550	87（5）[1]	1.744
２００８年（平成20年）	5,077,238	102（3）[6]	2.065
２００９年（平成21年）	5,287,101	107（3）[0]	2.107
２０１０年（１～９月）（平成22年）	3,999,981（速報値）	102（6）[2]	1.929
		61（3）[1]	1.525

（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２２年は、１月～９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	32	1	33	11	0	11	43	1	44
20～29歳	508	28	536	45	4	49	553	32	585
30～39歳	477	12	489	24	2	26	501	14	515
40～49歳	177	1	178	11	1	12	188	2	190
50～69歳	74	0	74	6	0	6	80	0	80
合計	1268	42	1310	97	7	104	1365	49	1414

※ 昭和61年～平成22年9月（昭和61年については年途中から集計し、平成22年7月～9月については速報値で集計）

２．都道府県別（献血地別）

県別	61年 (件)	62年 (件)	63年 (件)	元年 (件)	2年 (件)	3年 (件)	4年 (件)	5年 (件)	6年 (件)	7年 (件)	8年 (件)	9年 (件)	10年 (件)	11年 (件)	12年 (件)	13年 (件)	14年 (件)	15年 (件)	16年 (件)	17年 (件)	18年 (件)	19年 (件)	20年 (件)	21年 (件)	22年 (件)	合計 (件)	構成割合 (%)	ブロック別 陽性献血件数 (件)	ブロック別 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	3	2	1	37	2.6		
2. 青森			2									1					1	1	2	1	1	1		1	2	13	0.9		
3. 岩手										1				1										3		5	0.4		
4. 宮城							1	1				1	1	1		1	1	1	1	2				2		13	0.9	北海道	
5. 秋田												1		1		1	1	1	1				1		1	4	0.3	・東北	
6. 山形													1				1							1		3	0.2		
7. 福島							1					2		1	1			1							1	8	0.6	83	5.9
8. 茨城					1	1	4	2			1	2		1	2	1	1				1	1	1	5	1	25	1.8		
9. 栃木					3	1				2	1	1		1		3		1		1	4	2	1	1	1	23	1.6		
10. 群馬					1	1		1				1		1	3	1		2		3		2	1	3		20	1.4		
11. 埼玉		1				1	1	2	1	2	3	3	3	3	3	3	3	3	5	2	1	3	2	8	2	55	3.9		
12. 千葉							1	6	2	2	3	7	2	4	5	4	5	3	3	2	2	6	9	5	3	74	5.2	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	20	441	31.2		
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	80	5.7	718	50.8
15. 新潟				1		1		1						1		2			1		2			2		11	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	0.4	甲信越	
19. 山梨					1	1										1										4	0.3		
20. 長野							1	1			2						1				1	1				7	0.5	40	2.8
21. 岐阜							1									1			1				1	2	1	7	0.5		
22. 静岡					1	3			1					1	1		1	1				4		2	1	16	1.1	東海	
23. 愛知		1			3	2		3	1	1			4	3	2	3	2	2	4	4	5	4	10	4	2	60	4.2		
24. 三重											1	1	1		1			1		2				1		8	0.6	91	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		1	1	26	1.8		
27. 大阪	1	1	1	1	3		1		4	2	1	8	14	6	8	10	10	15	17	19	17	28	26	13	11	215	15.2		
28. 兵庫						2			1	2				2	1	1	4	5	3		3	3	4	3		34	2.4	近畿	
29. 奈良										1	2	3	1		1		1	1	1	1			1	1		13	0.9		
30. 和歌山																			2				1	1		4	0.3	299	21.1
31. 鳥取									1							1				1			1		1	5	0.4		
32. 島根						1															1				3	5	0.4		
33. 岡山									2								2	1		2	2	3	1			13	0.9	中国	
34. 広島							2	1	1				1				2	6		2	2	2	1	4		23	1.6		
35. 山口					1						2											1	1			4	0.3	50	3.5
36. 徳島										1				1						1		1	1	1		6	0.4		
37. 香川							1												1			1	2	2	1	8	0.6	四国	
38. 愛媛													1	1	2	3	2	2		1	1	2		1		16	1.1		
39. 高知																		1			1		1	1	1	6	0.4	36	2.5
40. 福岡							1		2	2	2	1	1	1	1	2	4	2	2		3	1	3	2	3	33	2.3		
41. 佐賀																										0	0.0		
42. 長崎																	2					1	1	2		6	0.4		
43. 熊本						1				2	1			1	2		1	2		1	1	2	2	2	1	19	1.3		
44. 大分									1									2	1							4	0.3	九州	
45. 宮崎														2						2		1	1	1		7	0.5	・沖縄	
46. 鹿児島							1						2			1	1		1		1	1	2			10	0.7		
47. 沖縄		1									1							2	3		1	5	1	3	1	18	1.3	97	6.9
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	61	1414	100	1414	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22年については、1月～6月の確定値と7月～9月の速報値で集計

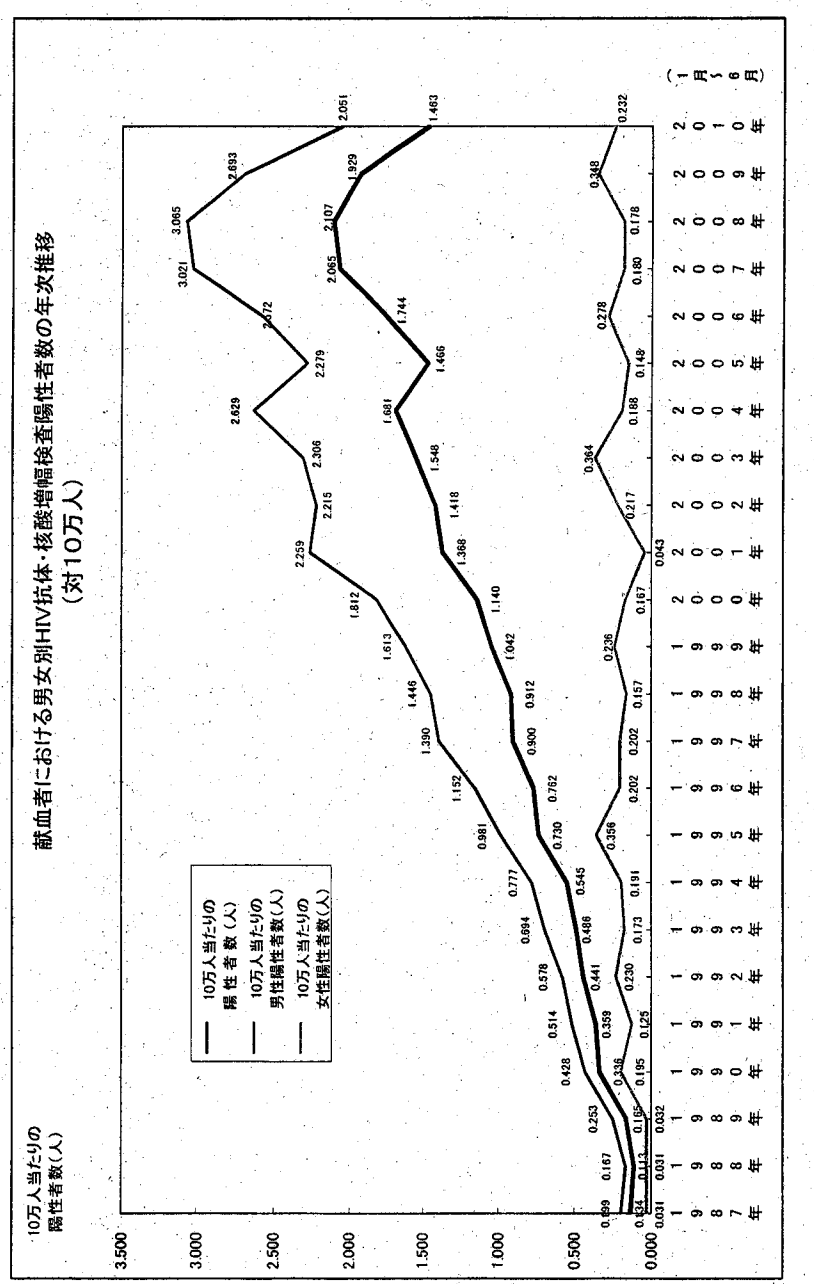
ブロック別ＨＩＶ抗体・核酸増幅検査陽性献血者

	平成18年 献血者 (人)	陽性 (件)	10万人当たり (件)	平成19年 献血者 (人)	陽性 (件)	10万人当たり (件)	平成20年 献血者 (人)	陽性 (件)	10万人当たり (件)	平成21年 献血者 (人)	陽性 (件)	10万人当たり (件)	平成22年（1月～9月）（速報値） 献血者 (人)	陽性 (件)	10万人当たり (件)
北海道・東北	674,411	3	0.445	647,438	4	0.618	651,215	5	0.768	677,073	9	1.329	515,973	5	0.969
関東	1,548,970	37	2.389	1,559,391	36	2.309	1,621,408	40	2.467	1,705,070	42	2.463	1,277,955	29	2.269
北陸・甲信越	337,810	4	1.184	330,485	4	1.210	335,848	0	0.000	340,901	3	0.880	255,466	0	0.000
東海	540,167	5	0.926	545,248	8	1.467	562,610	11	1.955	584,495	9	1.540	445,013	4	0.899
近畿	817,075	25	3.060	807,758	30	3.714	833,556	33	3.959	863,744	20	2.316	660,336	12	1.817
中国	335,666	5	1.490	316,087	5	1.582	316,509	13	1.264	329,443	4	1.214	248,739	4	1.608
四国	164,763	2	1.214	161,533	4	2.476	166,332	4	2.405	173,914	5	2.875	133,839	2	1.494
九州・沖縄	568,986	6	1.054	571,610	11	1.924	589,760	10	1.696	612,461	10	1.633	462,660	5	1.081
合計	4,987,857	87	1.744	4,939,560	102	2.065	5,077,238	107	2.107	5,287,101	102	1.929	3,999,981	61	1.525

年齢別HIV抗体・核酸増幅検査陽性献血者

	平成18年			平成19年			平成20年			平成21年			平成22年（1月～6月）（確定値）		
	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		人	件		人	件		人	件		人	件	
16才～19才	381,352	2 (1)	0.524	324,414	5	1.541	308,019	2	0.649	295,811	3	1.014	144,478	3	2.076
20才～29才	1,188,738	29 (2)	2.440	1,135,102	38 (2)	3.348	1,141,746	41	3.591	1,139,991	37 (1)	3.246	553,900	11 (1)	1.986
30才～39才	1,361,658	43 (2)	3.158	1,369,241	35 (1)	2.556	1,391,141	50 (1)	3.594	1,414,747	42 (3)	2.969	695,754	19 (1)	2.731
40才～49才	1,048,055	9	0.859	1,088,410	17	1.562	1,171,449	11 (1)	0.939	1,272,397	17 (2)	1.336	667,625	4	0.599
50才～59才	766,625	3	0.391	770,663	5	0.649	785,280	3 (1)	0.382	841,168	3	0.357	432,682	2	0.462
60才～	241,429	1	0.414	251,720	2	0.795	279,603	0	0.000	322,987	0	0.000	171,853	0	0.000
合　計	4,987,857	87 (5)	1.744	4,939,550	102 (3)	2.065	5,077,238	107 (3)	2.107	5,287,101	102 (6)	1.929	2,666,292	39 (2)	1.463

（注）陽性件数の（）内女性



資料4－1

XMRVの疫学に関する主な文献一覧(平成22年5月18日作成、平成22年11月24日改訂)

血液事業部会運営委員会委員　岡田　義昭

【前立腺癌関係】

文献番号	文献名	XMRVの陽性率			検出法(組織)	報告国	要約
		前立腺がん	慢性疲労症候群	健康人			
1	Urisman A, et al., PLoS Pathog. 2006 Mar;2(3):e25. Identification of a novel Gammaretrovirus in prostate tumors of patients homozygous for R462Q RNASEL variant.	9/86 10.5% (遺伝子の型による内訳) QQ 8/20 40%　RQ 0/14　0% RR 1/52 1.9%	-	-	RT-PCR (前立腺)	米国	DNAアレイによって前立腺がん組織から新たなウイルス(XMRV)を発見した。RNaseLにホモ型変異(QQ)にもつ前立腺癌の40%からXMRVが検出されたが、変異がない前立腺癌(RR)では1.9%であった。
2	Fischer N, Hellwinkel O, Schulz C, Chun FK, Huland H, Aepfelbacher M, Schlomm T. J Clin Virol. 2008 Nov;43(3):277-83. Prevalence of human gammaretrovirus XMRV in sporadic prostate cancer	1/87 1.2% (非家族性)	-	1/70 1.42%	RT-PCR (前立腺)	ドイツ	非家族性の前立腺がん組織からXMRVの検出が試みられた。その結果、欧州北部においてはほとんど検出されなかった。但し、本研究において、RNaseLにホモ型変異(QQ)をもつサンプルは6%未満であったことに注意を要する。
3	Hohn O, Krause H, Barbarotto P, Niederstadt L, Beimforde N, Denner J, Miller K, Kurth R, Bannert N. Retrovirology. 2009 Oct 16;6:92. Lack of evidence for xenotropic murine leukemia virus-related virus(XMRV) in German prostate cancer patients	0/589 0% (PCR) 0/146　0% (抗体)	-	0/5 0% (抗体)	PCR、RT-PCR (前立腺) ELISA(血清)	ドイツ	589例(76例の RNaseLホモ型変異型を含む)の前立腺癌組織からDNAとRNAを抽出し、核酸増幅法を用いてXMRVの遺伝子の有無を調べたが検出できなかった。また、血清中からもXMRVに反応する抗体は検出できなかった。
4	Schlaberg R, Choe DJ, Brown KR, Thaker HM, Singh IR. Proc Natl Acad Sci U S A. 2009 Sep 22;106(38):16351-6 XMRV is present in malignant prostatic epithelium and is associated with prostate cancer, especially high-grade tumors	14/233 6.2% PCR 54/233 23% ウイルス抗原	-	2/101 2% PCR 4/101 4% ウイルス抗原	PCR (前立腺) 組織染色 (前立腺)	米国	233例の前立腺癌中14例からPCR法によってXMRV遺伝子が検出できた。RNaseLの変異とは関連がなかった。XMRVのタンパクは上皮細胞に存在していた。
13	Danielson B.P.,Ayala G.E.,and Kimata J.T. JID.2010 Nov.202:1470-77 Detection of xenotropic murine leukemia virus-related virus in normal and tumor tissue of patients from the southern United States with prostate cancer is dependent on specific polymerase chain reaction conditions	32/144 22.2%	-	-	PCR (前立腺)	米国 (南部)	米国の南部にある州での前立腺癌患者からXMRV遺伝子の検出を行なった。前立腺癌の生検検体から *DNA*を抽出し、*PCR*を実施(*env*領域)した。32例が陽性であった。内28例は正常組織と癌組織を独立に検討でき、18例は両方とも陽性であった。XMRV陽性例ではRNASEL遺伝子の変異やgleason score(病理組織分類)との間に有意な差は認められなかった。
14	Aloia AL, Sfanos KS, Isaacs WB, Zheng Q, Maldarelli F, De Marzo AM, Rein A; Cancer Res; Published OnlineFirst October 21, 2010 XMRV: A New Virus in Prostate Cancer?	*0/約800 0%*	-	-	PCR (前立腺) 組織染色 (前立腺)		約800の前立腺検体について、リアルタイムPCRと免疫組織染色を用い、XMRVの検出を試みた。その結果、前立腺癌にXMRVは見られなかった。XMRVは実際にはヒトには感染を起こしていない可能性がある。もし感染していても、このデータは前立腺癌との因果関係を支持しない。

XMRVの疫学に関する主な文献一覧(平成22年5月18日作成、平成22年11月24日改訂)

血液事業部会運営委員会委員　岡田　義昭

【慢性疲労症候群関係】

文献番号	文献名	XMRVの陽性率			検出法(組織)	報告国	要約
		前立腺がん	慢性疲労症候群	健康人			
5	Lombardi VC, Ruscetti FW, Das Gupta J, Pfost MA, Hagen KS, Peterson DL, Ruscetti SK, Bagni RK, Petrow-Sadowski C, Gold B, Dean M, Silverman RH, Mikovits JA. Science. 2009 Oct 23;326(5952):585-9 *Detection of an infectious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome*	-	68/101 67%	8/218 3.7%	PCR (末梢単核球)	米国	慢性疲労性症候群(CFS)患者の67%からXMRV遺伝子が検出され、XMRVに感染したCFS患者の細胞や血漿中に感染性ウイルスが存在した。また、一部の症例ではウイルスと抗体が共存していた。 健常人の3.7%からもXMRVが検出された。CFS由来のXMRVは塩基配列が前立腺癌由来のものとクラスターを形成していた。
6	Erlwein O, Kaye S, McClure MO, Weber J, Wills G, Collier D, Wessely S, Cleare A. PLoS One. 2010 Jan 6;5(1):e8519. Failure to detect the novel retrovirus XMRV in chronic fatigue syndrome	-	*0/186 0%*	-	PCR (全血)	イギリス	慢性疲労性症候群186例を対象に全血から核酸増幅法によるXMRV遺伝子の検出を行ったが、検出できなかった。
7	Groom HC, Boucherit VC, Makinson K, Randal E, Baptista S, Hagan S, Gow JW, Mattes FM, Breuer J, Kerr JR, Stoye JP, Bishop KN. Retrovirology. 2010 Feb 15;7:10 Absence of xenotropic murine leukaemia virus-related virus in UK patients with chronic fatigue syndrome	-	0/136 0% DNA 0/140 0% RNA	0/95 0% DNA 0/141 0% RNA	PCR(全血) RT-PCR(血清)	イギリス	全血及び血清から核酸を抽出し、核酸増幅法を用いてXMRVの遺伝子を検出したが、慢性疲労性症候群及び健常人から検出されなかった。
8	van Kuppeveld FJ, de Jong AS, Lanke KH, Verhaegh GW, Melchers WJ, Swanink CM, Bleijenberg G, Netea MG, Galama JM, van der Meer JW. BMJ. 2010 Feb 25;340:c1018 Prevalence of xenotropic murine leukaemia virus-related virus in patients with chronic fatigue syndrome in the Netherlands: retrospective analysis of samples from an established cohort	-	0/32 0% RNA	0/43 0% RNA	RT-PCR (末梢単核球)	オランダ	1991～1992年に凍結保存されていた末梢単核球からRNAを抽出し、核酸増幅法によってXMRV遺伝子を検出したが、慢性疲労性症候群及び健常人から1例も検出されなかった。
9	Switzer WM, Jia H, Hohn O, Zheng HQ, Tang S, Shankar A, Bannert N, Simmons G, Hendry RM, Falkenberg VR, Reeves WC, Heneine W; Retrovirology 2010, 7:57 Absence of evidence of Xenotropic Murine Leukemia Virus-related virus infection in persons with Chronic Fatigue Syndrome and healthy controls in the United States	-	0/51 0% DNA	0/56 0% DNA	PCR (末梢単核球) 免疫学的試験	米国	米国カンザス州とジョージア州のCFS患者51名とコントロール56名の血清について、PCRと抗体検査が行われた。その結果、いずれからもXMRVは検出されなかった。この結果は、XMRVとCFSの関係を支持しない。
10	Lo SC, Pripuzova N, Li B, Komaroff AL, Hung GC, Wang R, and Alter H.J.PNAS.2010,107.1470-77 Detection of MLV-related virus gene sequences in blood of patients with chronic fatigue syndrome and healthy blood donors	-	32/37 86.5% DNA (XMRVとは異なるウイルス)	3/44 6.8% DNA (XMRVとは異なるウイルス)	PCR (末梢単核球)	米国	既に報告されているgag領域のプライマーを用いて37人のCFS末梢血を解析したところ、32人からマウス白血病に類似したレトロウイルスが検出された。塩基配列からは、XMRVよりもpolytropic(多種指向性)マウス内因性レトロウイルスに類似していた。
11	Barnes E.,Flanagan P.,Brown A.,Robinson N.,Brown H.,McClure M.,Oxenius A.,Collier J.,Weber J.,Gunthard H.F.,Hirschel B.,Fidler S.,Phillips R.,and Frater J.　JID.2010 Failure to detect xenotropic murine leukemia virus-related virus in blood of individuals at high risk of blood-borne viral infection	-	0/151 0% DNA 0/79 0% RNA	-	PCR (末梢単核球) RT-PCR (血漿)	英国 西ヨーロッパ	英国と西ヨーロッパの HIV-1感染者163人(慢性期84人、急性期79人)とHCV感染者67人(慢性期)において、慢性感染者からは DNA、急性期にある感染者からはRNAを抽出し、NAT検査を実施したが、XMRVの遺伝子は検出できなかった。さらにgagに対するT細胞の反応性も63人で検討したが、反応性は認められなかった。以上から、英国や西ヨーロッパでは血液や性的感染リスクを持つヒトでのXMRV感染率は高くなかった。
12	Hnrich T.J.,Li J.Z.,Felsenstein D.,Kotton C.N.,Plenge R.M.,Pereyra F.,Marty F.M.,Lin N.H.,Grazioso P.,Crochiere D.M.,Eggers D.,Kuritzkes D.R.,and Tsibris A.M.N.JID.2010 Xenotropic mueine leukemia virus-related virus prevalence in patients with chronic fatigue syndrome or chronic immunomodulatory conditions	-	0/198 0% DNA	0/95 0% DNA	PCR (末梢単核球)	米国	ボストン周囲にある大学病院において、XMRV感染の頻度を調べるためにCFS32人、HIV感染者43人、幹細胞及び臓器移植患者26人、関節リュウマチ(RA)患者97人、RAのコントロールの患者95人計230人から DNAを抽出しNAT検査を行なった。XMRVの遺伝子は検出できなかった。

文献9

RETROVIROLOGY

RESEARCH Open Access

Absence of evidence of Xenotropic Murine Leukemia Virus-related virus infection in persons with Chronic Fatigue Syndrome and healthy controls in the United States

William M Switzer*[1], Hongwei Jia[1], Oliver Hohn[2], HaoQiang Zheng[1], Shaohua Tang[1], Anupama Shankar[1], Norbert Bannert[2], Graham Simmons[3], R Michael Hendry[1], Virginia R Falkenberg[4], William C Reeves[4] and Walid Heneine[1]

Abstract

Background: XMRV, a xenotropic murine leukemia virus (MuLV)-related virus, was recently identified by PCR testing in 67% of persons with chronic fatigue syndrome (CFS) and in 3.7% of healthy persons from the United States. To investigate the association of XMRV with CFS we tested blood specimens from 51 persons with CFS and 56 healthy persons from the US for evidence of XMRV infection by using serologic and molecular assays. Blinded PCR and serologic testing were performed at the US Centers for Disease Control and Prevention (CDC) and at two additional laboratories.

Results: Archived blood specimens were tested from persons with CFS defined by the 1994 international research case definition and matched healthy controls from Wichita, Kansas and metropolitan, urban, and rural Georgia populations. Serologic testing at CDC utilized a Western blot (WB) assay that showed excellent sensitivity to MuLV and XMRV polyclonal or monoclonal antibodies, and no reactivity on sera from 121 US blood donors or 26 HTLV-and HIV-infected sera. Plasma from 51 CFS cases and plasma from 53 controls were all WB negative. Additional blinded screening of the 51 cases and 53 controls at the Robert Koch Institute using an ELISA employing recombinant Gag and Env XMRV proteins identified weak seroreactivity in one CFS case and a healthy control, which was not confirmed by immunofluorescence. PCR testing at CDC employed a *gag* and a *pol* nested PCR assay with a detection threshold of 10 copies in 1 ug of human DNA. DNA specimens from 50 CFS patients and 56 controls and 41 US blood donors were all PCR-negative. Blinded testing by a second nested gag PCR assay at the Blood Systems Research Institute was also negative for DNA specimens from the 50 CFS cases and 56 controls.

Conclusions: We did not find any evidence of infection with XMRV in our U.S. study population of CFS patients or healthy controls by using multiple molecular and serologic assays. These data do not support an association of XMRV with CFS.

Background

Chronic fatigue syndrome (CFS) is a complex illness that affects between 0.5 and 2 percent of adults in the U.S. [1,2]. CFS is characterized by a severe debilitating fatigue lasting at least six consecutive months that is not alleviated with rest. Individuals with CFS also report various cognitive, sleep and musculoskeletal pain disturbances, and symptoms similar to those of infectious diseases [3]. At least a quarter of those suffering from CFS are unemployed or receiving disability because of the illness; the average affected family forgoes $20,000 annually in lost earnings and wages; and, the annual value of lost productivity in the United States is at least $9 billion [2,4-6]. Diagnostic, treatment, and prevention strategies have proven difficult to devise because the etiology, pathophysiology and risk factors for CFS remain unclear [3,7].

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Because the symptoms characterizing CFS resemble those of infectious diseases, many studies have investigated a viral etiology in CFS. However, involvement of several viruses including human herpes virus-6 (HHV-6), Epstein-Barr virus (EBV), various enteroviruses, and the human T-lymphotropic virus type 2 (HTLV-2) has not been conclusively proven [3,7-10]. In October 2009, Lombardi *et al.* reported finding a gammaretrovirus called xenotropic murine leukemia virus-related virus (XMRV) in peripheral blood mononuclear cell (PBMC) DNA from about 67% (68/101) of CFS patients compared to only 3.6% (5/218) of healthy persons using PCR testing [11]. Virus isolation and antibody detection were also reported in some CFS patients [11].

XMRV is phylogenetically related to the xenotropic murine leukemia viruses (MuLV) sharing about 94% nucleotide identity across the viral genome [12]. XMRV was initially identified in prostate tissues from about 10% of prostate cancer patients using microarray and PCR analysis [12]. XMRV prevalence in this study was higher in patients with an inherited mutation in the RNase L gene [12]. More recent studies examining XMRV prevalence in prostate tissues of patients with prostate cancer from the US and Europe have reported both negative and positive findings [13-15], highlighting the need for more studies to assess the role of XMRV in prostate cancer.

Confirmation of an association and etiologic role of XMRV in CFS is important because it could provide a useful diagnostic test and might lead to new treatment interventions. However, two recent studies of CFS patients from the United Kingdom using PCR testing alone or together with serologic testing reported negative XMRV results in 186 and 170 CFS patients, respectively [16,17]. XMRV was also not found by PCR testing of 32 CFS patients and 43 matched controls from the Netherlands [18]. Additional studies of different patient cohorts, including those from the US, are critical to better evaluate both a possible association of XMRV with CFS and a potential geographic link.

We describe here results from the first US study following the initial report by Lombardi *et al.* [11]. Testing of 51 specimens from CFS patients and 56 matched and healthy controls from the US was performed independently in three laboratories for XMRV DNA by using several PCR tests and for anti-XMRV antibodies using different serological assays.

Results

Absence of XMRV antibodies in persons with CFS and healthy controls

Serologic testing at CDC was performed with a newly developed WB assay using a strategy employed successfully for assessing human infection with other zoonotic retroviruses [19,20]. The WB test used lysate from polytropic MuLV (PMLV)-infected HeLa cells as antigen. PMLV and XMRV are highly related. They share between 87 and 93% nucleotide identity across the genome with XMRV and also have 88 - 97% and 88 - 91% amino acid identity to XMRV Gag and Env proteins, respectively. Partial Gag (123 aa) and Env (55 aa) sequences from our polytropic HeLa isolate share 96% and 90% identity to XMRV, respectively. Thus, excellent antigenic cross-reactivity between XMRV and our polytropic HeLa isolate is expected. Specimens were tested for reactivity in parallel against control antigens from uninfected HeLa cell lysates. Positive seroreactivity was defined as detection of bands in the infected lysates corresponding to known viral antigens and a lack of similar reactivity in uninfected lysates to exclude nonspecific reactivity. Four available antisera demonstrated good antigenic reactivity to Gag and/or Env proteins (Figures 1 and 2): Goat anti-MuLV polyclonal antisera to whole virus and to p69/71 Env proteins, rabbit anti-XMRV polyclonal antiserum to whole virus, and rat monoclonal antibody to the Env of spleen focus forming virus (SFFV), a polytropic MuLV, that reacts with gp69/71 Env of polytropic and xenotropic MuLV [21]. The anti-XMRV antiserum was used previously to detect XMRV in prostate cancer tissues by immunohistochemistry [13]. The anti-SFFV antibody was used by Lombardi *et al.* in a flow-based antibody competition assay to detect antibodies to XMRV Env in CFS patients [11]. All positive control antisera were reactive at high titers to various Gag and/or Env proteins (Figures 1 and 2). The anti-MuLV whole virus antiserum and the anti-XMRV polyclonal antiserum both reacted to the p68/p80 Gag precursor and p30 Gag proteins at titers of 1:32,000 and 1:64,000 respectively (Figures 1 and 2). The polyclonal anti-gp69/71 Env antiserum and the anti-SFFV monoclonal antibody reacted with the Env gp69/71 doublet proteins (Figures. 1 and 2) at a titer of 1:8,000 and 1:32,000, respectively (Figures. 1 and 2). The same pattern of reactivity was seen using both the anti-MuLV whole virus and anti-XMRV antisera though a higher level of nonspecific reactivity was observed to the HeLa lysates with the XMRV antisera (Figures 1 and 2). No specific reactivity was observed for the pre-immune goat sera and to uninfected HeLa lysates (Figures 1 and 2). 1:500 dilutions of the whole virus and gp69/71 antisera and a 1:50 dilution of pre-immune goat sera were then used as positive and negative controls for testing patient samples in the WB assay, respectively.

Plasma samples from 51 CFS cases and 53 healthy controls were diluted 1:50 and examined for seroreactivity to bands corresponding to Gag (p30 or p68/80) and/or Env (gp69/71 or p15E) proteins present in only the infected lysate and not the uninfected lysate. We also tested sera from 26 retrovirus-positive specimens (13 HTLV-1/2,

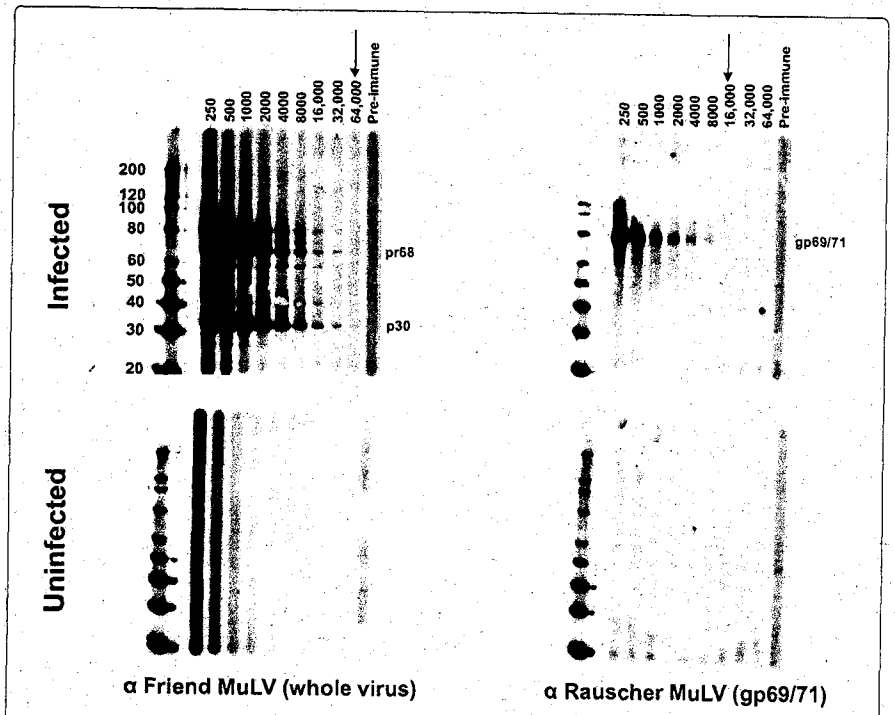

Figure 1 Titration of polyclonal MuLV goat antisera in Western blot (WB) assay. Antibody titers of positive control anti-sera and reactivity of pre-immune sera to polytropic MuLV-infected (upper panel) and uninfected (lower panel) HeLa cell crude cell lysates in WB testing. Specific antisera tested are located at the bottom of each WB. Arrows indicate observed titers for each antiserum. Fr, Friend; Ra, Rauscher. Locations of reactivity to specific viral proteins are indicated. Env (gp69/71), envelope; TM (p15E), transmembrane; MA (p15), matrix; Gag (pr68/80); CA (p30), capsid. Molecular weight markers (kD) are provided on the left of the WBs in the upper panels. Sizes of expected viral proteins are provided in each WB in the upper panels.

seven HIV-1, and six dual HIV-1/HIV-2 seropositive patients) and observed no reactivity to XMRV proteins (data not shown) confirming a lack of cross-seroreactivity. In addition, we tested archived sera from 121 anonymous US blood donors; all were negative (data not shown). Plasma samples from the 51 CFS patients and 53 healthy controls all tested negative for XMRV antibodies in this assay. Plasma samples were not available from three healthy controls. Typical WB results of CFS persons are shown in Figure 3. Every plasma specimen demonstrated some level of background reactivity, but without evidence of specific reactivity to Gag and/or Env proteins (Figure 3). For example, plasma from a CFS person showed reactivity to two proteins about 65 and 69 kD in size in the infected cell lysate but reacted non-specifically to proteins of the same size in the uninfected antigen and was thus considered seronegative (lane 2 of Figure 3). There were no clear differences in nonspecific WB seroreactivity observed in healthy persons compared to persons with CFS (data not shown).

Blinded serologic testing of these same CFS and control specimens was also performed at the Robert Koch Institute (RKI) in Germany using ELISAs containing recombinant XMRV Gag and Env proteins [14]. Plasma from 51 CFS cases and 53 healthy controls were not reactive in the recombinant XMRV Gag ELISA using either the N- or the C-terminus of the protein [14]. Two specimens, one each from a CFS patient (G9) and healthy control (G6), were weakly reactive in the recombinant XMRV Env ELISA with optical densities (OD) slightly above the

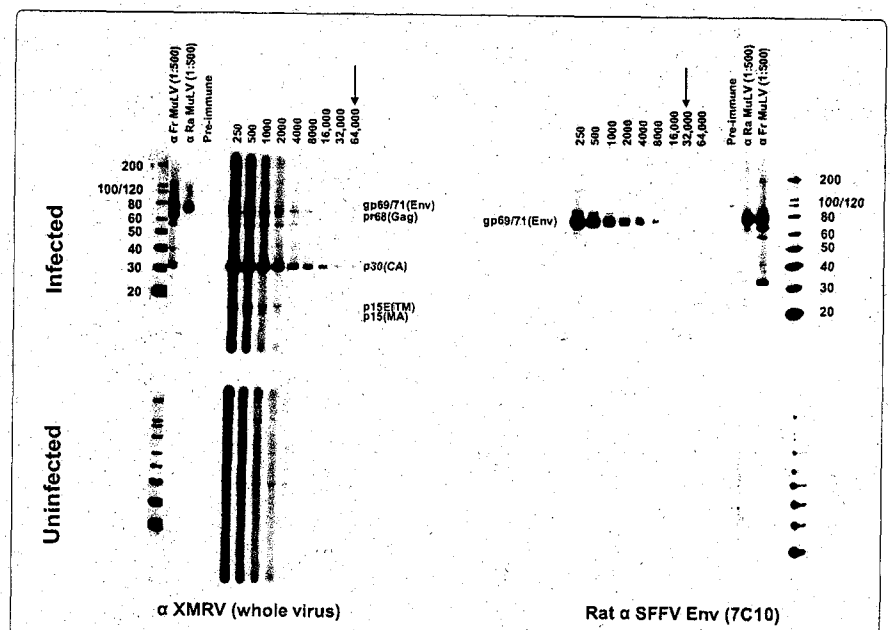

Figure 2 Titration of polyclonal XMRV rabbit and monoclonal spleen focus forming virus (SFFV) envelope rat antisera in Western blot (WB) assay. Antibody titers of positive control anti-sera and reactivity of pre-immune sera to polytropic MuLV-infected (upper panel) and uninfected (lower panel) HeLa cell crude cell lysates in WB testing. Specific antisera tested are located at the bottom of each WB. Arrows indicate observed titers for each antiserum. Fr, Friend; Ra, Rauscher. Locations of reactivity to specific viral proteins are indicated. Env (gp69/71), envelope; TM (p15E), transmembrane; MA (p15), matrix; Gag (pr68/80); CA (p30), capsid. Molecular weight markers (kD) are provided on the left of the WBs in the upper panels. Sizes of expected viral proteins are provided in each WB in the upper panels.

assay cutoff of 0.2 OD units (Figure 4) [14]. However, both specimens were negative by IFA testing using 293T cells expressing either XMRV Gag or Env proteins and were thus considered negative. Two blinded positive control specimens each consisting of goat polyclonal MuLV whole virus antisera diluted 1:100 in pre-immune goat sera both tested positive in the recombinant Gag ELISAs but were negative in the Env ELISA. These results are consistent with the seroreactivity of these polyclonal antisera to only Gag proteins in the WB assay. Five undiluted pre-immune goat sera all tested negative in both the Gag and Env ELISAs. These "external" positive and negative controls were included as a separate set of specimens and were all correctly detected in a blinded fashion. Testing of the blinded human and goat control specimens was performed separately since different secondary antibody conjugates are used for these different specimens. Internal positive and negative controls were also included in each run and performed as expected. Like the WB testing, the goat anti-MuLV whole virus and anti-MuLV p70 polyclonal antisera gave titers of 1:64,000 and 1:6,400 in the Gag and Env ELISAs, respectively.

Absence of XMRV sequences in PBMC DNA from persons with CFS and healthy controls

We used two PCR assays at CDC to detect XMRV DNA. The first assay was a nested *gag* PCR test used previously to identify XMRV sequences in prostate cancer patients and CFS patients [11,12]. The second nested PCR assay was designed on highly conserved polymerase (*pol*) sequences within xenotropic and other MuLV strains. Serial, ten-fold dilutions of full-length XMRV(VP62) plasmid (kindly provided by Robert Silverman) in a background of human DNA (PBMC or whole blood) showed that the nested *gag* and *pol* PCR tests each detected 10 XMRV copies in different experiments on subsequent

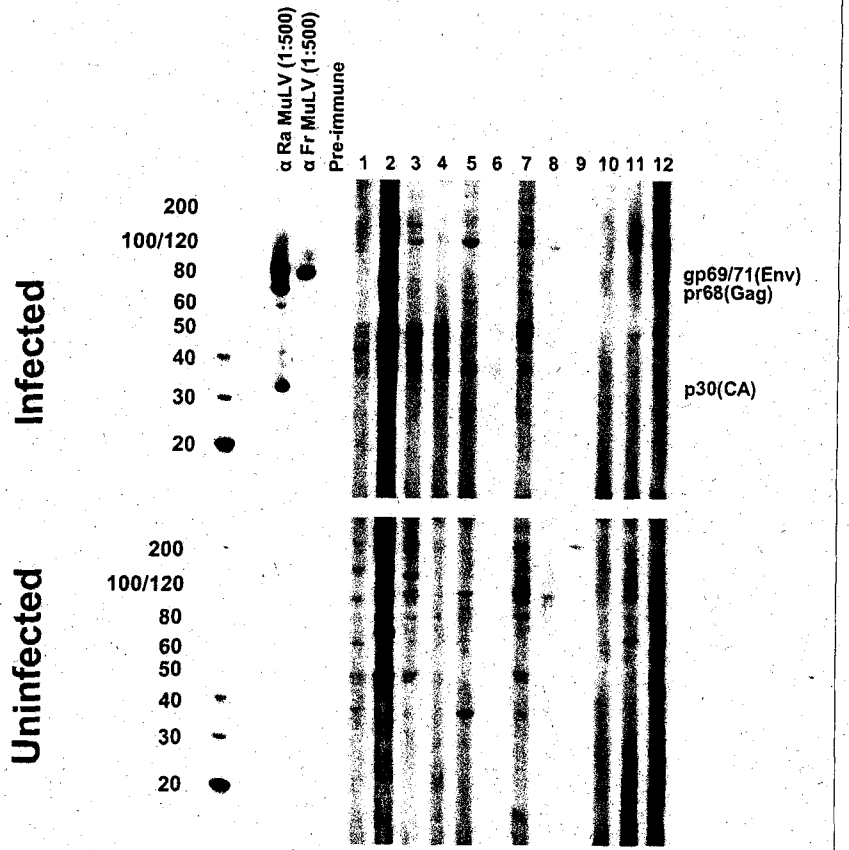

Figure 3 Absence of XMRV antibodies in CFS patients by Western blot (WB) analysis. Representative WB results for CFS cases from Wichita and Georgia identified after unblinding. Determination of MuLV specific reactivity is determined by comparison of observed seroreactivity to polytropic MuLV-infected HeLa antigens and uninfected HeLa antigens in upper and lower panels, respectively. Lanes 1 - 4 and 5 - 8 are plasma from CFS cases from the population based studies in Georgia and Wichita, respectively; lanes 9 - 12 are physician-referred CFS cases from the Georgia Registry study. MuLV positive and negative goat serum controls are labelled.

days (34/34 (100%) and 32/34 (94.1%), respectively). These results show that both PCR assays have an excellent sensitivity for detecting XMRV in one ug of DNA specimen. PBMC DNA from 41 anonymous US blood donors was also tested and found to be negative in both PCR assays. These 41 blood donors are distinct from the US blood donors whose plasmas were tested in the WB test.

PCR testing of β-actin sequences was positive for all clinical specimens confirming the integrity of the DNA and an absence of PCR inhibitors. Representative β-actin PCR results are shown in Figure 5. Subsequent XMRV

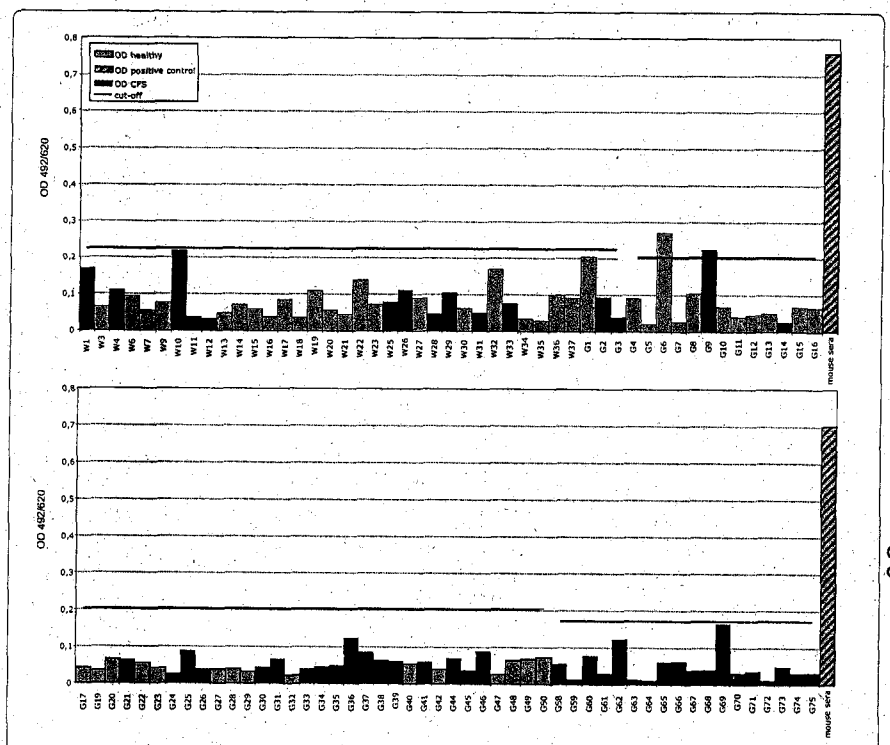

Figure 4 Absence of XMRV antibodies in CFS patients and healthy persons by ELISA using recombinant XMRV proteins. Representative XMRV Envelope (Env) ELISA results for 50 CFS cases and 49 healthy persons identified after unblinding. Specimens coded with W and G1-G50 are from the population-based study in Wichita and Georgia, respectively; specimens G59 - G75 are from physician-referred CFS cases from the Georgia Registry study. Specimens from a healthy control and a person with CFS, coded as G6 and G9 respectively, were weakly seroreactive in this test but were not confirmed by either Western blot or immunofluorscence testing. Human sera were diluted 1:200. The human negative control serum was obtained from a healthy volunteer previously determined to be seronegative. The polyclonal mouse Env antiserum was diluted 1:100. Assay cut-off was determined by the mean of the test samples plus three standard deviations.

testing showed that XMRV *gag* and *pol* sequences were not detected in 1 ug of PBMC (n = 31) or whole blood (n = 19) DNA from the CFS patients or in 1 ug PBMC DNA from the 56 healthy controls. A representative Southern blot of the XMRV *pol* PCR testing of persons with CFS is shown in Figure 5. Matching DNA was not available from one CFS case.

Blinded PCR testing performed at an independent institution (Blood Systems Research Institute (BSRI), CA) using a second nested PCR assay for XMRV *gag* DNA sequences, with a sensitivity of 3 copies per reaction, was also negative using 100 ng DNA specimens from all 50 CFS cases and 56 healthy controls (data not shown). 250 ng of DNA from the Georgia Registry patients also tested negative using this nested *gag* PCR test (Figure 6). Four blinded, "external" control specimens, included with the panel of human specimens and spiked with 4, 40, 400, and 4000 XMRV plasmid copies in 100 ng of human DNA, were all detected by this testing (data not shown).

Discussion

We found no evidence of infection with XMRV among persons with CFS or matched healthy controls from the US by testing with multiple serologic and PCR assays performed independently in three laboratories blinded to the clinical status of the study participants. Our results con-

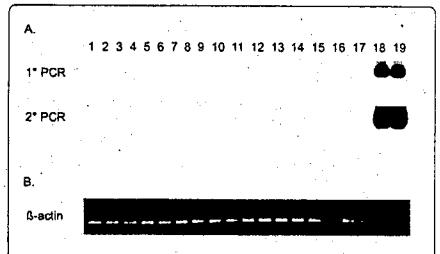

Figure 5 Absence of XMRV polymerase (*pol*) sequences in CFS patients. A. Representative nested *pol* PCR results using PBMC DNA specimens from persons with CFS identified after unblinding. Lanes 1 - 5, 6 - 10, and 11 - 14 are results for persons with CFS from Wichita, Georgia, and the Georgia registry studies, respectively; lanes 15 and 16, water only controls; lane 17, negative human PBMC DNA control; lanes 18 and 19, assay sensitivity controls consisting of 10^1 and 10^3 copies of XMRV VP62 plasmid DNA diluted in a background of 1 ug of human PBMC DNA, respectively. B. Semi-quantitative β-actin PCR results for PBMC DNA specimens above in lanes 1 - 14; lane 15, water control; lanes 16 - 19, 10-fold dilutions of blood donor PBMC DNA starting at 0.1 ug as a positive assay control.

trast with the high rate of XMRV detection reported by Lombardi *et al.* among both CFS patients and controls, but are in agreement with recent data reported in two large studies in the UK and a smaller study in the Netherlands that could not detect XMRV sequences in CFS patients and one UK study that also failed to detect specific XMRV neutralizing antibody responses in CFS [11,16-18]. Combined, these negative data do not support XMRV as the etiologic agent of the majority of CFS cases.

Several possibilities could explain these discordant results, including technical differences in assays used for the testing in each study. However, the inability of four

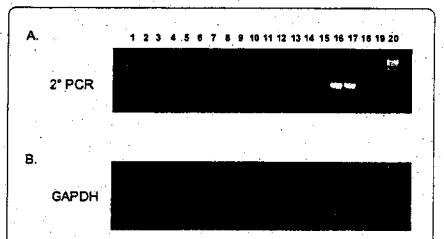

Figure 6 Absence of XMRV *gag* sequences in CFS patients. A. Representative nested *gag* PCR results from patients from the Georgia Registry identified after unblinding. Lanes 1 and 20, 100-bp ladder; lanes 2 - 15 are results from CFS patients; lanes 16 - 18 assay sensitivity controls consisting of 10, 3 and 1 copies of XMRV VP62 plasmid DNA diluted in a background of 250 ng of human PBMC DNA; lane 19, water control. B. GAPDH PCR results for same PBMC DNA specimens above.

independent laboratories to replicate the high XMRV prevalence in CFS cases reported by Lombardi *et al.* cannot be explained by minor differences in assays used in each study. In addition, testing at CDC utilized the nested XMRV *gag* PCR assay used by Lombardi *et al.* and Urisman *et al.* to identify XMRV infection in CFS and prostate cancer patients, respectively [11,12]. Further, to improve assay sensitivity, we used 1 ug of input DNA which is 4-5 times higher than that used by others [11-13,16,17], all while maintaining an assay sensitivity of 10 copies. To ensure that our testing would not miss genetically diverse XMRV or MuLV strains, we also used a sensitive nested PCR assay with conserved *pol* gene primers and found that this testing was also negative confirming the absence of XMRV/MuLV sequences. While PBMC DNA was used in the majority of specimens, 1 ug whole blood DNA was also used in testing 19 CFS cases. This input DNA represents about 350 ng of PBMC DNA which is similar to the amount used by others [11-13,15,16], thus not affecting the sensitivity of our results. The negative PCR findings were confirmed by an independent laboratory with a second nested *gag* PCR assay which provided additional evidence for the absence of XMRV sequences among CFS cases and controls. The primary PCR amplification used in this second test is also that used by Lombardi *et al.* which when combined with a nested PCR step has a 3-copy detection threshold.

Antibody responses particularly to Gag and Env proteins are hallmarks of immune responses to retroviral infections including experimental XMRV infection of macaques [22]. We used a new WB assay to test for anti-XMRV antibodies and showed by using both monoclonal antibodies and polyclonal antisera that this assay detected specifically, and with high titers, reactivity to both XMRV and MuLV Gag and Env proteins. We were unable to detect antibodies to XMRV Gag and Env in any of the CFS and controls specimens by using this WB assay. Likewise, negative results were obtained in a second, independent laboratory by using XMRV-specific ELISA-based and IFA assays. Thus, the observed negative serologic results for all CFS patients reflect an absence of antibody responses and active XMRV infection. Although limited, the negative WB serology observed in 56 healthy controls and 121 blood donors also suggests that the XMRV seroprevalence in this population is not high. Screening of larger numbers of US blood donors using a high throughput ELISA followed by confirmation in a WB test also showed uncommon seropositivity (~0.1%) [22]. More studies, however, are needed to determine the prevalence of XMRV in healthy populations.

One current limitation of our study, and of others performing serologic and PCR testing for XMRV, is the absence of bona fide positive and negative control specimens from infected and uninfected humans to determine

the analytical sensitivity and specificity of the detection assays. Until panels of well-characterized clinical specimens become available, assay validation will be limited to reagents generated experimentally, such as polyclonal and monoclonal antibodies, XMRV plasmids, and XMRV-infected cells.

The selection criteria with which persons with CFS were included in these various studies may also help to explain the incongruent XMRV findings. The study by Lombardi *et al.* used samples from the *Whittemore Peterson Institute National Tissue Repository* reported to contain specimens from well-characterized cohorts of CFS [11]. Yet, the paper provides no information regarding the repository or concerning the nature of these cohorts other than that they were collected from private medical practices in several regions of the U.S. where clusters of CFS have been documented [11]. An absence of details of the CFS cases and controls in this report makes it difficult to replicate and interpret their findings. In contrast, patients in the UK and Netherland studies were typical of CFS patients seen in specialist clinical services in those countries and resemble persons seen in other specialist CFS services in the US and Australia [16-18]. Almost half of the UK CFS patients described onset of their illness as related to an acute viral disease [16,17]. Thus, they would appear quite comparable to those in the study by Lombardi *et al.* Similarly, our study also failed to detect XMRV infection in 18 CFS patients referred to a fatigue registry by health care providers in Georgia and included three persons who reported sudden onset to their illness. Our study is the first to evaluate XMRV infection in persons with CFS and healthy controls from the general populations of Wichita and Georgia. These CFS cases are different from CFS patients seen in general practice and referral clinics; of the participants from the population-based study in Georgia, only half had consulted a physician because of their fatigue, about 16% had been diagnosed with CFS, and 75% described an insidious onset to their illness that had no obvious relation to an acute infectious disease. Nonetheless, results from our general population cohort extend the examination of XMRV in CFS to persons whose illness developed gradually, for the most part, rather than acutely. Our negative findings, in conjunction with those in Europe [16-18], indicate no discernable association of XMRV with a wide spectrum of CFS cases. The negative results for CFS patients and controls from the US in the current study also do not support a continental clustering of XMRV infection suggested by the absence of infection in the UK and Netherlands [16-18]. However, our findings may not be generalizable beyond our study populations because XMRV infection rates may vary in different regions or locales.

CFS is a diagnosis of exclusion based on self-reported symptoms and requires careful medical and psychiatric evaluations to rule out conditions with similar clinical presentation. Our study and the negative reports from the UK and the Netherlands evaluated patients for exclusionary conditions and defined CFS according to criteria of the 1994 International CFS Research Case Definition [23] or the earlier Oxford case definition [24]. The Lombardi *et al.* study specifies that samples were selected from patients fulfilling the 1994 international CFS case definition [23] and the 2003 Canadian Consensus Criteria for CFS/ME [25]. Lombardi *et al.* did not specify if patients were evaluated for exclusionary conditions, or if the study subjects met both definitions, or which patients met either CFS definition. The 1994 International CFS case definition and the Canadian Consensus Criteria are different and do not necessarily identify similar groups of ill persons. Most notably, the Canadian Criteria include multiple abnormal physical findings such as spatial instability, ataxia, muscle weakness and fasciculation, restless leg syndrome, and tender lymphadenopathy. The physical findings in persons meeting the Canadian definition may signal the presence of a neurologic condition considered exclusionary for CFS and thus the XMRV positive persons in the Lombardi *et al.* study may represent a clinical subset of patients [11].

CFS is a complex disease with various clinical subtypes proposed which could also account for differences in the results obtained in each study [11,16-18]. While there is still no universal agreement on a precise clinical presentation encompassing CFS illness, defining patient characteristics in studies of CFS etiology or pathogenesis remains crucial for making comparisons across various research conclusions.

Conclusions

In our study population of CFS and healthy persons from the US, we did not find any evidence of infection with XMRV using PCR and serologic methods performed independently in three laboratories blinded to the clinical status of the study participants. These results do not support an association of XMRV with CFS.

Methods

Study population and specimen preparation

The CDC Institutional Review Board reviewed and approved all study protocols. All participants were volunteers and provided informed consent. Laboratory testing of the samples was performed anonymously and blinded to clinical status.

Details of our two study populations have been described previously [2,26,27]. Briefly, between 2002 and 2003 we sampled adults 18 to 59 years old from Wichita, Kansas [26,27] and between 2008 and 2009 we sampled adults 18 to 59 years old from metropolitan, urban, and rural Georgia [2]. In both studies, we used random digit-dial screening interviews to classify household residents as either well or having symptoms of CFS. A follow-up

detailed telephone interview was administered to all individuals with symptoms and to a probability sample of those without symptoms. Based on the detailed interview, those meeting criteria of the 1994 International CFS Research Case Definition [23] were classified as CFS-like and other respondents classified as either unwell (not CFS-like) or well. All CFS-like individuals were recruited and a random sample of those who were unwell but not CFS-like, and a set of matched (sex, age, race/ethnicity, geographic) well people were recruited for a 1-day clinical evaluation.

We also tested specimens from CFS cases identified in a CDC Health Care Provider-based Registry of Unexplained Fatiguing Illnesses and CFS (unpublished). Between October 2008 and December 2009, healthcare providers practicing in Bibb County, GA referred adolescents and adults 12 - 59 years old who met criteria for unexplained fatiguing illness (fatigue for > 1 month), and having at least one other core CFS symptom during that period (unrefreshing sleep, problems with cognition or memory, joint or muscle pain in extremities), and did not have an exclusionary medical or psychiatric condition. All referred patients underwent a telephone screening interview to document fatigue lasting > 6 months, and the presence of at least one core symptom and no exclusionary conditions. Patients meeting these criteria underwent the same 1-day clinical evaluation as persons from our population-based studies, described in detail below.

Clinical assessment

Clinical evaluations involved: 1. Administration of standardized questionnaires to measure the 3 domains of the 1994 CFS case definition [23]: the Multidimensional Fatigue Inventory (MFI) to measure 5 dimensions of fatigue [28] the Medical Outcomes Survey Short Form 36 (SF-36) to evaluate 8 dimensions of functional impairment [29]; and the CDC Symptom Inventory to evaluate occurrence/frequency/severity of the 8 CFS-accompanying symptoms [30]; 2. A standardized physical examination conducted by a specifically trained physician who also reviewed past medical history, review of systems, and current medications/supplements; 3. Collection of blood and urine for routine clinical analyses [23,31]; 4. A standardized psychiatric evaluation conducted by specifically trained psychiatric interviewers - Diagnostic Interview Schedule (DIS) in Wichita [32] and the Structured Clinical Interview for DSM-IV Disorders (SCID) in Georgia [33].

The physician's evaluation and routine clinical laboratory tests served to identify medical conditions considered exclusionary for CFS, specified in the 1994 case definition [23] as further clarified by the International CFS Study Group in 2003 [31]. The psychiatric interview served to identify current psychiatric disorders considered exclusionary for CFS, which included current melancholic depression, current or lifetime bipolar disorder or psychosis, substance abuse within 2 years and eating disorders within 5 years [23,31].

Illness classification

Following clinical evaluation, participants who had no exclusionary medical or psychiatric conditions were diagnosed with CFS if they met criteria of the 1994 international case definition [23] as quantified by the CDC Symptom Inventory and ancillary criteria of the MFI and SF-36 [26,31]. We used the MFI to assess fatigue status [28]. For classification as CFS, those with a score ≥ well-population medians on the general fatigue or reduced activity scales of the MFI were considered to meet fatigue criteria of the 1994 international case definition. Functional impairment was assessed by the medical outcomes survey short form-36 (SF-36) [29]. For classification as CFS, those with a score ≤ 25th percentile of population norms in the physical function or role physical, or social function, or role emotional subscales of the SF-36 were considered to have substantial reduction in activities as specified in the 1994 definition. Those who met at least one but not all 1994 criteria were considered unwell not CFS. Those who met none of the criteria were considered well.

Specimens were available from 89 persons (33 CFS and 56 well controls) from the population-based case-control studies and 18 CFS persons from the Registry study described above. Subjects were included based on availability of specimens, and comprised 11 of 43 persons with CFS and 26 of 53 healthy controls from Wichita, KS and 22 of 32 persons with CFS and 30 of 51 healthy controls from Georgia. Persons with CFS and healthy controls had similar mean ages, similar predominance of females and white race, and had a similar mean body mass index (BMI) (Table 1). Subjects with CFS had been ill on average 13.9 years (median 11.15 yrs, range 3 - 40 yrs), were severely fatigued (MFI General Fatigue 16.5, range 10 - 20; MFI Reduced Activity 12.8, range 4 - 20) and severely impaired (SF-36 physical functioning 65.5, range 10-100); SF-36 bodily pain 48.8, range 12 - 84), and 3/33 (9%) reported sudden onset to their illness. Clinical and demographic characteristics of subjects with specimens available for this study did not differ from those persons who did not have ample specimen volumes and case-control matching was maintained.

18 of 38 persons enrolled in the Registry study had a diagnosis of CFS and were available for the current study. These provider-referred CFS patients had a mean age of 42.8 years (SEM = 2.85 years), and were predominantly white [17/18, (94.4%)] and female [16/18 (88.99%)]. They had suffered fatigue for an average of 9.4 years (range: 1 - 35 years) and 3/18 (16.7%) reported sudden onset to their illness.

Table 1: Distribution of demographic variables by CFS case-control status among persons from the combined Wichita and Georgia case-control population-based studies.

	CFS		Well		
Demographic Factor	**Wichita, KS (N = 11)**	**Atlanta, GA (N = 22)**	**Wichita, KS (N = 26)**	**Atlanta, GA (N = 30)**	**p-value[2,3,4]**
Age					
Mean ± SEM[1]	46.7 ± 3.32	47.7 ± 4.69	51.6 ± 5.1	46.1 ± 5.48	p = 0.51
Sex [n (%)]					
Female	8 (72.7)	20 (90.9)	21 (80.8)	25 (83.3)	p = 0.74
Male	3 (27.3)	2 (9.1)	5 (19.2)	5 (16.7)	
Race [n (%)]					
White	10 (90.9)	18 (81.8)	25 (96.2)	27 (90)	
Black	0 (0)	3 (13.6)	1 (3.8)	3 (10)	p = 0.69
Other	1 (9.1)	1 (4.6)	0	0 (0)	
Body Mass Index					
Mean ± SEM	27.6 ± 3.3	28.2 ± 4.7	29.2 ± 5.1	26.3 ± 5.5	p = 0.76

1. SEM, standard error of the mean
2. t-test was used to compute probabilities for comparisons of mean age and mean body mass index between study groups.
3. Chi square test was used to compute the probability for comparison of the distribution of sex between cases and controls.
4. Fisher's exact test was used to compute the probability for comparison of the distribution of race between the study groups, and was based on Blacks and Whites only.

Specimen collection, processing, storage

Fresh whole blood was collected in either CPT Vacutainer tubes containing sodium citrate and a blood separation reagent (Becton Dickinson, NJ, USA) for the Georgia and Wichita studies or in PAXgene tubes for the Georgia CFS Registry study and transported to CDC. Blood was also collected in PAXgene tubes for two persons from the Georgia population-based study. PAXgene tubes were gently inverted 5 times, stored overnight at -20°C, and then transferred to -70°C until DNA isolation was performed. PBMCs and plasma were immediately isolated by centrifugation of the CPT tubes. PBMCs were stored in liquid nitrogen under conditions designed to maintain viability. Plasma was aliquoted and stored at -80°C within 4 hours of blood collection. For samples collected from persons living in Wichita, KS and from the Georgia CFS Registry study, whole blood was also collected in EDTA Vacutainer tubes. Plasma was recovered from the EDTA-treated blood by centrifugation at 15,000 × g for 20 minutes and aliquoted and frozen at -80°C until use. Plasma samples were aliquoted again when thawed for WB testing; the remaining aliquots were re-frozen at -80°C.

DNA was extracted from cryopreserved PBMCs or frozen whole blood with the Qiagen blood DNA minikit or Qiagen PAXgene Blood DNA kit (Qiagen, Valencia, CA), respectively, then aliquoted and stored frozen at -80°C. All PBMC samples had viabilities > 90% when they were thawed for DNA isolation. Nucleic acid concentrations were determined by spectrophotometry using the Nanodrop instrument (Thermo Scientific, Wilmington, DE). For the PCR testing at CDC, 1 ug of PBMC or whole blood DNA was used. Integrity of the DNA specimens was determined using β-actin PCR as previously described [34]. Matching plasma or DNA was not available from three healthy persons from Wichita, KS and one CFS case from Georgia, respectively. All specimen preparation, tissue culture, and PCR testing was done in physically isolated rooms to prevent contamination of specimens.

Serologic Assays

HeLa cells were infected with supernatant from the murine macrophage cell line RAW264.7 (ATCC, Manassas, VI) known to express polytropic and ecotropic MuLV (PMLV and EMLV, respectively). To characterize the isolate that replicated in HeLa cells, a 166-bp RNA sequence containing the variable region C of the envelope (Env) surface protein was PCR-amplified from infected HeLa cell tissue culture supernatants. Phylogenetic analysis of

the *env* sequence showed that the isolate was a PMLV by clustering tightly with other PMLV, and not EMLV (data not shown). XMRV and PMLV are highly related sharing between 87 - 94% nucleotide identity across their genomes and 88 - 97% and 88 - 91% amino acid identity to complete Gag and Env proteins, respectively. Indeed, partial Gag (123 aa) and Env (55 aa) sequences from our polytropic HeLa isolate share 96% and 90% identity to XMRV, respectively. Thus, the high amino acid relatedness supports the use of this isolate for WB serologic testing. Infected and uninfected HeLa crude cell lysates were prepared for WB testing as previously described [35]. Protein concentrations of the lysates were determined using the BioRad DC Protein Assay (Hercules, CA). Plasma or serum samples were diluted 1:50 and reacted separately to 150 ug of infected and uninfected cell lysates overnight at 4°C after protein separation through 4-12% polyacrylamide gels and transfer to nytran membranes, as previously described [35,36]. Seroreactivity in human specimens was detected using peroxidase-conjugated protein A/G (Pierce, Rockford, IL) and chemiluminescence (Amersham, Uppsala, Sweden) [35,36].

Since validated XMRV-positive human sera are not currently available, we used experimentally derived polyclonal antisera and monoclonal antibodies to assess antigenic reactivity of the WB assay. These reagents included goat polyclonal antisera to MuLV (whole virus and gp69/71Env, respectively) available at ATCC (VR-1537 and VR-1521, respectively), and a rabbit anti-XMRV polyclonal antiserum (kindly provided by Ila Singh) and a rat anti-SFFV (7C10) monoclonal antibody (kindly provided by Sandra Ruscetti) used previously to detect XMRV protein expression and antibodies in prostate cancer and CFS patients, respectively [11,13,21]. Peroxidase-conjugated protein A/G or anti-rat antibody (Sigma, St. Louis, MS) was used to detect bound goat, rabbit, and rat antibodies, respectively. Sensitivity of the assay was estimated using two fold serial dilutions of the MuLV, XMRV, and SFFV polyclonal and monoclonal antibodies. Cross-reactivity of the WB assay on HIV and HTLV positive plasma was evaluated on 13 HTLV-1/2 positive, 7 HIV-1-positive, and six HIV-1/HIV-2 dual positive plasma. In addition, sera from 121 HIV and HTLV seronegative anonymous US blood donors collected in 1998 were tested.

An aliquot of coded plasma from the CFS and healthy controls was tested at RKI by an ELISA using recombinant Gag and Env proteins used recently to investigate XMRV infection in German prostate cancer patients [14]. Briefly, recombinant proteins were coated overnight on microtiter plates at room temperature in equimolar amounts. The plates were blocked with 2% Marvel milk powder in phosphate buffered saline (PBS) for 2 h at 37°C, washed three times with PBS, 0.05% Tween 20. Patient plasma diluted 1:200 in PBS with 2% milk powder and 0.05% Tween20 were added into each well and incubated for 1 hour at 37°C. Each well was again washed three times and a 1:1000 dilution of a goat anti-human IgG-HRP conjugate (Sigma Aldrich, Munich, Germany) in PBS, 2% milk powder, 0.05% Tween 20 (Serva, Heidelberg, Germany) was added. Following incubation for 1 hour at 37°C, each well was again washed three times, and chromogen ortho-phenylendiamin (OPD) in 0.05 M phosphate-citrate buffer, *pH 5.0 containing* 4 µl of a 30% solution of the hydrogen peroxide substrate per 10 ml was added. After 5-10 minutes the color development was stopped by addition of sulphuric acid and the absorbance at 492 nm/620 nm was measured in a microplate reader. Positive controls included mouse anti-Gag and Env antisera and pre-immune sera diluted 1:50 in PBS with 2% milk powder and 0.05% Tween20. In addition, a separate set of goat sera was also tested in a blinded fashion and included external positive and negative controls consisting of dilutions of the MuLV whole virus, gp69/71 goat polyclonal antisera, or pre-immune goat sera, respectively. Detection of antibody reactivity in the goat sera was done by using rabbit anti-goat HRP conjugate (Dako, Hamburg, Germany).

Samples reactive by ELISA testing were then re-tested using an immunofluorescence assay (IFA) [14]. Briefly, plasma specimens were diluted 1:200 in blocking buffer and tested against 293T cells expressing codon optimized synthetic full-length genes of the XMRV *env* or *gag* under control of the CMV promoter and bound to glass slides, as described in detail previously [14]. Following incubation for 60 min at 37°C, the slides were washed extensively with PBS and secondary antibodies conjugated to fluorophores were added for 30 min. After thorough washing steps with PBS, the cells were mounted in Mowiol and viewed on a Zeiss (LSM510) confocal laser-scanning microscope.

Detection of XMRV sequences

DNA specimens were screened by PCR at the CDC with an XMRV-specific *gag* and a polymerase (*pol*) assay that detects xenotropic and polytropic MuLV. The XMRV specific assay uses the primers GAG-O-F and GAG-O-R and GAG-I-F and GAG-I-R for the primary and nested PCRs, respectively, and conditions as previously described [11,12]. This is the same nested PCR test used by Urisman *et al.* and Lombardi *et al.* to detect 413-bp XMRV *gag* sequences in prostate cancer and CFS patients, respectively [11,12]. The primers and probes of the generic *pol* PCR assay were designed from an alignment of complete XMRV and prototypical xenotropic, polytropic, and ecotropic MuLV genomes available at GenBank (accession numbers: **xenotropic (XMLV):** XMRV VP35 = DQ241301, XMRV VP62 = DQ399707, XMRV VP42 = DQ241302, XMRV WPI-1106 = GQ497344, XMRV WPI-1178 = GC497343, MuLV DG-75 = AF221065; MuLV MTCR = NC_001702, mERV Chr 9 = AC121813, mERV Chr 4 = AL627077, mERV Chr 1 = AC083892; **polytropic (PMLV):** mERV Chr 7 = AC167978, mERV Chr 7 = AC127565, mERV Chr 12 = AC153658; **ecotropic (EMLV):** MuLV AKV = J01998, MuLV BM5eco = AY252102.1, Moloney MuLV = J02255, Rauscher MuLV = NC_001819, Friend MuLV = X02794). The external external XPOLOF (5' CCG TGC CCA ACC CTT ACA ACC TCT 3') and *XPOLOR* (5' CCG AGG TTC CCT AGG GTT TGT AAT 3') and internal primers XPOLIF (5' TCC ACC CCA CCA GTC AGC CTC TCT 3') and XPOLIR (5' AAG TGG CGG CCA GCA GTA AGT CAT 3') were used to generically detect 216-bp XMLV/XMRV *pol* sequences. All assays were optimized to achieve the highest sensitivity in detecting XMRV VP62 plasmid DNA in one ug of genomic DNA. One ug of human DNA was used as input for the PCR tests. PCR products were visualized by electrophoresis in an ethidium bromide-stained 1.8% agarose gel. To further increase the sensitivity and specificity of the PCR assays, amplified *gag* and *pol* sequences were confirmed by Southern blot analysis using the biotinylated oligoprobes XGAGP2 (5' ACC TTG CAG CAC TGG GGA GAT GTC 3'), and XPOLP (5' TTG ATG AGG CAC TGC ACA GAG ACC 3') and chemiluminescence detection. The detection limit of the assays was evaluated using 10-fold dilutions of XMRV VP62 plasmid diluted in a background of one ug of genomic human DNA. Assay specificity was evaluated using PBMC DNA from 41 anonymous US blood donors screened negative for HIV and HTLV.

Nested PCR was also performed at BSRI using double blinded genomic DNA specimens in order to independently test for XMRV *gag* sequences. The first round was performed as previously described to detect XMRV in PBMC DNA of CFS patients [11]. Briefly, 100 - 250 ng of genomic DNA was amplified using outer gag primers 419F (5' ATC AGT TAA CCT ACC CGA GTC GGA C 3') and 1154R (5' GCC GCC TCT TCT TCA TTG TTC TC 3') at a final concentration of 0.3 µM, HotStart-IT FideliTaq Master Mix (USB Corporation, Cleveland, OH) and 1 *mM magnesium chloride*. PCR was performed using an initial denaturation step at 94°C for 4 minutes followed by 45 cycles of 94°C for 30 seconds, 57°C for 30 seconds and 72°C for 1 minute and a final extension step at 72°C for 2 minutes. Nested PCR was conducted using 1 µl of the first round DNA in the second round reaction. Nested primers 488F (5' GGG GAC GAG AGA CAG AGA CA 3') and 1107R (5' CAG AGG AGG AAG GTT GTG CT 3') were used at a final concentration of 0.3 µM and amplification was performed using HotStart-IT FideliTaq. PCR was performed using an initial denaturation step at 95°C for 90 seconds followed by 40 cycles of 95°C for 20 seconds, 58°C for 30 seconds and 72°C for 40 seconds and a final extension step at 72°C for 2 minutes. PCR contamination occurring during nested PCR was evaluated by including at least one third as many water controls as test samples in each PCR experiment and were always negative.

Using serial dilutions of a cloned fragment of XMRV *gag* as a positive control, the nested PCR assay could reliably detect at least 3 copies of DNA per reaction, even when spiked into genomic DNA prepared either from 293FT cells or donor PBMCs previously validated to be negative for XMRV. Controls of GAPDH (forward - 5' CAT GTT CCA ATA TGA TTC AC 3'; reverse - 5' CCT GGA AGA TGG TGA TG 3'; 75 ng genomic DNA, 3 minutes at 95°C followed by 45 cycles of 95°C for 20 seconds, 55°C for 45 seconds and 72°C for 30 seconds, followed by 1 cycle of 72°C for 2 minutes) were performed to ensure similar levels of genomic DNA input in each PCR reaction.

Competing Interests
The authors declare that they have no competing interests.

Authors' contributions
WMS, WCR, RMH and WH conceived and designed the study. WCR and VRF provided specimens and data on study population. HJ, HZ, ST, AS, GS, NB, and OH performed specimen testing and data analysis with WMS and WH. WMS, WCR and WH wrote the manuscript. All authors read and approved the final manuscript.

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文献１０

Detection of MLV-related virus gene sequences in blood of patients with chronic fatigue syndrome and healthy blood donors

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Chronic fatigue syndrome (CFS) is a serious systemic illness of unknown cause. A recent study identified DNA from a xenotropic murine leukemia virus-related virus (XMRV) in peripheral blood mononuclear cells (PBMCs) from 68 of 101 patients (67%) by nested PCR, as compared with 8 of 218 (3.7%) healthy controls. However, four subsequent reports failed to detect any murine leukemia virus (MLV)-related virus gene sequences in blood of CFS patients. We examined 41 PBMC-derived DNA samples from 37 patients meeting accepted diagnostic criteria for CFS and found MLV-like virus *gag* gene sequences in 32 of 37 (86.5%) compared with only 3 of 44 (6.8%) healthy volunteer blood donors. No evidence of mouse DNA contamination was detected in the PCR assay system or the clinical samples. Seven of 8 *gag*-positive patients tested again positive in a sample obtained nearly 15 y later. In contrast to the reported findings of near-genetic identity of all XMRVs, we identified a genetically diverse group of MLV-related viruses. The *gag* and *env* sequences from CFS patients were more closely related to those of polytropic mouse endogenous retroviruses than to those of XMRVs and were even less closely related to those of ecotropic MLVs. Further studies are needed to determine whether the same strong association with MLV-related viruses is found in other groups of patients with CFS, whether these viruses play a causative role in the development of CFS, and whether they represent a threat to the blood supply.

xenotropic murine leukemia virus-related virus | murine leukemia virus-like virus | viral gag gene sequence | polytropic | mouse mitochondria DNA PCR

Chronic fatigue syndrome (CFS) is a debilitating disorder defined solely by clinical symptoms (1) and the exclusion of other diseases; its distribution is wide and its cause is unknown. In many instances, the illness starts suddenly with an infectious-like syndrome. A number of objective immunological and neurological abnormalities have been found more often in patients with CFS than in healthy controls or in patients with other fatigue-inducing illnesses (2). Various microbial and viral infections have been implicated as possible triggers of CFS, including human herpesvirus-6, Epstein–Barr virus, enteroviruses, parvovirus B19, and the bacteria that cause Lyme disease and Q fever (2). However, no single agent has been associated with a large fraction of cases.

A recent study reported that a high percentage of patients with CFS are infected with a mouse leukemia retrovirus that has been designated xenotropic murine leukemia virus-related virus (XMRV) (3), a virus first identified in samples of human prostate cancer tissue about 4 y ago (4). However, two subsequent studies failed to find an infectious murine leukemia virus (MLV)-related virus in German prostate cancer patients (5, 6), and four recent studies from Europe and the United States have failed to detect XMRV or an MLV-related viral gene sequence in the blood of CFS patients using PCR (7–10).

In the mid-1990s, we obtained serum and whole-blood samples from CFS patients for the investigation of possible mycoplasmal infections (11). Whole-blood, peripheral blood mononuclear cell (PBMC), and plasma samples from 37 CFS patients in the mycoplasma studies were maintained in frozen storage at −80 °C. Twenty-five patients were from an academic medical center and 12 were referred by community physicians. Repeat blood samples were obtained from the academic medical center patients: four samples were obtained 2 y later and similarly kept in frozen storage, eight were obtained ~15 y later, in 2010, and processed for XMRV/MLV-related virus testing without being frozen.

By nested PCR assays targeting the MLV-related virus *gag* gene, using both the previously described primer sets (3, 4) and an in-house–designed primer set with highly conserved sequences from different MLV-like viruses and XMRVs, we examined DNA prepared from the blood samples of these 37 CFS patients for the presence of MLV-like virus *gag* gene sequences. In addition, RNA was prepared from the deep-frozen plasma samples of these patients and analyzed by RT–PCR assay. DNA extracted from frozen PBMC samples of 44 healthy volunteer blood donors was tested in parallel.

Results

MLV-Related Viral *gag* Gene Sequences Detected in the Blood of CFS Patients. By nested PCR assays, targeting the mouse retrovirus *gag* gene using either the previously reported PCR primer sets (first round: 419F/1154R; second round: GAG-I-F/GAG-I-R) (3, 4) or our in-house–designed PCR primer set (first round: 419F/1154R; second round: NP116/NP117) (Fig. 1), we detected a high frequency of MLV-related virus *gag* gene sequences in patients with CFS. The NP116/NP117 is an internal primer set with highly conserved sequences in different MLV-like viruses and XMRVs (Fig. S1). After the first round of nested PCR using primer set 419F/1154R, gel electrophoresis revealed positive PCR-amplified products with the predicted size of ~730 bp in 21 of 41 PBMC or whole-blood samples from 37 CFS patients (Fig. 1*A*). The nested PCR results produced by the second round of amplification using either the internal primer set GAG-I-F/GAG-I-R (with a predicted size of an ~410-bp product) or the internal primer set NP116/NP117 (with a predicted size of an ~380-bp product) were essentially identical. Overall, samples from 32 of 37 (86.5%) CFS patients revealed positive amplification products with the correct predicted sizes in the nested PCR (Fig. 1*B*). Of the 25 CFS patients who had been rigorously evaluated at the academic medical center, 24 (96%) were positive. On repeated testing 2 y

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The authors declare no conflict of interest.

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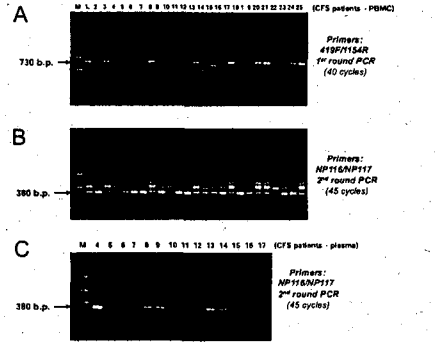

Fig. 1. MLV-related *gag* gene sequences detected in blood DNA from CFS patients. (*A*) Results of PBMC DNA from CFS patient samples 1–25 (of 41 samples examined) amplified after the first round of nested PCR using a previously published primer set (419F/1154R); targeting *gag* gene. (*B*) Results of PBMC DNA from the 25 CFS samples after completing the second round of nested PCR using an in-house–designed PCR primer set (NP116/NP117). (*C*) MLV-related *gag* gene RNA sequences are detected in plasma of CFS samples by RT-nested PCR. Results of RT-nested PCR for RNA derived from the plasma samples of CFS patients 4–17 are shown. The positions of expected sizes of the "positive" PCR amplicons are indicated by arrows. M, DNA ladder size markers. All positive PCR amplicons with the expected size have been confirmed by DNA sequencing.

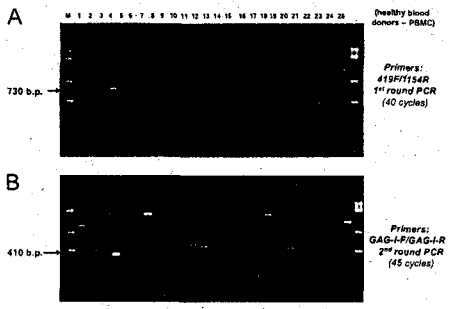

Fig. 2. MLV-related *gag* gene sequences detected in normal blood donors by nested PCR. (*A*) Results of PBMC DNA from blood donors 1–25 (of 44 donors examined) amplified after the first round of nested PCR using primer set 419F/1154R. Lane 4: PBMC DNA from BD22 has a positive target PCR amplicon confirmed by sequencing. (*B*) Results of PBMC DNA from the 25 normal blood donors after the second round of nested PCR using PCR primer set GAG-I-F/GAG-I-R (4). Sequencing of the PCR bands with size ~413 bp revealed that lane 4 (BD22), lane 7 (BD26), and lane 9 (BD28) were MLV-like virus *gag* gene sequences; lane 8 (BD27) was a human sequence. The positions of expected sizes of the positive PCR amplicons are indicated by arrows. M, DNA ladder size markers.

later of four of the academic center patients, all four remained positive. On repeated testing of eight academic center patients ~15 y later (in 2010), seven remained positive. All PCR products with the correct predicted size were retrieved from the gel and analyzed by DNA sequencing. Their DNA sequences were all confirmed to be those of MLV-related virus *gag* genes. The alignments of all of the sequences obtained from PCR products of ~730 bp are shown in Fig. S1. All of the positive PCR products amplified from the CFS patients' samples using primer set 419F/1154R were 746 bp in length. All of the positive PCR products amplified from the patients' blood samples using primer set GAG-I-F/GAG-I-R and primer set NP116/NP117 were 413 and 380 bp in length, respectively.

In 42% of samples, we also detected and sequence-confirmed the presence of MLV-related viral RNA in the frozen plasma samples of these CFS patients, using an RT–PCR assay (Fig. 1*C*). With one exception, all of the patients who tested positive for viral RNA *gag* gene sequences in the plasma samples also tested positive in the DNA prepared from PBMCs and/or whole blood. On the other hand, only about half of the patients with MLV-related virus *gag* gene sequences detected in PBMC DNA also had viral *gag* RNA sequences detected in the plasma.

MLV-Related Viral *gag* Gene Sequences Detected in the Blood of Healthy Volunteer Blood Donors. DNA originating from 44 healthy volunteer blood donors was tested in parallel by nested PCR (Fig. 2). The nested PCR testing using the MLV-related virus *gag* gene-specific primer sets could produce many side products from human DNA (Figs. 1 and 2). We sequenced all of the PCR-amplified DNA bands from the 44 control samples of blood donors having molecular sizes close to that of the predicted PCR products from the target XMRV *gag* gene in the first round of PCR (Fig. 2*A*) and in the second round of nested PCR (Fig. 2*B*). After the first round of amplification in nested PCR, a positive PCR product with the predicted size of ~730 bp was found in PBMC DNA from 1 of 44 blood donor controls (lane 4, Fig. 2*A*). This ~730-bp PCR product amplified from the blood donor (BD22) was confirmed by DNA sequencing as an MLV-related virus *gag* gene sequence of 745 bp (Fig. S1). Overall, we found 3 of 44 (6.8%) blood donors' PBMCs (BD22, BD26, and BD28) to be positive for the MLV-related virus *gag* gene sequences by completing both rounds of nested PCR (Fig. 2*B* and Fig. S2).

MLV-Related Viral *env* Gene Sequences Detected in the Blood of a CFS Patient and a Healthy Blood Donor. PBMC DNA from all of the CFS patients and healthy blood donors was also tested by PCR, targeting various regions of the MLV-related viral *env* gene. The MLV-related viral *env* gene segment of 240 bp was amplified and confirmed by sequencing from one healthy donor (BD-26) by a semi-nested PCR using the primer set 5922F/6273R in the first round of amplification and 5922F /6173R in the second round of amplification. The MLV-related viral *env* gene segment of 206 bp was amplified and confirmed by sequencing from 1 CFS patient by a nested PCR using primer set 5922F/6273R in the first round of amplification and 5942F/6159R in the second round of amplification (*SI Materials and Methods*).

Phylogenetic Analyses of MLV-Related Virus *gag* and *env* Gene Sequences. Multiple sequence alignment (MSA) and phylogenetic analysis of the MLV-related virus *gag* gene sequences amplified from 21 CFS patient samples and one blood donor (BD22) are shown in Fig. S1 and Fig. 3*A*, respectively. There were three different MLV-related retroviral *gag* gene sequences identified by PCR in the blood samples of the CFS patients and a fourth variant was detected in blood donor BD22. The sequences in all four variants were more closely related to the sequences of polytropic mouse endogenous retroviruses (mERVs) than to those of XMRVs. Although variations were observed, the majority (18/21, 86%) of CFS patient samples had the same viral *gag* gene sequence (CFS type 1), whereas 2/21 had a different, but similar, viral *gag* gene sequence (CFS type 2), and a third distinct sequence (CFS type 3) was found in the remaining CFS case. Phylogenetic analysis using the 746-nt sequences obtained revealed that CFS type 1, CFS type 2, and CFS type 3 formed a cluster that is clearly separable from the cluster formed by the newly reported XMRVs (Fig. 3*A*). Interestingly, the 745-nt virus *gag* gene sequence of donor BD22 (with a 1-nt deletion in the alignment) could not be included in either the cluster of CFS type 1/CFS type 2 or the cluster of

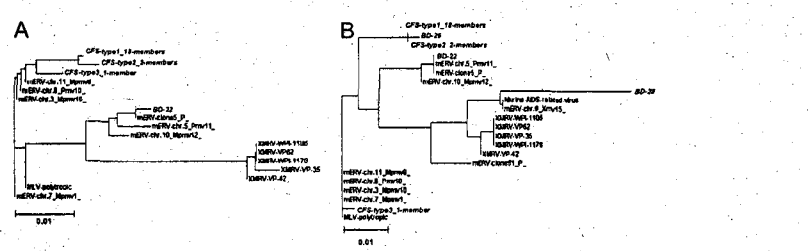

Fig. 3. Phylogenetic trees corresponding to the MSAs shown in Figs. S1 and S2 were generated by the ClustalW2 program using the neighbor-joining method (*Materials and Methods*). (*A*) Phylogenetic analysis based on the 746-nt *gag* gene nucleotide sequences amplified from blood samples of CFS patients and BD-22 of the corresponding MSA in Fig. S1. (*B*) Phylogenetic analysis based on the 380-nt *gag* gene sequences amplified from blood samples of CFS patients and healthy blood donors using the primer set NP116/NP117 of the corresponding MSA in Fig. S2.

XMRVs. The viral *gag* gene sequences of CFS type 3 and blood donor BD22 appear to be phylogenetically more closely related to polytropic mERVs or modified polytropic mERVs (Fig. 3*A*).

Sequence analysis of the shorter fragments of the viral *gag* gene amplified from blood of 36 out of 41 CFS patient samples and 3 out of 44 blood donor samples after the second round of nested PCR similarly confirms that there are significant variations among the MLV-like *gag* gene sequences. Fig. S2 shows sequence alignment of the 380-nt segments of viral *gag* genes amplified from three blood donors (BD22, BD26, and BD28), patients with CFS types 1–3, XMRVs, and other closely related mERVs/polytropic MLVs. As an example, the viral *gag* gene sequence identified in BD28, but not sequences of BD22 and BD26, has a prominent 21-nt deletion that is uniquely present in polytropic mERV clone 51 (Fig. S2). In phylogenetic analysis, the 380-nt segment of the *gag* gene sequence found in BD26 appears to be closely related to those of CFS types 1 and 2. However, similar to the analytic result with the 746-nt product, the 380-nt *gag* gene sequences of BD22 and BD28 again cannot be included in either the cluster of CFS type 1/CFS type 2 or the cluster of XMRVs (Fig. 3*B*). On the other hand, when the protein sequences coded by the *gag* gene sequences identified in the CFS patients and blood donors are compared with those of a wider range of exogenous and endogenous MLVs, they are most similar to those of polytropic MLVs and XMRVs (Fig. S3). A ClustalW Gag protein tree again reveals that MLV-like virus *gag* proteins have much more similarity to those of modified-polytropic and polytropic mERVs or to those of XMRVs, but are very different from those of exogenous ecotropic MLVs (Fig. 4).

The sequence alignment and the phylogenetic analysis of the MLV-related virus *env* gene sequences obtained from both the CFS patient and healthy blood donor revealed that they were also more closely related to those of polytropic or modified polytropic MLVs than to those of XMRVs (Fig. S4).

Testing for the Presence of Mouse DNA in Patient and Blood Donor *Samples Positive for MLV-Like gag Gene Sequences.* Mouse DNA contains endogenously many closely related proviruses of MLVs. Hence, contamination of the blood samples or reagents by mouse DNA could have produced falsely positive PCR results. Although we took great precautions to prevent potential contamination in the laboratory, and although multiple negative controls were always included in each assay, we took additional steps to confirm that no mouse DNA had contaminated the assays or the clinical samples prepared in this study. We estimated that there were about 200–1,800 mitochondrial DNA (mtDNA) copies per mammalian cell. A highly sensitive PCR assay targeting mouse-specific mtDNA was developed (*Materials and Methods*) to exclude any possible minute mouse DNA contamination in the assay system and in the clinical samples with positive amplified *gag* gene products.

The first round of the semi-nested PCR (40 cycles) used primer set mt15982F/mt16267R and could detect 10 fg of mouse DNA in the presence of 35 ng of human background DNA. By comparison, when studied in parallel under the same assay conditions, the first round of nested PCR (40 cycles) against the MLV *gag* gene required ~10 pg of mouse DNA to detect viral gag gene sequences (Fig. 5*A*). Thus, the first round of mouse-specific mtDNA PCR assay could detect a positive mtDNA signal at a mouse DNA concentration 1,000-fold below the concentration required to detect a positive *gag* signal. The second round of mouse mtDNA semi-nested PCR, using primer set mt16115F/mt16267R, could consistently amplify the target 153-bp amplicon from 2.5 fg of *mouse DNA mixed with 35 ng of human DNA* (Fig. 5*B*). By comparison, the second round of the nested *gag* gene PCR produced positive ~400-bp amplicons from 500 fg of mouse DNA mixed with 35 ng of human background DNA in the reaction. Thus, the second round of mouse mtDNA semi-nested PCR had a sensitivity hundreds-fold higher than that of the second round of the MLV *gag* gene nested PCR in amplifying mouse DNA.

Using this highly sensitive PCR assay for mouse-specific mtDNA, we examined all of the blood samples that were found positive for MLV-like virus *gag* gene sequences from both CFS patients and healthy controls for evidence of mouse DNA contamination. PBMC DNA (30–40 ng) from the CFS patients and the healthy blood donors, as well as serial dilutions from 50 to 1 fg of mouse DNA mixed with 35 ng of human DNA as the

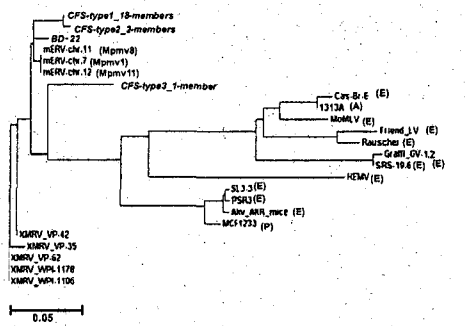

Fig. 4. Phylogenetic analysis of protein sequences based on the alignment shown in Fig. S3. CFS types 1, 2, and 3 and BD-22 and MLVs *gag* protein sequences are compared. Gag protein sequences starting from the AUG initiation codon are allgned with those of relevant endogenous as well as *exogenous MLVs. Sequences of MLVs are referred to as polytropic (P), ecotropic (E), amphotropic (A), or modified polytropic (Mpmv). MoMLV, Moloney murine leukemia virus; HEMV, hortulanus endogenous murine virus.*

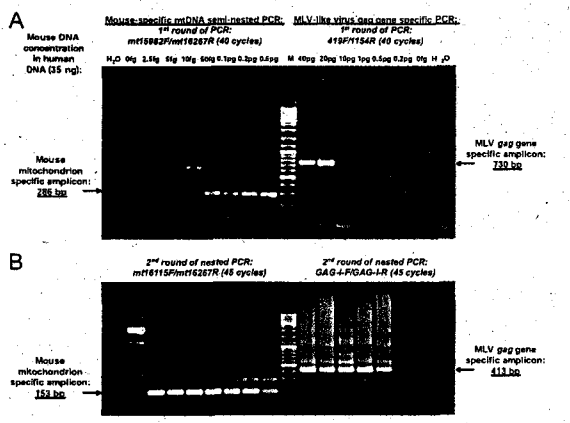

Fig. 5. Comparison of sensitivity in amplifying mouse DNA by the semi-nested PCR targeting mouse-specific mtDNA and by the nested PCR targeting MLV-like virus *gag* gene. Serial dilutions of mouse spleen DNA (from 40 pg to 2.5 fg) were spiked into 35 ng of total human PBMC DNA and compared in parallel for the mouse DNA detection sensitivity of the two PCR assays. (*A*) In the first round of the mtDNA-specific PCR assay, 10 fg or more of mouse DNA could be detected in the presence of 35 ng of human DNA by producing the 286-bp target product. In the first round of MLV *gag* gene nested PCR assay, 10 pg or more of mouse DNA could be detected in the presence of 35 ng of human DNA by producing the ~730-bp target product. (*B*) In the second round of mouse-specific mtDNA semi-nested PCR, the 153-bp target amplicon could consistently be amplified from 2.5 fg of mouse DNA. In the second round of *gag* gene-specific nested PCR, the 413-bp target product could be amplified from 0.5 pg or more of mouse DNA. Lane 0 fg: 35 ng of human DNA without spiking any mouse DNA. Lane H_2O: No DNA template. M: 100-bp DNA ladder mix. Primers and PCR cycle numbers used in each round of amplification for both of the assays are shown at the top of each gel.

positive templates, were tested in parallel. No mouse DNA was found in the PCR mix nor in the blood samples of CFS patients and blood donors that tested positive for the MLV-like virus *gag* gene sequences. Fig. 6 shows the results of the two rounds of mouse-specific mtDNA semi-nested PCR testing in DNA from PBMCs of four CFS patients (patients 8, 17, 20, and 25) with positive 746-bp amplicons in the first round of the nested PCR targeting the MLV-like virus *gag* gene, as well as from three blood donors (BD22, BD26, and BD28) who tested positive and two donors (BD21 and BD23) who tested negative for MLV-like *gag* gene sequences.

Discussion

Detection of MLV-Related Nucleic Acid Sequences. Our laboratory detected MLV-related virus *gag* gene sequences in DNA from PBMC and whole-blood samples from 32 of 37 (86.5%) CFS patients, compared with 3 of 44 (6.8%) volunteer blood donors, using a two-round nested PCR. Following only one round of PCR amplification, 21 of the 41 CFS patients' DNA samples were found positive compared with only 1 of 44 donor samples. In every instance throughout these studies, the "positive" result by PCR (an amplicon of the predicted size) was confirmed by sequencing.

In four CFS patients from whom two samples were obtained, 2 y apart, the *gag* gene sequences were detected on both occasions. Further, *gag* gene sequences were still detectable in seven of eight CFS patients from whom fresh samples were obtained ~15 y after they were initially found to be MLV *gag* gene positive. In one *gag*-positive CFS patient and one *gag*-positive blood donor, MLV-related *env* gene sequences also were detected by PCR. However, we were unable to PCR amplify and determine the MLV-related *env* gene sequences in the majority CFS patients, possibly because of the low copy number and the greater genetic variability in the *env* gene compared with the *gag* gene.

In the CFS patients, plasma samples revealed MLV-related virus *gag* gene sequences in 42% when tested by RT–PCR for viral RNA. Whereas all but one patient whose plasma tested positive for viral RNA also tested positive in PBMCs for viral DNA, only half of the cases in which MLV-related virus *gag* gene sequences were detected in PBMCs had detectable RNA sequences in plasma. Thus, accurate determination of the prevalence of these agents in patients and donors requires cellular DNA for analysis.

Sequence Variability. Previous reports of XMRV isolates from patients with CFS and with prostate cancer and from individuals in different geographic locations have described very similar nucleic acid sequences (3, 4, 12), a feature believed to be a unique characteristic of XMRVs (13). However, our analysis revealed three different types of MLV-related virus *gag* gene sequences in CFS patients. In all three groups, the sequences were more closely related to the sequences of polytropic mERVs than to XMRVs and were more distant from the sequences of ecotropic MLVs (Fig. 3). Moreover, viral *gag* gene sequences with significant variations from both the cluster of CFS type 1/CFS type 2 and the cluster of XMRVs were identified in at least two blood donors (BD22 and BD28); phylogenetic analysis revealed the latter sequences to be more closely related to those of polytropic or xenotropic mERVs (Fig. 3*B*). It is unclear whether the sequence variations of the viral genes identified in the CFS patients and healthy blood donors have any significance in viral pathogenesis or disease development.

The MLV-like virus *gag* gene sequences in the CFS patients and blood donors had a deletion of 9 nt in the 5′ *gag* leader region and did not have the 24-nt deletion in this region reported in XMRVs. Internal deletions of 9 nt similar to what we have identified in the CFS patients and the blood donors are known to be present in the region that encodes the glycosylated Gag protein (GlycoGag) in some infectious endogenous (ecotropic) MLVs and exogenous (xenotropic) MLVs, such as AKV and DG-75 (14, 15). Many previous studies have shown that the nonstructural GlycoGag of MLVs plays a critical role in viral pathogenesis and in vivo infectivity (16–19). In this context, the MLV-like virus *gag* gene sequences identified in most of our CFS patients (both CFS type 1 and CFS type 2) appear to have an intact GlycoGag in-frame with the matrix and are consistent with the gene sequences of infectious MLVs. Unfortunately, the sequences presently obtained in the study are still a bit short and lack the alternative start codon CUG. Slight extension of the 5′ leader sequence will be needed to confirm the intact nature of GlycoGag.

Could Our PCR Results Have Been Falsely Positive? Voisset and coauthors (20) recently reviewed the pitfalls encountered in the identification of new retroviruses ("tumor viruses"). False-positive results can occur for a variety of reasons. Viral gene sequence-specific PCR primers can nonspecifically amplify nucleic acid sequences that differ from the target sequence. For this reason, we sequenced every positive PCR product (every amplicon of the predicted size) and confirmed MLV-related gene sequences in every instance.

Although contamination must always be a concern for any PCR-based study, several pieces of evidence argue against the possibility that the PCR products identified in our study are the

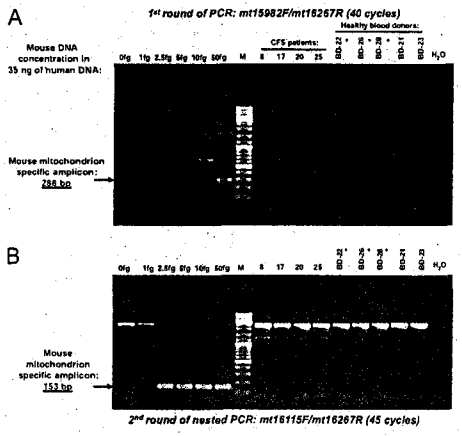

Fig. 6. Testing of CFS patients' and healthy blood donors' samples positive for MLV-like *gag* gene sequences for the presence of mouse DNA contamination using the semi-nested PCR assay targeting mouse-specific mtDNA. Serial dilutions of mouse DNA were spiked into 35 ng of human DNA and used as the controls of the assay sensitivity. The first round of mouse mtDNA semi-nested PCR (*A*) detected 10 fg of mouse DNA, and the second round of the semi-nested PCR (*B*) detected 2.5 fg of mouse DNA in the presence of 35 ng human background DNA. No evidence of mouse DNA contamination could be found by either round of mouse mtDNA semi-nested PCR in the PBMC DNA (35 ng) of CFS patients (patients 8, 17, 20, and 25); three blood donors (BD-22, BD-26, BD-28) tested positive and two blood donors (BD-21 and BD-23) tested negative for the MLV-like virus *gag* gene sequences. Healthy blood donors' samples positive for MLV-like *gag* gene sequences are labeled by asterisks. Lane 0: 35 ng of human DNA without spiking any mouse DNA. Lane H_2O: No DNA template. M: 100-bp DNA ladder mix.

result of laboratory contamination. First, every clinical sample that tested positive for the MLV-like virus *gag* gene sequences was tested for evidence of mouse DNA contamination using a semi-nested PCR for mouse-specific mtDNA that was exponentially more sensitive in detecting mouse mtDNA than MLV-related *gag* sequences (Fig. 5). Any detection of MLV *gag* that was caused by contamination with mouse DNA also would have detected mouse mtDNA by PCR, thereby identifying the *gag* result as falsely positive. In fact, no positive signal was detected by the mtDNA semi-nested PCR assay in any of the reaction mixtures or in the DNA of clinical samples examined in the study, thus excluding possible contamination by mouse DNA.

Second, we addressed the possibility that the clinical samples or the assay system might have been contaminated. The blood samples were obtained in clinical laboratories that never worked with mice or retroviral vectors and were drawn through sterile needles into vacuum tubes that remained unopened until testing. The laboratory in which PCR testing was performed also had never worked with murine cells, tissues or serum samples, or MLV vectors. Finally, because repeated entry into samples would increase the chance of contamination, we emphasize that sample vials from both patients and normal donors had never been entered before our testing.

Third, there were at least six different MLV-related *gag* gene sequences amplified from the blood samples of CFS patients and blood donors. Typically, contamination would be manifest as the same sequence in all or most samples. Moreover, the sequences that we observed all had significant variations from the previously reported exogenous MLVs or viral vectors. Furthermore, during this study more than 300 negative controls set up for multiple PCR amplification assays targeting the MLV *gag* gene were performed, and all were negative.

Finally, a new set of blood samples was recently obtained from 8 of the original 25 patients followed in an academic medical center. Testing of the repeat blood samples, ~15 y after the index sample, showed that seven of eight patients examined had detectable MLV-*gag* gene sequences. Significant variations of MLV-like virus *gag* gene sequences amplified from the freshly obtained blood samples were identified as would be expected in retroviral infections, but not from contamination.

The ultimate proof of low-grade infection by MLV-related viruses in humans may rely on demonstrating the integration of the viral genes into the human genome (20). The identification of provirus integration sites will take more time and effort to investigate, given that we estimate only one virus gene copy in every 400–4,000 nucleated PBMCs. Also, previous work with XMRV indicates that integration sites are quite variable (21) and the same may be true for the polytropic mouse endogenous retroviruses, which are predominant in this study.

Why Have Other Studies Come to Different Conclusions? Although we find evidence of a broader group of MLV-related viruses, rather than just XMRV, in patients with CFS and healthy blood donors, our results clearly support the central argument by Lombardi et al. (3) that MLV-related viruses are associated with CFS and are present in some blood donors. However, four recent studies have failed to confirm the PCR results reported by Lombardi (7–10). There are various possible explanations for this disparity. As stated in the reports, there could be a difference in the prevalence of these infectious agents in CFS patient populations in different geographic areas. This argument is somewhat less plausible since the publication of a recent negative study with subjects from the United States (10). Nevertheless, the heterogeneity in *gag* gene sequences that we observed suggests that geographic differences in different MLV-related viruses may be considerable and could affect both the sensitivity and the specificity of molecular amplification using standard primer sets.

Indeed, it is possible that the PCR primers used in various studies may have different sensitivity in detecting the diverse group of MLV-related virus *gag* gene sequences that we found in the clinical samples. The 5′ *gag* leader sequence of previously described XMRVs represents the most divergent segment of the XMRV genome in comparison with the genomes of the other MLVs (4). In particular, there is evidently a unique 15-nt deletion in the 5′ *gag* leader region in all of the XMRVs previously identified in patients with prostate cancer and CFS (3, 4). To detect XMRVs in human samples with better sensitivity and specificity, some studies used a PCR primer spanning this unique deletion as the "XMRV-specific" primer (6). However, none of the viral *gag* gene sequences amplified from the blood samples of CFS patients and blood donors in our study has this particular deletion (Fig. S1). As a consequence, such primers might have been insensitive in detecting the MLV-related *gag* gene sequences that we have identified.

Finally, it is also quite possible that there is heterogeneity in the patients diagnosed with CFS in different studies. CFS is a syndrome defined exclusively by a group of nonspecific symptoms and thus has an ill-defined phenotype. Future studies should adhere to consensus case definitions such as that developed by the Centers for Disease Control and Prevention (CDC) (1). Conversely, putative "healthy" control subjects should explicitly deny the presence of those symptoms that constitute the case definition of CFS. Furthermore, even bona fide cases of CFS may have different viral or other etiologies.

Further Considerations. The finding of XMRV or MLV sequences in persons with CFS or other diseases does not constitute definitive proof of viral infection. However, in the study of Lombardi et al. (3) and studies reviewed subsequently by Silverman et al. (22) the evidence for XMRV infection in humans not only involved detection of viral nucleic acids using PCR, but also reported the detection of

viral antigens, detection of anti-viral antibodies, the ability to culture the virus in a prostate cancer cell line, the detection of gamma retrovirus particles by electron microscopy, and transmission of infection to macaques. In sum, none of the four studies that have failed to confirm the PCR evidence reported by Lombardi et al. (3), nor our own study, has attempted to fully replicate that study.

It remains to be shown that the association that we have found, using the methods that we have described, can be generalized to a larger group of patients with CFS. Indeed, we suspect that the association will be lower in CFS cases identified through *community-based surveys, as contrasted to cases seen at academic* medical centers. Even if subsequent studies confirm an association between MLV-like viruses and CFS, that will not establish a causal role for these viruses in the pathogenesis of this illness. For example, such a high frequency of infections with *MLV*-related viruses in patients with CFS could reflect an increased susceptibility to viral infections due to an underlying CFS-related immune dysfunction, rather than a primary role for these viruses in the pathogenesis of CFS.

Finally, the finding of MLV-related virus *gag* gene sequences in nearly 7% of healthy volunteer blood donors in our study and of XMRV in 3.7% of healthy controls in the study of Lombardi et al. (3) raises additional issues. The possibility that these agents might be blood-transmitted and pathogenic in blood recipients warrants extensive research investigations of appropriately linked donor–recipient cohorts.

Materials and Methods

Samples from CFS Patients and Healthy Controls. Initially, we tested 41 whole-blood samples that had been obtained for culture isolation of mycoplasmal agents in the mid-1990s. We maintained whole-blood, PBMC, and plasma samples from CFS patients in frozen storage at −80 °C. Of the 41 patient samples, 29 were collected from 25 patients by one of us (A.L.K.) at the Chronic Fatigue Research Center, Brigham and Women's Hospital (Boston, MA). Four of the CFS patients also had blood obtained on a second occasion ~2 y later. Most of the patients were from the New England area; none were related, and virtually none had any regular social contact. Each of the 25 patients was systematically evaluated with a standardized history (supplemented by a patient questionnaire), physical examination, and battery of laboratory tests. Each met the 1988 CDC criteria for CFS, and 21 also met the 1994 CDC criteria. The average age of the patients at the time of venepuncture was 44.4 y; 4 were male and 21 were female. All of the patients signed informed consent documents approved by the Institutional Review Board of Brigham and Women's Hospital. A new set of blood samples was obtained in 2010 from 8 of the original 25 patients followed in the academic medical center. The blood samples were processed for PCR study without first being frozen. The other 12 samples from CFS patients were sent by individual clinicians taking care of patients in the mid-1990s who were given the diagnosis of CFS. We do not have details regarding the methodology by which the referring clinicians established the diagnosis of CFS. The samples had also been sent in the mid-1990s and stored at −80 °C. Frozen PBMC samples from 44 normal blood donors from the Washington, DC, area were collected in 2003–2006 and stored at the Department of Transfusion Medicine, Clinical Center, National Institutes of Health. All patient and control samples were coded and tested in parallel. Details of the preparation of blood samples and DNA/RNA isolation are described in *SI Materials and Methods*.

XMRV/MLV *gag* Nested PCR. The nested PCR for the *gag* gene was performed according to the protocols described previously (3, 4) with minor modifications. Three primer sets used in the study are as follows: 419F and 1154R (3), GAG-I-F and GAG-I-R (4), NP116 forward, and NP117 reverse. The NP116/NP117 was an in-house–designed primer set based on the highly conserved sequences found in different MLV-like viruses and XMRVs (Fig. S1). Primer sequences and details of PCR sensitivity and specificity quality controls can be found in *SI Materials and Methods*.

Phylogenetic Analysis. To generate the neighbor-joining phylogenetic tree, the viral *gag* gene sequences obtained from blood samples of patients with CFS, normal blood donors, as well as all of the closely related MLV *gag* gene sequences selected from the National Center for Biotechnology Information (NCBI) database by BLAST querying with the *gag* gene sequences obtained in the PCR study (*SI Materials and Methods*) were aligned with ClustalW2 (http://www.ebi.ac.uk/tools/clustalw2) using default settings. The analysis produced the same phylogenetic trees with or without consideration of the sequence gaps in alignment.

Mouse Mitochondrial DNA Assay. The complete mtDNA sequences of humans and mice were downloaded from GenBank and aligned using ClustalW. Sequence alignment revealed the 439 bp of the 3′ end of mouse mtDNA (beyond 15,862 bp, according to the coordinates of BALB/c mouse; accession no. AJ512208) were not present in human mtDNA. Primer sets were designed for a semi-nested, mouse-specific mtDNA PCR based on the sequence in this region of mouse mtDNA using Primer-Blast from NCBI. The external PCR primers (*SI Materials and Methods*) were designated as mt15982F and mt16267R, which would produce a predicted amplicon of 286 bp. The internal primers of the semi-nested PCR were designated as mt16115F and mt16267R, which would produce a predicted amplicon of 153 bp from mouse mtDNA. PCR system and setup were the same as for the *gag* gene-nested PCR study. However, PCR conditions were slightly different: 4 min at 94 °C; (30 s at 94 °C, 30 s at 55 °C, 1 min at 72 °C) × 40–45 cycles; 10 min at 72 °C.

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BRIEF REPORT

Failure to Detect Xenotropic Murine Leukemia Virus–Related Virus in Blood of Individuals at High Risk of Blood-Borne Viral Infections

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(See the article by Danielson et al, on pages 1470–1477, the brief report by Henrich et al, on pages 1478–1481, and the editorial commentary by Kearney and Maldarelli, on pages 1463–1466.)

A xenotropic murine leukemia virus–related virus (XMRV) has recently been reported in association with prostate cancer and chronic fatigue syndrome, with a prevalence of up to 3.7% in the healthy population. We looked for XMRV in 230 patients with human immunodeficiency virus type 1 or hepatitis C infection. XMRV was undetectable in plasma or peripheral blood mononuclear cells by polymerase chain reaction targeting XMRV *gag* or *env*. T cell responses to XMRV Gag were undetectable in peripheral blood mononuclear cells by ex vivo gamma interferon enzyme-linked immunospot assay. In our cohorts, XMRV was not enriched in patients with blood-borne or sexually transmitted infections from the United Kingdom and Western Europe.

Xenotropic murine leukemia virus-related virus (XMRV) is a gammaretrovirus which has been linked with prostate cancer [1, 2] and chronic fatigue syndrome [3]. XMRV was first described in prostate tumor samples from the United States [1, 2], but the virus could not be detected in a subsequent survey of 589 German patients with prostate cancer [4]. Two other surveys in Europe [5] and in Ireland [6] also failed to find the virus. The reason for the discrepancy is not clear. More recently, XMRV was detected in 67% of patients with chronic fatigue syndrome in the United States [3], however 4 additional studies examining cases of chronic fatigue syndrome diagnosed in Europe and the United States identified no cases of XMRV infection [7–10].

In one of these, Groom et al [8] also looked for neutralizing antibodies to XMRV and found that ~1% of serum samples tested had evidence of neutralization that could not be dismissed as nonspecific binding. These and other data suggest the prevalence of XMRV in the healthy population may lie between 1% and 3.7% [3, 8]. This has potential implications for blood transfusion recipients and is currently under investigation by the Centers for Disease Control and Prevention and the Department of Health and Human Services in the United States.

The route of transmission of XMRV has not been elucidated. We identified individuals who were at high risk for either sexually transmitted or blood-borne infections, according to whether they were infected with human immunodeficiency virus type 1 (HIV-1) or hepatitis C. We tested plasma and peripheral blood mononuclear cells (PBMCs) to reveal whether these patients were at increased risk of XMRV infection.

Methods. Two cohorts of HIV-1–infected individuals were tested for XMRV, 1 for patients with acute and 1 for those with chronic HIV-1 infection (Table 1). The cohort of patients with chronic infection included Swiss participants from the Swiss-Spanish Intermittent Therapy Trial, described elsewhere [11]. All patients were not receiving therapy at the time of sampling and had received a median duration of 26 months (range, 8.5–44.5 months) of highly active antiretroviral therapy at study entry. The median time not receiving therapy for the cohort was 14 months (range, 3–19 months). The second HIV-1–infected group of patients were recruited from a study of acute HIV-1 infection at St. Mary's Hospital, London [12]. Plasma samples were obtained from these individuals prior to receipt of antiretrovirals. For both cohorts, plasma samples and PBMCs were stored in −80°C freezers and liquid nitrogen, respectively, for up to 6 years.

The cohort of hepatitis C–positive individuals was recruited from the John Radcliffe hospital, Oxford, United Kingdom (Table 1). All patients had chronic infection and were treatment

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Table 1. Patient Cohort Characteristics

Characteristic	Patient cohort		
	Chronic HIV-1 infection[a] (n = 133)	Acute HIV-1 infection[a] (n = 101)	HCV infection (n = 67)
Sex, ratio male:female	92:41	92:9	53:14
Age, median years (range)	40 (22–68)	31 (20–67)	50 (29–81)
Available for testing	84	79	67
CD4 count pretherapy, median cells/μL (range)	359 (1–1035)	510 (90–1480)	...
Viral RNA pretherapy, median $\log_{10}$ copies/mL (range)	4.41 (2.23–6.11)	4.95 (1.90–5.95)	...
Antiretroviral therapy			
Received dual therapy	10	0	...
Received triple therapy	123	0	...
Route of acquisition or risk factor			
MSM	50 (38)	89 (88)	...
Heterosexual	60 (45)	12(12)	...
IVDU	23 (17)	0 (0)	51 (76)
Blood transfusion	...	...	10 (15)
Other[b]	...	...	9 (13)

NOTE. Data are no. or no. (%) of patients, unless otherwise indicated. HCV, hepatitis C virus; HIV-1, human immunodeficiency virus type 1; IVDU, intravenous drug user; MSM, men who have sex with men.

[a] Patients with chronic HIV-1 infection were from the Swiss-Spanish Intermittent Therapy Trial cohort, and those with acute HIV-1 infection were from St Mary's Hospital acute HIV-1 cohort.

[b] Other includes tattoos (6), acupuncture (1), blood product recipient (1), and vaccination (1). Three patients in the HCV cohort had >1 risk factor.

naive. Enzyme-linked immunospot (ELISPOT) assays were performed using cryopreserved PBMCs (n = 12) or whole blood (n = 2), when they were performed fresh. For the 67 polymerase chain reactions (PCR), DNA was extracted from cryopreserved PBMCs (n = 11), fresh PBMCs (n = 20), or whole blood (n = 36). All patients gave informed consent for enrollment in their respective studies, according to the relevant ethics committees.

DNA was extracted from 3×10^6 to 5×10^6 PBMCs or whole blood (Puregene DNA extraction kit), and 200–800 ng were used in each PCR reaction. Patient plasma samples (1 mL) underwent ultracentrifugation at 21,185 g at 4°C for 1 h and was resuspended in 140 μL of plasma. Viral RNA was extracted (Qiagen viral RNA extraction kit) and converted to complementary DNA (cDNA) with use of random decamers and Superscript II reverse transcriptase (Invitrogen). When amplifying genomic DNA, a house-keeping gene (hGAPDH) was incorporated. For each run, 2 negative water controls and a separate positive control XMRV DNA were included (XMRV plasmid VP-62, a generous gift from Dr. Robert Silverman), of which PCR reactions were able to detect at least 5 plasmid copies, determined by absorbance measurements at 260 nm.

To amplify XMRV, we targeted the *gag* and *env* genes with use of nested PCR reactions described elsewhere [1, 3]. For the *gag* PCR, we used 2 μL of DNA or cDNA template, 5μL of 10× reaction buffer (Invitrogen), 1 μL of 50 mmol/L $MgCl_2$, 1 μL of 10 mmol/L dNTPs, 0.75 μL of 20 μmol/L primer GAG-O-F (5′-CGCGTCTGATTTGTTTTGTT-3′), 0.75 μL of 20 μmol/L primer GAG-O-R (5′-CCGCCTCTTCTTCATTGTTC-3′), 0.2 μL of Platinum Taq (Invitrogen), and water to make up a 50-μL final volume. Conditions were incubation at 95°C for 4 min, followed by 35 cycles of 94°C for 30 s, 52°C for 30 s, and 72°C for 45 s, and a final incubation of 72°C for 7 min. From this first-round reaction, 3 μL were used in a nested second round with use of 5 μL of 10× reaction buffer (Invitrogen), 1.5 μL of 50 mmol/L $MgCl_2$, 1 μL of 10 mmol/L dNTPs, 0.75 μL of 20μmol/L primer GAG-I-F (5′-TCTCGAGATCATGGGACAGA-3′), 0.75 μl of 20 μmol/L primer GAG-I-R (5′-AGAGGGTAAGGGCAGGGTAA-3′), 0.2 μL of Platinum Taq (Invitrogen), and water to make up a 50-μL reaction volume. Conditions for the second round were an incubation at 95°C for 4 min, followed by 35 cycles of 94°C for 30 s, 54°C for 30 s, and 72°C for 45 s, and a final incubation of 72°C for 7 min. The resulting fragment was 413 bp in size. For the GAPDH PCR reaction, the primers used were HGAPDH-F (5′-GAAGGTGAAGGTCGGAGTC-3′) and HGAPDH-R (5′-GAAGATGGTGATGGGATTTC-3′), with the same reaction conditions as the first round Gag PCR.

The *env* PCR reaction included 2 μL of DNA or cDNA template, 5 μL of 10× reaction buffer (Invitrogen), 1 μL of 50 mmol/L $MgCl_2$, 1 μL 10 mmol/L dNTPs, 0.75 μL of 20 μmol/L primer 5922F (5′-GCTAATGCTACCTCCCTCCTGG-3′), 0.75 μL of 20 μmol/L primer 6273R (5′-GGAGCCCACTGAGGAATCAAAACAGG-3′), 0.2μL Platinum Taq (Invitrogen), and water to make up a 50-μL reaction volume. Reaction conditions were an incubation at 95°C for 4 min, followed by 45 cycles

Table 2. Assay Results for the Identification of Xenotropic Murine Leukemia Virus–Related Virus (XMRV)

Cohort,[a] assay	Disease stage	Sample	No. of samples tested	No. of positive results
HIV-1 infection				
Gag PCR	Chronic	DNA	84	0
Env PCR	Chronic	DNA	84	0
Gag RT-PCR	Acute	RNA	79	0
Env RT-PCR	Acute	RNA	77	0
ELISPOT	Acute	PBMCs	49	0
HCV				
Gag PCR	Chronic	DNA	67	0
Env PCR	Chronic	DNA	67	0
ELISPOT	Chronic	PBMCs	14	0

NOTE. HIV-1, human immunodeficiency virus type 1; PBMC, peripheral blood mononuclear cell; PCR, polymerase chain reaction; RT-PCR, reverse transcription PCR.

[a] Patients with chronic HIV-1 infection were from the Swiss-Spanish Intermittent Therapy Trial cohort, patients with acute HIV-1 infection were from St Mary's Hospital acute HIV-1 cohort, and patients with chronic HCV were from the John Radcliffe Hospital viral hepatitis clinic.

of 94°C for 30 s, 57°C for 30 s, and 72°C for 45 s, and then a final incubation of 72°C for 7 min. From this first-round reaction, 3 μL were used in a nested second round reaction with the following conditions: 5 μL of 10× reaction buffer (Invitrogen), 1.5 μL of 50 mmol/L $MgCl_2$, 1μL of 10 mmol/L dNTPs, 0.75 μL of 20 μmol/L primer 5942F (5′-GGGGACGATGACAGACACTTTCC-3′), 0.75 μL of 20 μmol/L primer 6200R (5′-CCCATGATGATGATGGCTTCCAGTATGC-3′), 0.2 μL Platinum Taq (Invitrogen), and water to make up a 50-μL reaction volume. Conditions for the second round were an initial incubation at 95°C for 4 min, followed by 35 cycles of 94°C for 30 s, 57°C for 30 s, and 72°C for 45 s, and a final incubation of 72°C for 7 min. The resulting fragment was 259 bp in size.

Reactions were performed under strict conditions to avoid contamination. Master mix reagents were prepared in DNA-free areas in hoods subject to UV decontamination. DNA templates were prepared and added to the reaction in separate hoods. PCR products were run on 1% agarose gels stained with ethidium bromide in a third area, away from PCR machines and hoods.

For ex vivo T cell ELISPOT assays, 96-well plates were coated with anti-interferon-γ immunoglobulin G, to which peptide pools and between 100,000 and 200,000 PBMCs were added, in duplicate. After incubation, biotinylated interferon-γ antibody was added. Following addition of a streptavidin-labelled chromogen, the number of reactive cells was counted. Sixty-six overlapping peptides covering the XMRV Gag protein were synthesized as 18-mer peptides, overlapping by 10 amino acids and tested in pools containing 11 peptides (final concentration, 2 μg/mL of each peptide). Assay results were considered to be positive if the mean number of spot-forming units in the test wells was greater then the mean plus 3 times the standard deviation of the negative control well spot-forming units. Assays with high background (>10 spots/well) in the negative control wells were excluded.

Results and discussion. We tested DNA extracted from PBMCs from 84 patients who were chronically infected with HIV-1. For all patients, the PCR for hGAPDH was positive, confirming the presence of amplifiable DNA. We detected no XMRV DNA by either the *env* or *gag* PCR reactions from patient samples (Table 2), whereas in all reactions, 2 positive controls containing ~5 and ~50 copies of XMRV, respectively, amplified successfully. We tested plasma from 79 drug-naive individuals recently infected with HIV-1 by reverse transcription PCR. No patient samples were positive for XMRV *gag* or *env* RNA (Table 2). Genomic DNA extracted from the blood of 67 patients with hepatitis C virus (HCV) infection was tested for the presence of XMRV *gag* and *env* viral DNA. Positive XMRV controls and the hGAPDH house-keeping gene amplified successfully, but no samples were positive for XMRV (Table 2). In summary, of 230 patients tested, 0 (95% confidence interval, 0%–1.3%) had positive results by PCR for either XMRV *env* or *gag*.

Following the absence of positive results with PCR, we developed a novel ex vivo ELISPOT assay to identify T cell responses in PBMCs to the XMRV Gag protein. T cell ELISPOT assays are a sensitive tool for detecting immune responses to infecting pathogens and have become a routine component of clinical diagnostics, for example, to screen for latent *Mycobacterium tuberculosis* infection. The PBMCs tested in an ELISPOT assay can either be ex vivo or "cultured." We chose the ex vivo approach because it is both sensitive and specific for the detection of effector T cell responses to active infections with retroviruses and other pathogens, whereas cultured ELISPOT assays are more appropriate for detecting rare or weak non-infective memory responses, for example, those induced by

vaccines. Here, we used T cell ELISPOT assays with use of 18 amino acid overlapping peptides from XMRV Gag to look for evidence of XMRV infection in PBMCs from 49 patients with acute HIV-1 infection and 14 patients with HCV infection. We targeted the XMRV Gag gene, as in other retroviral infections, the Gag protein is highly expressed and is recognized by T cells [13]. In the original report of XMRV in patients with chronic fatigue syndrome, XMRV Gag was detectable by Western blot in lysates of patient PBMCs [3]. We found no positive responses in the 63 patients studied, giving an estimated prevalence of 0% (1-sided 95% confidence interval, 0%–4.7%), providing no evidence of enrichment for XMRV infection in this cohort. As controls, PBMCs were tested with phytohemaglutinin and FEC (influenza, Epstein-Barr virus, and human cytomegalovirus) antigens. Peptides that are 18 amino acids long will bind to both human leukocyte antigen class I and class II, and the assay is designed to detect both cytotoxic T cell (CD8) and T helper cell responses (CD4). Although widely used to detect T cells targeting retroviral peptides, this is the first time ELISPOT assays have been applied for the detection of responses to XMRV, and the result must be interpreted in the context of the lack of PCR-positive samples to act as positive controls. However, patient cells were responsive to other antigens, such as phytohemaglutinin or FEC, suggesting that the lack of responses simply reflects an absence of XMRV Gag-specific T cells in our cohort.

The identification of a previously unreported retrovirus in the human population may have profound implications. The 2 classes of known human retroviruses (HIV-1 and human T-lymphotropic virus) are pathogenic, and murine leukemia virus gene therapy vectors have caused insertional mutagenesis in children treated for X-linked immunodeficiency [14]. In our cohorts of patients with HIV-1 or HCV infection, we found no evidence of XMRV infection in blood, consistent with some of the studies on chronic fatigue syndrome [7–9]. XMRV DNA has been found within malignant prostate tissue, and XMRV can be cultured in a prostate cancer cell line, LNCaP. In vitro studies show that XMRV has an affinity for prostate cancer cells but is much less infectious for other cell types [15]. We are not aware of any studies in which the PBMCs of patients with XMRV-positive prostate cells have also been tested. It is therefore possible that in a compartmentalized infection, XMRV may not be represented in blood, and our data must be interpreted in this light. However, because all the studies of patients with chronic fatigue syndrome have used PBMCs, this is the context in which our study was set.

We hypothesized that if XMRV is present in the United Kingdom or Switzerland and is spread by either sexual or blood-borne routes, we might expect to see it enriched in the blood of patients with HIV-1 or HCV infection. We did not detect XMRV in any patients. Together, the results of PCR reactions for 230 patients and the paired ELISPOT assays are evidence that XMRV is not enriched in patients who are at risk of blood-borne and sexually transmitted infections in the United Kingdom and Western Europe.

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BRIEF REPORT

Xenotropic Murine Leukemia Virus–Related Virus Prevalence in Patients with Chronic Fatigue Syndrome or Chronic Immunomodulatory Conditions

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We investigated the prevalence of xenotropic murine leukemia virus-related virus (XMRV) among 293 participants seen at academic hospitals in Boston, Massachusetts. Participants were recruited from the following 5 groups of patients: chronic fatigue syndrome ($n = 32$), human immunodeficiency virus infection ($n = 43$), rheumatoid arthritis ($n = 97$), hematopoietic stem-cell or solid organ transplant ($n = 26$), or a general cohort of patients presenting for medical care ($n = 95$). XMRV DNA was not detected in any participant samples. We found no association between XMRV and patients with chronic fatigue syndrome or chronic immunomodulatory conditions.

Murine and human xenotropic viruses were first described >30 years ago [1]. The xenotropic murine leukemia virus–related virus (XMRV) was discovered in prostate tissue from patients with prostate cancer who were homozygous for a reduced-activity variant of RNase L [2–4]. RNase L gene abnormalities have been described in patients with chronic fatigue syndrome (CFS) [5], although these findings have not been verified in a more recent investigation [6].

A recent study in the United States found evidence of integrated XMRV DNA in 67% of subjects with CFS, compared with 3.7% of healthy controls [7]. Three subsequent CFS studies in European cohorts have not demonstrated the presence of XMRV DNA in subjects with CFS or healthy controls [8–10]. The explanation for these contrasting results is unknown.

We designed a cross-sectional cohort study to determine XMRV prevalence in a variety of North American clinic populations, including healthy subjects, participants with CFS, and participants with states of chronic immune activation or suppression. We incorporated 3 different polymerase chain reaction (PCR) primer sets from other XMRV studies into our testing strategy to determine whether differences existed in their ability to amplify XMRV DNA.

Methods. Samples were obtained from adult patients presenting to outpatient clinics, or from preexisting repositories and cohorts at Brigham and Women's Hospital, Massachusetts General Hospital, and Dana-Farber Cancer Institute. Participants prospectively enrolled had a prior diagnosis of human immunodeficiency virus (HIV) infection, hematopoietic stem-cell or solid organ transplant, or CFS. This study was powered to detect XMRV prevalence with an upper confidence limit of 1.2% for the combined cohorts.

All CFS participants enrolled met the Centers for Disease Control and Prevention revised CFS case definition (http://www.cdc.gov/cfs/cfsdiagnosis.htm). CFS participants had 10 mL of whole blood collected for peripheral blood mononuclear cell (PBMC) isolation and XMRV PCR testing and completed a questionnaire. Demographic and clinical information were obtained from chart review. Cryopreserved PBMCs and clinical information were obtained from HIV-infected patients from the International HIV Controllers Study [11–12]. Cryopreserved PBMC DNA and demographic data for rheumatoid arthritis participants and an age- and sex-matched cohort of participants who had presented for either inpatient or outpatient clinical care were obtained from the Crimson Biospecimen Core [13]. This study was approved by the relevant Institutional Review Boards.

For XMRV PCR amplification, human DNA was extracted from at least 5×10^6 PBMCs (Qiagen). PCR reaction conditions and primer sets are described in Table 1. First-round PCR amplifications were performed with 1 unit of Platinum

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Table 1. Xenotropic Murine Leukemia Virus–Related Virus (XMRV) and Human β-Globin (hBG) Oligonucleotide Primers and Polymerase Chain Reaction (PCR) Conditions

Reaction, primer	Sequence	Location[a]	PCR conditions
XMRV outer			
Forward outer [7]	5′-ATCAGTTAACCTACCCGAGTCGGAC-3′	424–448	94°C × 2 min; 40 cycles: 94°C × 30 s, 57°C × 30 s, 68°C × 60 s; 68°C × 5 min
Reverse outer [7]	5′-GCCGCCTCTTCTTCATTGTTCTC-3′	1154–1132	
XMRV inner no. 1			
Forward inner no. 1 [8]	5′-GACTTTTTGGAGTGGCTTTGT-3′	446–466	94°C × 2 min; 30 cycles: 94°C × 30 s, 55°C × 30 s, 68°C × 30 s; 68°C × 5 min
Reverse inner no. 1 [8]	5′-ACAGAAGAACAACAAAACAAATC-3′	571–549	
XMRV inner no. 2			
Forward inner no. 2 [4]	5′-TCTCGAGATCATGGGACAGA-3′	603–622	94°C × 2 min; 30 cycles: 94°C × 30 s, 60°C × 30 s, 68°C × 30 s; 68°C × 5 min
Reverse inner no. 2 [4]	5′-AGAGGGTAAGGGCAGGGTAA-3′	1015–996	
hBG			
Forward	5′-TGGTGGTCTACCCTTGGACC-3′	148–162	94°C × 2 min; 40 cycles: 94°C × 30 s, 55°C × 30 s, 68°C × 30 s; 68°C × 2 min
Reverse	5′-GAGGTTGTCCAGGTGAGCCA-3′	296–277	

[a] Locations in XMRV isolate VP62 (GenBank accession no. DQ399707) and hBG (GenBank accession no. NM000158.4) genes. XMRV gag-pro-pol gene starts at nucleotide location 613.

Taq DNA Polymerase High Fidelity (Invitrogen), 80–200 ng of PBMC DNA, and 200 nmol/L of the outer primers described by Lombardi et al (outer primer set) [7]. Nested PCRs were performed with first-round PCR product and 200 nmol/L of the inner primers described by either Erlwein et al (inner primer set 1) [8] or Urisman et al (inner primer set 2) [4]. PBMC DNA from participant S6 was retested using an additional cryopreserved DNA aliquot, the original Platinum Taq HF Polymerase reagents, and USB *Taq* Polymerase (Affymetrix).

Each PCR amplification run included a negative control and positive controls of 10, 100, and 1000 copies of full-length XMRV (isolate VP62), generously provided by Robert H. Silverman (Cleveland Clinic). To test the efficiency of DNA extraction and PCR amplification, increasing amounts of XMRV plasmid, starting at 1 copy, were added to 200 ng of PBMC DNA and to PBMCs prior to DNA extraction. Nested PCR amplification for each experiment was performed in triplicate for each primer set. To verify DNA integrity, human β-globin was amplified from each participant sample with a single-round PCR reaction that used the outer human β-globin primer set described by Erlwein et al [8]. All but 1 sample, from a general clinic participant, demonstrated β-globin amplification. This sample was excluded from our analysis. Laboratory personnel who performed PCR amplification were blinded to the identity of each sample.

For XMRV cloning and sequence analysis, DNA bands near the length of interest were purified with the QIAquick Gel Extraction kit (Qiagen). These amplicons were cloned into a pCR4-TOPO vector (Invitrogen) and were bidirectionally sequenced (ABI 3730 automated DNA sequencer; Applied Biosystems).

χ^2 testing with continuity correction was used to compare intergroup variation between nonparametric variables. Fisher's exact tests were used if expected counts were <5. One-way analysis of variance testing was performed to define intergroup comparisons of continuous variables; a Bonferonni correction was used in pair-wise tests of individual hypotheses (SPSS, version 10; SPSS). To estimate the probability of detecting XMRV in CFS participants on the basis of sample size, the Blyth-Still-Casella 95% exact confidence intervals for disease prevalence were calculated.

Results. PBMC samples from 293 participants were tested for the presence of XMRV DNA (Table 2). Thirty-two subjects who had previously received a CFS diagnosis and 26 patients who had undergone either hematopoietic stem-cell or solid organ transplant from participating outpatient hospital clinics were prospectively enrolled. Samples from 43 HIV-infected subjects were included, either from an existing sample cohort or enrolled prospectively from the Brigham and Women's Hospital infectious disease clinic. Twenty-two of the HIV-infected patients (51%) were virologically suppressed and receiving antiretroviral therapy; the remainder were chronically infected persons who were not receiving treatment. We tested 97 samples from patients with rheumatoid arthritis and 96 samples from age- and sex-matched controls obtained from the Crimson Biospecimen Core.

Participants with rheumatoid arthritis and those from the age- and sex-matched general clinic/hospital cohort had the

Table 2. Demographic Characteristics of Screened Participant Cohorts

Characteristic	Cohort					All subjects
	CFS	HIV	SOT/HCT	RA	RA-matched hospital control[a]	
No. of participants	32	43[b]	26[c]	97	95	293
Age, median years	46	46	56	66	62	58
Female sex, no. (%) of participants	21 (65.6)	8 (18.6)	12 (46.2)	83 (85.6)	84 (88.4)	208 (71.0)

NOTE. CFS, chronic fatigue syndrome; HCT, hematopoietic stem-cell transplant; HIV, human immunodeficiency virus; SOT, solid organ transplant.

[a] DNA obtained from discarded inpatient or outpatient phlebotomy samples, age- and sex-matched with the RA cohort.

[b] Twenty-two subjects currently receiving and 21 subjects not receiving antiretroviral therapy.

[c] Three HCT, 11 kidney, 2 lung, 2 heart, 7 liver, and 1 liver and kidney transplants. One liver transplant patient also had concurrent HIV infection.

highest percentage of women (>80%) within each group. The majority of CFS participants were women (66%), whereas the HIV-infected and hematopoietic stem-cell transplantation or organ transplantation cohorts contained fewer women (19% and 46%, respectively; significant intergroup difference, compared with CFS cohort; $P < .001$). Mean $CD4^+$ T lymphocyte counts for HIV-infected participants receiving or not receiving antiretroviral therapy were similar (566 and 554 cells/μL, respectively; $P = .89$); 95.5% of participants receiving antiretroviral therapy had plasma HIV RNA levels <50 copies/mL. A majority of transplant patients had undergone solid organ transplantation, including 11 kidney, 7 liver, 2 lung, 2 heart, and 1 liver/kidney transplants. One liver transplant recipient had underlying treated HIV infection. The remaining 3 transplant participants had undergone allogeneic hematopoietic stem-cell transplantation.

A majority of participants with CFS had daily symptoms (75.9%), stopped work as a direct result of CFS symptoms (69%), or experienced fever, lymphadenopathy, or swollen glands at the start of their illness (75%); 20.7% of participants had household contacts with similar symptoms or a diagnosis of CFS, and 7% noted a tick bite just prior to onset of their symptoms. The mean reported duration of symptoms for the CFS participants was 11.6 years.

With PCR analysis, we could reliably detect 10 copies of XMRV per 200 ng of PBMC DNA when control XMRV plasmid was added to PBMC aliquots either before or after DNA extraction. We were able to detect 1 copy of XMRV DNA in 2 of 3 assays when the VP62 plasmid was added to extracted PBMC DNA aliquots, a result consistent with the Poisson distribution. With use of the PCR strategy described, we did not detect XMRV *gag* DNA in any of the 293 participant samples. In 7 nested PCR reactions, DNA bands of a size similar to the expected XMRV *gag* PCR product were observed (data not shown). Cloning and sequence analysis of the PCR amplicon from participant S6, a subject with rheumatoid arthritis, demonstrated >99% sequence identity to a mouse endogenous retrovirus that was not XMRV [14]. Multiple repeat PCR amplifications with participant S6 PBMC DNA that used the original reagents, an additional aliquot of DNA, or another commercially available Taq polymerase failed to generate a PCR amplicon; this finding suggests that the original amplicon most likely resulted from contamination. Amplicons from the other 6 nested PCR reactions had sequences matching a portion of the human genome and shared no homology with XMRV.

Discussion. We assessed the prevalence of XMRV in a cohort of 293 American patients with CFS or chronic conditions associated with immune activation and/or immune deficiency and did not detect XMRV in any participant sample. Another report showed that XMRV DNA could be detected in patient samples after a single round of 45-cycle PCR [7]. Our XMRV amplification strategy used similar amounts of input PBMC DNA and identical primer sets as were used in other reports, and we increased the sensitivity of our methods by adding a nested PCR amplification that used 2 additional published XMRV primer sets [4, 7, 8]. These negative findings demonstrate that XMRV was not associated with any specific group that we investigated; the choice of PCR primers did not affect XMRV prevalence estimates. XMRV DNA could be present at levels below our detection threshold. However, we used PCR methodology that was comparable to other published methods that detected XMRV DNA in CFS and healthy control subjects [7, 15].

The upper limit of the 95% confidence interval around our CFS participant XMRV prevalence estimate (0%) was 9.5%. This result is similar to reports from Europe and suggests a far lower rate of XMRV infection, if any, in patients with CFS, compared with the initial report [7]. Regional differences in XMRV prevalence among CFS patients could reflect geographical clustering of XMRV infection and weakens the epidemiological link between XMRV infection and CFS.

To further characterize our CFS cohort and provide a basis of comparison to other CFS groups described in the literature, we administered a 43-item CFS questionnaire. Although our questionnaire may be confounded by recall bias, a majority of our CFS participants reported ongoing symptoms at the time

of study entry. These symptoms had been present for a mean of 12 years and were debilitating enough to cause the majority of participants to stop working.

Endogenous or latent viruses can become activated in patients with altered immune function. We explored the effect that immune activation or suppression could have on XMRV prevalence by including participants with rheumatoid arthritis, HIV infection (both treatment naive and virologically suppressed), and hematopoietic stem-cell and solid organ transplant. We did not identify an association between XMRV prevalence and immune status. Healthy individuals were not included per se in this study and preclude us from drawing conclusions about the prevalence of XMRV in the general population. Our rheumatoid arthritis age- and sex-matched hospital cohort may contain healthy patients that presented for routine clinical care, but we would reasonably expect greater morbidity in this control group, relative to the population at large in Boston. We did detect a mouse endogenous retroviral sequence in PBMC DNA from 1 participant, but we could not replicate this finding. Mouse endogenous retroviral sequences are not present in the human genome; reagent testing did not identify the source of this contaminating sequence.

In summary, we found no evidence of XMRV infection in a cohort of patients cared for at Boston-area hospitals and no association of XMRV with either CFS or chronic conditions with altered immune function. Further research should be performed to define the demographic and geographic distribution of XMRV and to clarify its relationship with chronic fatigue syndrome.

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Note added in proof. A recent study detected a high prevalence of murine leukemia virus–related virus DNA, but not XMRV DNA, in patients with CFS [16]. The outer PCR primers and a nested PCR primer set used in that are report are identical to primers used in our study.

MAJOR ARTICLE

Detection of Xenotropic Murine Leukemia Virus–Related Virus in Normal and Tumor Tissue of Patients from the Southern United States with Prostate Cancer Is Dependent on Specific Polymerase Chain Reaction Conditions

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(See the brief report by Henrich et al, on pages XXX–XXX, the brief report by Barnes et al, on pages XXX–XXX, and the editorial commentary by Kearney and Maldarelli, on pages XXX–XXX.)

Background. *There are questions regarding the prevalence of xenotropic murine leukemia virus–related virus* (XMRV) in patients with prostate cancer and its association with the *RNASEL* R462Q polymorphism. We therefore investigated whether XMRV infection could be found in patients with prostate cancer from the southern United States, and we sought to verify the association with the R462Q.

Methods. Prostate tissue specimens of 144 patients with prostate cancer from the southern United States were genotyped for R462Q by real time polymerase chain reaction allelic discrimination and were screened for XMRV proviral DNA by nested polymerase chain reaction specific for the *env* gene.

Results. The R462Q polymorphism was found at an allelic frequency of 0.33. XMRV was detected in 32 (22%) of the 144 patients. Patients were significantly more likely to test positive for XMRV in both tumor and normal tissue rather than either alone ($\kappa = 0.64$). A positive result for XMRV was not significantly correlated with the R462Q polymorphism ($P = .82$) or clinical pathological parameters of prostate cancer, including Gleason score ($P = .29$).

Conclusions. XMRV is detectable in normal and tumor prostate tissue from patients with prostate cancer, independent of R462Q. The presence of XMRV in normal tissue suggests that infection may precede cancer onset.

Prostate cancer is a leading cancer in men in Western countries, accounting for 25% of incident cancers in American men in 2009 [1, 2]. Despite the high prevalence and gravity of this disease, there are currently few suitable biomarkers to distinguish between cancers with high and low recurrence potentials and to determine whether patients require immediate therapeutic intervention or only periodic observation [3]. Such biomarkers for classifying prostate cancers into different treatment categories may depend on the underlying etiology of each case. Epidemiological evidence suggests that environmental factors, such as diet and infectious agents, may contribute to chronic inflammation of the prostate and tumorigenesis [2]. An infectious etiology for prostate cancer is supported by the linkage of hereditary prostate cancer to the common R462Q polymorphism in the *RNASEL* gene. The polymorphism, which has been reported to be more prevalent among familial patients with prostate cancer, results in a reduced-activity variant of the innate antiviral factor, ribonuclease L [4, 5]. In 1 study, the R462Q polymorphism was implicated in up to 13% of prostate cancer cases [4]. Correspondingly, xenotropic murine leuke-

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mia virus–related virus (XMRV) was discovered by searching for viruses in patients with prostate cancer homozygous for R462Q with a microarray (Virochip) designed to detect the most conserved regions of all viral families [6]. An expanded screen with patients with prostate cancer harboring wild-type *RNASEL* alleles indicated a strong correlation with the R462Q variant, thus establishing a connection between infection and the disease [6].

The linkage of XMRV to prostate cancer through the *RNASEL* R462Q polymorphism has become the subject of controversy because recent reports indicate that infection occurs independent of R462Q [7, 8]. Additional studies are needed to determine whether *RNASEL* genotype is a reliable indicator of susceptibility to XMRV infection. Furthermore, there is no agreement about the cell types infected in the prostate. XMRV was originally discovered exclusively in the nonmalignant stromal and hematopoietic cells adjacent to the carcinoma [6]. By contrast, another study found XMRV primarily in prostate carcinoma cells [7]. Additional studies are therefore needed to determine whether nonmalignant cells are susceptible to infection by XMRV to address whether infection may precede tumor initiation. Questions regarding the association of XMRV with prostate cancer have also arisen in light of recent studies that detect little to no presence of the virus in patients with prostate cancer [9, 10]. Interestingly, studies that detect XMRV in patients with prostate cancer were conducted in the United States, whereas those that do not detect the virus were conducted in Germany. These conflicting reports emphasize the need to confirm the presence of XMRV in prostate cancer and to define the geographic distribution of the virus.

Here, we conducted a retrospective study in which we screened a cohort of patients with prostate cancer that is unique from those of previous studies with respect to its location within the United States. Additionally, we selected for patients with a family history of prostate cancer to enrich for carriers of the R462Q polymorphism. The goals of the study were to confirm the presence of XMRV in patients with prostate cancer and to investigate the linkage of XMRV to the R462Q polymorphism. Here, we demonstrate that XMRV is present in 22% of patients with prostate cancer from the southern United States, that infection does not correlate with R462Q, and that reliable detection of viral DNA was dependent on particular conditions of polymerase chain reaction (PCR). Additionally, we show that XMRV is detectable in both normal and cancer tissues in the prostate, suggesting that the virus does not specifically target transformed cells and that infection may therefore precede cancer onset. If XMRV is shown to promote prostate cancer, it may prove to be a valuable biomarker for clinicians when considering treatment for patients.

METHODS

Prostate cancer cohort and tissue preparation. Frozen prostate tissue cores were obtained from a prostate cancer tissue bank at Baylor College of Medicine. Details of the donor patients have been described elsewhere [3]. All prostate tissues used in the study were derived from patients who underwent radical prostatectomy and had provided consent in accordance with the Baylor College of Medicine Institutional Review Board. No patients underwent preoperative treatment for their cancer. To enrich for carriers of the R462Q polymorphism in *RNASEL*, only tissues from patients having at least 1 first- or second-degree relative diagnosed with prostate cancer were selected for XMRV screening and *RNASEL* genotyping. In total, 144 patients were screened for XMRV and the R462Q polymorphism in *RNASEL*. For 57 of the 144 patients, both normal and tumor tissue were available for screening.

All prostate tissues were prepared for DNA extraction in a separate laboratory from the laboratory in which the infectious XMRV clone VP62 (GenBank accession number, NC_007815.1) was handled. DNA was extracted from sections of prostate biopsies with use of the QIAamp DNA Mini kit (Qiagen). All prostate tissue DNA samples were stored at −20°C immediately following extraction in a laboratory free of amplified or cloned DNA.

RNASEL *genotyping.* All patients were genotyped for *RNASEL* G1385A (R462Q) with use of the Applied Biosystems real-time PCR TaqMan single nucleotide polymorphism assay (Assay ID: C 935391_1_) with TaqMan Universal PCR Master Mix (Applied Biosystems). A 7500 real-time PCR system (Applied Biosystems) was used for amplification and analysis of *RNASEL* genotyping reactions containing 20 ng of prostate tissue DNA. Specimens of predetermined genotypes (homozygous wild-type, heterozygous, and homozygous variant) were used as controls for genotyping reactions. All patients were tested in duplicate.

Provirus screen. A nested PCR assay was developed to screen prostate tissue DNA for XMRV provirus. The first-round primers (5′-ACCAGACTAAGAACTTAGAACCTCG-3′ and 5′-AGCTGTTCAGTGATCACGGGATTAG-3′) amplify an 888-bp region containing the 5′ terminus of *envelope* (*env*). The nested, second-round primers (5′-GAACAGCATGGAAAGTCCAGCGTTC-3′ and 5′-CAGTGGATCGATACAGTCTTAGTCC-3′) amplify a 653-bp region encompassing the 3 variable regions (VR) of *env*, VRA, VRB, and VRC. First-round reactions contained 650 ng of prostate tissue DNA, 2.5 mmol/L $MgCl_2$, 800 μmol/L of dNTPs, 100 ng of each primer, and 1.5 units of AmpliTaq Gold DNA polymerase (Applied Biosystems) in a 50-μL total volume. Two microliters of first-round reactions were transferred to 48 μL of a PCR master mix containing 100 ng of each second-round primer and the same concentrations of each of the components of the first-round reactions. Thermocycling conditions were as follows: 94°C for 5 min; 35 cycles of 94°C for 30 s, 56°C for 1.5 min, and 72°C for 1 min; and ending with 72°C for 10 min. The master mixes for each set of PCR reactions were tested for sensitivity and nucleic acid contamination by incorporation of positive and negative controls, respectively. The positive control consisted of 3 separate reactions, each with 100 ng of DNA isolated from XMRV-infected LNCaP cells diluted 1 to 1×10^3 in uninfected LNCaP cells. The master mix was considered to be adequately sensitive only if all 3 positive control reactions had positive results. Negative controls consisted of 3 separate reactions with H_2O in place of DNA template and 3 separate reactions of 650 ng of uninfected LNCaP DNA. After thermocycling, second-round reactions were electrophoresed on agarose gels containing ethidium bromide and were visualized under ultraviolet light. All tissues were screened in triplicate, and patients/tissues were considered to be positive if ≥1 PCR reactions had positive results.

Cell culture and generation of PCR sensitivity controls. The LNCaP human prostate carcinoma cell line was used to test the sensitivity of the PCR assay and to generate XMRV stocks. XMRV has been shown to infect and replicate within this cell line [11]. LNCaP cells were cultured in RPMI 1640 (Invitrogen), 10% heat-inactivated fetal bovine serum (Sigma), glutamine, and penicillin and streptomycin (Invitrogen) and were incubated at 37°C with 5% CO_2.

To generate PCR sensitivity controls, LNCaP cells were transfected with an infectious XMRV clone (VP62), a generous gift from Robert Silverman (Cleveland Clinic) [11]. One day after transfection, the cells were washed with phosphate-buffered saline and were supplied with fresh media. Two days posttransfection, the conditioned media was passed through a 0.45-μm syringe filter and was used to infect a new stock of LNCaP cells. The infected cells were cultured for 40 days, splitting them 1:10 every 5–7 days. The infected cells and a separate stock of uninfected LNCaP cells were washed with phosphate-buffered saline, trypsinized, and mixed together at ratios of 1:100, $1:1 \times 10^3$, $1:1 \times 10^4$, and $1:1 \times 10^5$ (infected cells:uninfected cells). Without further culturing, total cellular DNA was extracted from the cell mixtures with use of the QIAamp DNA Mini kit (Qiagen). Extracted DNA was used as template to test the sensitivity of the XMRV *env* nested PCR assay.

To test for VP62 plasmid contamination in prostate specimens, a set of 4 primers were designed for nested PCR. The 2 forward primers (5′-TCTGGCTAACTAGAGAACCCACTG-3′ and 5′-AATACGACTCACTATAGGGAGACC-3′) were specific to the multiple cloning site of pCDA3.1(-) (Invitrogen). The 2 reverse primers (5′-AAGGTAACCCAGCGCCTCTTC-3′ and 5′-GTTACGGTCTGTCCCATGATCTC-3′) were specific to the 5′ terminus of VP62 *gag*. The VP62 nested PCR assay was found to be capable of detecting 10 plasmids diluted in 600 ng of uninfected LNCaP DNA in 3 of 3 samples and 1 plasmid in 600 ng of uninfected LNCaP DNA in 1 of 3 samples (data not shown).

Cloning and sequencing of patient-derived PCR products. Positive PCR reactions were electrophoresed on agarose gels and were extracted using the Qiaex II Gel Extraction Kit (Qiagen), according to the manufacturer's instructions. Extracted

Table 1. Summary of *RNASEL* Genotyping and Xenotropic Murine Leukemia Virus–Related Virus Screen

Variable	Screen result
Total no. of patients	144
Positive PCR result, no. (%) of patients[a]	32 (22.2)
R462Q allelic frequency	
Total patients, frequency of R462Q alleles	0.33
Patients with positive PCR result, frequency of R462Q alleles[b]	0.297
Genotypic distribution	
Wild-type RR, no. (%) of patients	66 (45.8)
Heterozygous RQ, no. (%) of patients	61 (42.4)
Homozygous variant QQ, no. (%) of patients	17 (11.8)

NOTE. PCR, polymerase chain reaction.

[a] Patients who had positive results by PCR for xenotropic murine leukemia virus–related virus regardless of tissue type.

[b] R462Q allelic frequency for the 32 patients with positive results for xenotropic murine leukemia virus–related virus DNA.

This figure is available in its entirety in the online version of the *Journal of Infectious Diseases*.

Figure 1. Xenotropic murine leukemia virus–related virus *env* polymerase chain reaction fragment nucleotide sequence alignment.

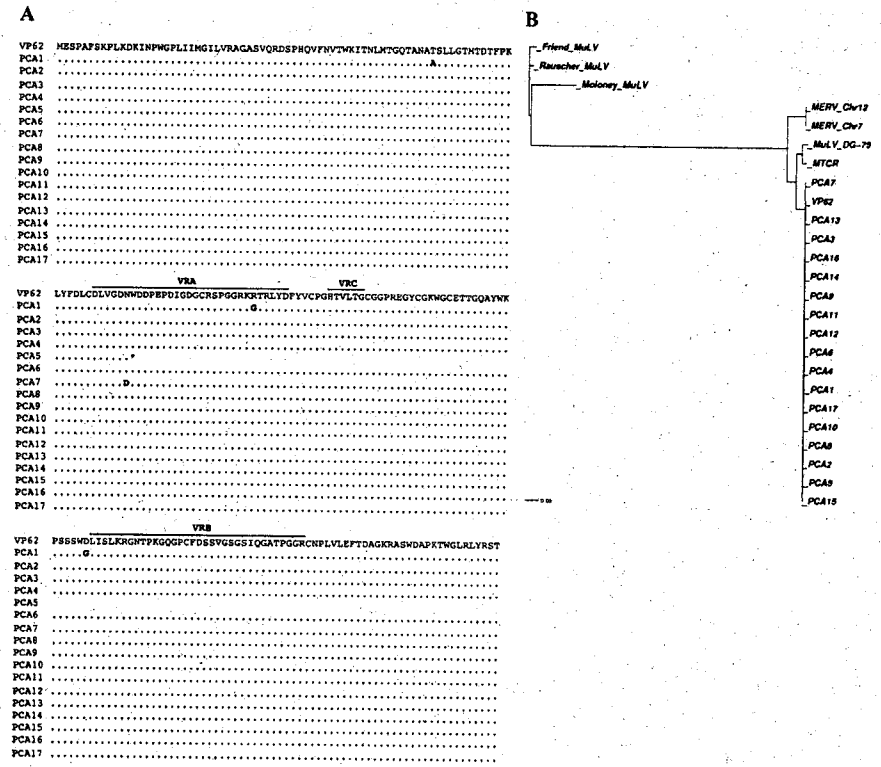

Figure 2. Sequence analysis of patient-derived polymerase chain reaction (PCR) products. *A*, Comparison of the predicted Env protein sequences from the PCR products of 17 patients with the Env sequence of the xenotropic murine leukemia virus–related virus (XMRV) clone VP62; variable regions (VR) A, B, and C are indicated; *dots* indicate identical residues; and a stop codon is indicated by an *asterisk*. *B*, Phylogenetic tree of the XMRV patient clones, compared with other murine retroviruses.

PCR products were cloned into pCR2.1-TOPO with use of the TOPO TA Cloning Kit (Invitrogen), according to the manufacturer's protocol. The cloned PCR sequences were propagated in NEB 10-beta (New England BioLabs) *Escherichia coli* and were isolated with the QIAprep Spin Miniprep Kit (Qiagen), and the sequences of the DNA inserts were determined.

Phylogenetic analysis. *Env* sequences were aligned using Clustal and were trimmed to the same length with gaps. The maximum likelihood tree of *env* sequences was generated using PhyML [12].

Statistical analysis. Statistical analyses were performed using Stata, version 10 (StataCorp). Correlation between XMRV positivity and tissue type was analyzed by measuring the simple kappa coefficient. Correlations between XMRV positivity and Gleason score or seminal vesicle invasion were analyzed using Fisher's exact test. Correlation between XMRV positivity and extracapsular extension or surgical margin invasion was assessed using the χ^2 test.

Accession numbers. Sequences of cloned XMRV *env* genes were deposited in GenBank under accession numbers GU812341–GU812357. Accession numbers from GenBank for other viral sequences are as follows: DG-75, af221065;

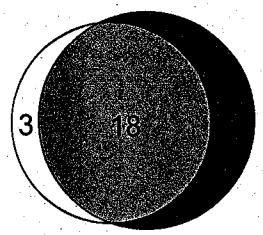

Figure 3. Distribution of xenotropic murine leukemia virus–related virus (XMRV) between normal and cancer tissue of 57 patients with prostate cancer. The *white circle* represents patients from whom XMRV DNA was detected by polymerase chain reaction in normal tissue, the *dark gray* circle represents patients from whom XMRV DNA was detected by polymerase chain reaction in tumor tissue, and the *light gray* overlap represents patients for whom both tissue types had positive results for XMRV DNA. Twenty-nine of the 57 patients had negative results for XMRV DNA. Patients were found to be more likely to have positive results for both tissue types (simple κ coefficient, 0.64).

Raucher MuLV, NC_001819; Friend MuLV, M93134; Moloney MuLV, NC_001501.1; MERV Chr12, ac153658; MTCR, NC_001702; and MERV Chr7, ac127565.

RESULTS

Distribution and frequency of* RNASEL *R462Q. To investigate the linkage of XMRV infection to *RNASEL* R462Q, we obtained prostate tissue specimens from patients with prostate cancer to screen for the virus and the R462Q polymorphism in *RNASEL*. Because XMRV was originally found to be strongly associated with R462Q, we screened patients with prostate cancer with a family history of prostate cancer, which are reported to have an increased R462Q allelic frequency [4–6]. In total, 144 prostate cancer cases were screened by a real-time PCR-based allelic discrimination assay for the R462Q polymorphism in *RNASEL*. We found there to be 66 (45.8%) wild-type (RR) individuals, 61 (42.4%) heterozygotes (RQ), and 17 (11.8%) individuals homozygous for the Q variant (Table 1). The allelic frequency for R462Q was determined to be 0.33, which is intermediate in comparison to other described prostate cancer cohorts of unselected or sporadic cases (allelic frequency of R462Q, 0.25 and 0.38) [4, 7, 10].

XMRV is detected in patients with prostate cancer. A highly-sensitive, nested PCR assay for XMRV *env* was developed to screen patients for XMRV infection. The PCR assay was found to be capable of detecting a single copy of VP62 plasmid (data not shown). We also tested the sensitivity of the PCR assay in the context of integrated provirus. We found that XMRV provirus could be detected at a dilution of 1 infected human prostate carcinoma cell per 1×10^4 uninfected cells in 3 of 3 samples with use of 600-ng of DNA ($\sim 1 \times 10^5$ cells). With the same quantity of DNA, the nested PCR assay was found to be capable of detecting XMRV provirus in 1 infected cell per 1×10^5 uninfected cells in 1 of 3 samples (data not shown). Importantly, assuming XMRV provirus is present at a frequency of 0.15%–1.5%, which has been estimated in other reports, our nested PCR assay is greater than 15–150 times more sensitive than that which is minimally required to detect the virus [6, 7]. Thus, the nested PCR assay is a highly sensitive method to detect XMRV provirus. Each patient specimen was screened in triplicate with use of 650 ng of prostate tissue DNA. In total, 32 (22%) of 144 patients were found to have positive results for XMRV by PCR (Table 1). The majority of tissue specimens that were positive for XMRV had positive results in only 1 or 2 of 3 replicates (data not shown).

To confirm that XMRV was detected, the 653-bp *env* PCR products were sequenced from 17 patients with positive results (nucleotide sequence alignment is presented in Figure 1). In comparison to reference strain VP62, 3 of the 17 sequences encoded nonsynonymous nucleotide differences, representing a total of 5 amino acid differences (Figure 2*A*). With respect to all 17 predicted Env peptide sequences, differences from VP62 were observed at a rate of 0.14%, ranging from 0% to 1.4% (patient PCA1). The high degree of sequence identity with VP62 suggests that positive PCR results for the tissue specimens were attributable to the presence of XMRV DNA. This was confirmed by phylogenetic analysis of the sequences (Figure 2*B*). Additionally, we tested for the presence of contaminating VP62 plasmids in the DNA isolated from the patient tissue specimens with use of a nested PCR assay targeting the pcDNA3.1(-) multiple cloning site–XMRV genome junction. We found no evidence for contamination in specimens with positive results by PCR for XMRV *env* (data not shown).

XMRV is present in cancer and normal tissues. For 57 of the 144 patients, both normal and tumor prostate tissues were available for screening, whereas only tumor tissue was available for the remaining 87 patients. In this subset of patients, XMRV was detected in 21 (36.8%) of 57 normal tissue samples and in 25 (43.9%) of 57 tumor tissue samples. The virus was de-

Table 2. Xenotropic Murine Leukemia Virus–Related Virus Screening by Nested Polymerase Chain Reaction (PCR) for *env*

PCR result	*RNASEL* genotype			Total
	RR	RQ	QQ	
Positive	16	13	3	32
Negative	50	48	14	112
All[a]	66 (24.2)	61 (21.3)	17 (17.6)	144 (22.2)

NOTE. Data are no. or no. (%) of specimens. RR, homozygous wild-type; RQ, heterozygous; QQ, homozygous R462Q variant.

[a] The numbers in parentheses are percentages that indicate the proportion of xenotropic murine leukemia virus–related virus PCR-positive specimens.

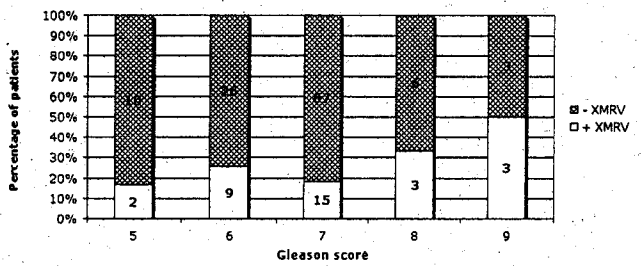

Figure 4. Xenotropic murine leukemia virus–related virus (XMRV) infection is not significantly correlated with Gleason score. Numbers of infected patients (*light gray*) and uninfected patients (*dark gray*) are graphed according to Gleason score. No association between detection of provirus and Gleason score was found ($P = .29$, by Fisher's exact test; $P = .30$, by 2-sample t test).

tected exclusively in the normal tissue of 3 patients and was detected exclusively in tumor tissue of 7 patients, whereas 18 patients had provirus detected in both tissue types (Figure 3). Statistical analysis of these results indicate that patients were more likely to harbor provirus in both normal and tumor tissue rather than one or the other (κ coefficient of agreement, 0.64), suggesting that XMRV does not specifically target tumor tissue in the prostate.

XMRV infection does not correlate with R462Q, Gleason score, or other pathological parameters of prostate cancer. We investigated whether XMRV infection is enriched among carriers of the R462Q polymorphism of *RNASEL* in this cohort. XMRV was detected in 24.2%, 21.3%, and 17.6% of wild-type (RR), heterozygous (RQ), and homozygous variant (QQ) patients, respectively (Table 2). However, infection was not found to be significantly associated with the R462Q polymorphism of *RNASEL* ($P = .82$, by χ^2 test).

XMRV infection is reportedly associated with higher Gleason score prostate cancers [7]. We therefore examined whether XMRV infection correlates with tumor grade. The patients in our study consisted of 12, 35, 82, 9, and 6 patients with Gleason scores of 5, 6, 7, 8, and 9, respectively. Although there appears to be a trend between XMRV infection and increasing Gleason score in Figure 4, no statistically significant association was found ($P = .29$, by Fisher's exact test). Furthermore, we examined whether XMRV infection correlates with seminal vesicle invasion, extracapsular extension, and surgical margin invasion, which are indicators of spreading prostate cancer (Table 3). However, we found no significant correlation between XMRV infection and seminal vesicle invasion ($P = .33$, by Fisher's exact test), extracapsular extension ($P = .59$, by χ^2 test), or surgical margin invasion ($P = .89$, by χ^2 test).

DISCUSSION

Our screen of patients with prostate cancer confirms the presence of XMRV among patients with prostate cancer in the United States. We detected XMRV DNA in normal and tumor tissue, indicating that nonmalignant cells may be susceptible to infection. In agreement with recent studies, we find no correlation between the presence of XMRV infection and the R462Q polymorphism of *RNASEL*, confirming that the population at risk of infection is not confined to homozygous carriers of the Q variant [7, 8].

Interestingly, 3 independent studies, including 2 surveys of German prostate tissue specimens and a screen of English chronic

Table 3. Statistical Analysis of Xenotropic Murine Leukemia Virus–Related Virus Positivity Versus Clinical Pathological Parameters of Spreading Prostate Cancer

	Positive clinical pathology		Negative clinical pathology			
Parameter	PCR positive, no. (%) of patients	PCR negative, no. of patients	PCR positive, no. (%) of patients	PCR negative, no. of patients	Test	*P*
ECE	11 (25.6)	32	21 (21.4)	77	χ^2	.59
SVI	5 (33.3)	10	27 (21.4)	99	Fisher's exact	.33
SMI	8 (23.5)	26	24 (22.4)	83	χ^2	.89

NOTE. Patients either scored positive or negative for clinical pathological parameters of prostate cancer and were either positive or negative by PCR for xenotropic murine leukemia virus–related virus. ECE, extracapsular extension; SMI, surgical margin invasion; SVI, seminal vesicle invasion.

fatigue syndrome patients, found little to no evidence of XMRV infection [9, 10, 13]. However, in agreement with studies performed in the United States, we found the presence of XMRV in prostate cancer tissues [6, 7, 14]. It is possible that XMRV is mostly absent from the European population. If so, it would be interesting to uncover the reason for this geographic distribution. Alternatively, the inability to detect XMRV in Europe may possibly reflect genetic differences between American and European strains. However, this seems unlikely considering the high degree of sequence conservation among XMRV isolates and the variety of primer target sequences used for detection among the studies in Europe [6, 8–10, 13]. Additionally, the failure to detect XMRV may be attributable to differences in the detection techniques employed. We have found that detection of XMRV required rather specific conditions. For instance, at least 600 ng of prostate tissue DNA was necessary for reliable detection with our PCR assay. XMRV was detected in 3.2% of the patients when we initially used 100–140 ng of prostate tissue DNA, compared with 22.2% of the patients when we used 650 ng. Additionally, we found that detection of XMRV from patient specimens, but not from LNCaP cells infected in vitro, depended on the gene targeted in the PCR assay. We were unable to detect XMRV in the patient tissue samples by nested PCR with primers specific for the *gag* and *pol* genes, regardless of whether 100 or 650 ng of DNA was used as template. We found the *gag* primers to be at least 10-fold less sensitive than the *env* primers, and the *pol* primers tended to amplify a competing region from the human genome (data not shown). It is unclear whether these deficiencies account for the inability to detect XMRV in patient samples or whether XMRV is mainly present as an incomplete provirus in the cells of these patients. Nonetheless, the difficulty associated with detecting XMRV in patient samples may perhaps explain studies that do not detect the virus among large cohorts.

We found our nested PCR assay for XMRV *env* to be capable of detecting 1 infected cell per 1×10^5 uninfected LNCaP cells in 1 of 3 samples with use of 600 ng of DNA. The fact that the PCR-positive tissue specimens tested positive in only 1 or 2 of 3 replicates may indicate that XMRV provirus is present at a very low copy number. This interpretation would be consistent with another report [7]. Alternatively, it is possible that the quality of the tissue specimens was low because of preservation, handling, and the duration of storage prior to DNA isolation. However, we were able to genotype the patients for R462Q with use of 20 ng of DNA without difficulty.

Our finding that XMRV can be detected in the normal tissue of patients with prostate cancer suggests that nonmalignant cells may also be susceptible to XMRV infection. If this is correct, XMRV infection may precede and possibly participate in the process of tumorigenesis. There is currently little evidence to suggest that XMRV employs any traditional mechanisms for transforming cells. The virus harbors no known oncogenes, and a clonal integration pattern indicative of insertional mutagenesis has not been observed in prostate cancer specimens. In accord with other studies, we predict a proviral copy number of far less than 1 per cell, arguing against insertional mutagenesis as a mechanism of transformation [6, 7].

A limitation of our PCR-based screen is that it does not identify the infected cell types. It is possible that the XMRV we detected was exclusively from nonmalignant cells, because tumor tissue consists of both malignant and nonmalignant cell types. It is important to note that XMRV may promote tumorigenesis through paracrine and cell-cell interactions. Prostate cancer has been shown to depend on the biology of the surrounding stromal microenvironment, and a reactive stromal phenotype has been shown to promote cancer progression [3, 15–18]. It would be interesting to determine whether XMRV elicits the conversion of prostate stromal cells to a reactive phenotype, regardless of the cell type infected.

We did not find a correlation between XMRV infection and various clinical pathological parameters of prostate cancer, including seminal vesicle invasion, extracapsular extension, and surgical margin invasion. Similar to another report, which found a correlation with higher Gleason scores, we observed a slight trend in favor of increasing Gleason score [7]. However, our results were not statistically significant. Additional studies with a greater number of patients will be required to evaluate a correlation between XMRV infection and Gleason score.

In conclusion, our data support a hypothesis that XMRV is endemic to North America. However, further investigation into the association of XMRV with prostate cancer and other human diseases is needed. If established as an agent of human disease, XMRV may prove to be an important biomarker for selecting a suitable course of treatment.

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文献１４

XMRV: A New Virus in Prostate Cancer?

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Abstract

Several recent papers have reported the presence of a gammaretrovirus, termed "XMRV" (xenotropic murine leukemia virus-related virus) in prostate cancers (PCa). If confirmed, this could have enormous implications for the detection, prevention, and treatment of PCa. However, other papers report failure to detect XMRV in PCa. We tested nearly 800 PCa samples, using a combination of real-time PCR and immunohistochemistry (IHC). The PCR reactions were simultaneously monitored for amplification of a single-copy human gene, in order to confirm the quality of the sample DNA and its suitability for PCR. Controls demonstrated that the PCR assay could detect the XMRV in a single infected cell, even in the presence of a 10,000-fold excess of uninfected human cells. The IHC used two rabbit polyclonal antisera, each prepared against a purified MLV protein. Both antisera always stained XMRV-infected or –transfected cells, but never stained control cells. No evidence for XMRV in PCa was obtained in these experiments. We discuss possible explanations for the discrepancies in the results from different laboratories. It is possible that XMRV is not actually circulating in the human population; even if it is, the data do not seem to support a causal role for this virus in PCa.

Introduction

In 2006, a new retrovirus was reported to be associated with prostate cancer (PCa) (1). It was recognized as a murine leukemia virus (a member of the gammaretrovirus genus), and was termed "XMRV", or "xenotropic murine leukemia virus-related virus". (Murine leukemia viruses [MLVs] are found in mice; xenotropic MLVs cannot infect mouse cells, but can generally infect human cells.)

Association of a virus with this important cancer could have enormous implications for detection, prevention, and treatment of PCa, just as the discovery of the role of human papillomavirus in cervical cancer has revolutionized our approach to this disease. Accordingly, many laboratories have begun testing for the presence of XMRV in PCa patients. Remarkably, the same virus was also reported in patients with chronic fatigue syndrome (CFS) (2).

Retroviruses are relatively simple RNA-containing viruses. Their unique properties include the copying of their RNA into double-stranded DNA at the time of infection ("reverse transcription") and the integration of this DNA copy into the chromosomal DNA of the infected cell. Once inserted, this DNA is replicated with the chromosome, and will thus be present in the cell and its descendants into the indefinite future.

The methods that have been used to detect XMRV include nucleic acid hybridization; PCR and reverse transcription-PCR (RT-PCR); fluorescence in situ hybridization (FISH); immunohistochemistry (IHC); screens for anti-viral antibodies in patient sera; and virus cultivation. These diverse methods have only given concordant

results in a handful of cases. The field was recently reviewed (3), and the state of the science was also covered in a 1 ½ day meeting held on the NIH campus in September, 2010.

XMRV was initially reported to be more prevalent in prostate cancer tissues from men with homozygous germline RNase L mutations (R462Q) than in men without this QQ genotype (1). In this study, both *in situ* hybridization and IHC found the virus in approximately 1% of stromal cells, but not in tumor cells. A subsequent study reported that 14 of 233 prostate tumors were positive for XMRV by real-time PCR and 54 of 233 were positive by IHC (4). Surprisingly, a number of cases were positive by IHC, but negative by PCR. The IHC in this study localized XMRV proteins primarily in malignant epithelial cells rather than stromal cells, and positive staining correlated with high tumor grade. No association with RNase L variants was found in this study. Finally, another study reported that 8 of 20 prostate cancer patients with the QQ RNASEL genotype and 3 of 20 with the RQ or RR genotypes were positive in a serum neutralization assay and that 5 of 7 tested tumors were positive by FISH in a subset of stromal cells; FISH and serum positivity correlated with nested PCR results (5). One strong indication that XMRV has infected some human cells in some prostate tumors is the finding of XMRV sequences integrated into human DNA (6, 7).

In contrast, several studies have reported the absence, or extremely low prevalence, of XMRV in PCa. These include a study of 338 samples representing tumor, normal, and BPH tissues from 200 prostate cancer patients using a highly sensitive nested PCR assay; 105 prostate tumors using nested RT-PCR; 589 prostate tumors using nested

PCR and nested RT-PCR as well as 146 serum samples using an ELISA assay; and 130 prostate tumors and control tissue samples using RT-PCR(8-11).

At this point, it would be hard to overstate the discrepancies between different laboratories on the basic question of whether XMRV is actually present in the human population, and there is no understanding of its possible role in disease. In an effort to resolve the discrepancies concerning the prevalence of XMRV in PCa, we have tested for the virus using both real-time PCR and IHC, with two antisera specific for different viral proteins; our results are described below. Methods are detailed in Supporting Information.

Results

We developed a real-time PCR assay for detection of XMRV sequences in PCa tissues. The quality and concentration of the sample DNAs were confirmed by a duplex PCR procedure, in which the same PCR wells were simultaneously tested for XMRV and for CCR5, a single-copy nuclear gene. For a positive control, we tested the genomic DNA (gDNA) of 22Rv1 cells, an XMRV-infected PCa cell line (12). 22Rv1 gDNA was diluted into 293T or HeLa cell gDNA (typical results shown in Fig. 1). We could routinely detect XMRV sequences in 10 pg of 22Rv1 gDNA (Fig. 1A, blue line), even in the presence of 100 ng or more of background human gDNA. Tests of 1 pg (orange and pink lines) were occasionally positive, but viral sequences were never detected in 0.1 pg of 22Rv1 gDNA (data not shown). Tests with the XMRV plasmid VP62 as standard indicate that there are ~ 15 copies of XMRV per diploid genome in 22Rv1 gDNA, a number similar to that reported by Knouf et al. (12) (data not shown). Thus our assay can always detect ~20 copies of XMRV DNA, and can occasionally detect ~ 2 copies. The

CCR5 assays in the same PCR wells were uniformly positive (Fig. 1A). All assays included parallel tests of 293T or HeLa gDNA alone (XMRV negative control, Fig. 1B, red line) and *E. coli* gDNA (CCR5 negative control, Fig. 1B, green line). Using this duplex assay, we screened DNA from 161 prostatic adenocarcinomas, including 12 that had been micro-dissected and 10 that were metastases (Table 1). In all cases CCR5 was successfully amplified, confirming the quality of the DNA preparation, but there was no amplification from the XMRV primers in any of the cases (Fig. 1C).

Similar real-time PCR assays were also performed on 54 of these samples using a primer-probe set directed at a highly conserved region within the Gag gene. In addition, nested RT-PCR was performed on 41 additional cases using the primer set described in Urisman et al. (1) and nested PCR was used on 24 cases using the primers of Hohn et al. (9). In all of these tests, XMRV sequences could be efficiently detected in the RNA or DNA from infected cells, but no positive results were obtained with any of the PCa samples (data not shown).

The MLV proteins $p30^{CA}$ and $gp70^{SU}$ are cleavage products of the viral Gag and Env polyproteins, respectively. We tested the ability of the MLV30 and MLV70 antisera to react with XMRV proteins. As shown in Fig. S1 in Supplementary Information, both antisera reacted with the expected viral proteins in virus particles (the MLV30 blot shows that some uncleaved Gag polyprotein, $Pr65^{Gag}$, as well as the normal cleavage product $p30^{CA}$, is present in the virus particles). Thus, these antisera cross-react with the corresponding polypeptides from XMRV. Western blots with MLV30 and MLV70 using lysates from 22Rv1 cells were similarly positive (data not shown), but not using lysates

of the human PCa cell lines DU145 and PC3, which were negative for XMRV by PCR (data not shown).

By IHC both sera showed clear and reproducible staining of 293T cells transfected with the VP62 clone of XMRV (Fig. 2B) but never stained 293T cells that had been transfected with an empty plasmid (Fig. 2A). Additionally, both antisera stained 22Rv1 cells (Fig. 2C), indicating that the staining did not require the overexpression typically associated with transient transfection. A total of 596 prostatic adenocarcinomas and 452 benign prostate tissue specimens, prepared either as full tissue sections or as tissue microarrays (TMAs), were analyzed with MLV30 and/or MLV70 (Table 1). Many of the prostatic tissues evaluated from these cancer patients included areas of acute and chronic inflammation, atrophy, benign prostatic hyperplasia and high grade prostatic intraepithelial neoplasia (Table 1). Each experiment included positive and negative controls, which always gave results as in Figs. 2A and 2B. However, no staining of prostate tissue samples was ever observed with either antiserum (Fig. 2D).

Discussion

We used a real-time PCR assay capable of detecting XMRV sequences in DNA from a very small number of infected cells, even in the presence of a vast excess (more than 10,000-fold) of uninfected cell DNA. We also performed IHC with two antisera, each specific for a different MLV protein, under conditions where the sera reproducibly stained XMRV-containing cells but not identically treated control cells. Taken together, the two assays surveyed nearly 800 prostate tumors, including microdissected tumor specimens; metastatic tumor tissue; and intermediate and high-grade primary tumors. No

signs of XMRV infection were found in any of these tests. The results suggest that the prevalence of XMRV in prostate tumors may be far lower than has been reported previously.

How can our negative results be reconciled with the positive reports from other laboratories? It has been suggested that XMRV might be present in North American, but not European, prostate tumors (9). However, our samples, like those of Schlaberg et al. (4), were from North American men. Also, while we did not select RNase L R462Q homozygotes for analysis, the number of cases we examined was high enough to include a substantial number of these individuals. Another possibility is that XMRV was present in our samples, but we failed to detect it because the viral sequences were somewhat different from the published XMRV sequences. While little variation in XMRV sequences has been observed to date (the reported sequences are ~ 97% identical), this could potentially explain our negative PCR results. However, we used several primer sets, some against highly conserved MLV sequences, and still saw no MLV signals. Further, unlike PCR primers, the sera we used in our IHC assays are both broadly reactive, since they were generated using Mo–MLV proteins but reacted with the XMRV proteins in our positive controls (Mo-MLV and XMRV are 82 % identical at the amino-acid level). Thus it seems extremely improbable that sequence polymorphisms can explain our failure to detect XMRV by IHC.

It could also be proposed that infected cells are present at such a low level in virus-positive tumors that the samples we tested were too small to contain infected cells. (Contrary to this, Schlaberg et al. initially reported that positive samples contained 1-10 XMRV copies per 660 cells; 660 diploid cells contain ~ 5 ng of DNA, while we tested

amounts ranging from 25 to 1000 ng (4)). This might explain the negative IHC results with tissue microarrays, but seems unlikely in the >100 tumors for which we analyzed standard slides, which generally contain more than 10^5 cells.

Finally, another conceivable explanation for the staining seen by Schlaberg et al. (4) is that the anti-XMRV serum used in their experiments contains antibodies directed against cellular proteins, in addition to the antibodies against XMRV proteins. The XMRV used as immunogen by Schlaberg et al. was apparently produced in human cells. It is thus difficult to exclude the possibility that human proteins were present in the virus preparation used as immunogen. HIV-1 virus particles are known to incorporate a wide variety of proteins from the virus-producing cells (13), so that these proteins are impossible to remove from the virus; indeed, early vaccine trials with simian immunodeficiency virus were confounded by this phenomenon (14, 15). Incorporation of major histocompatibility complex proteins into MLV particles has also been reported (16). We received PCa tissue sections (kindly provided by Dr Ila Singh, University of Utah) from a number of cases from specimens used by Schlaberg et al. (4). Based on their results with the anti-XMRV antiserum, these samples were predicted to be IHC-positive. However, the sections did not stain with our MLV30 or MLV70 antisera (data not shown). While we cannot fully explain the discrepancies in staining results, Switzer et al. have also demonstrated that under immunoblotting conditions, the anti-XMRV antiserum (4) reacts with proteins in uninfected HeLa cells (17).

Many laboratories have used PCR to detect XMRV in clinical samples. However, the extraordinary sensitivity of this technique magnifies the risk of finding false positives, as well as the ability to find authentic positives. The risk is compounded by the

widespread use of mice in biomedical research. Every mouse cell contains, in its DNA, ~ 100 MLV genomes, termed "endogenous viruses". These genomes reflect past infections of germ cells and the resulting integration of the viral sequences into the mouse germline. As PCR is capable of amplifying and detecting a single molecule of viral DNA, this means that, for example, (depending, of course, on the specificity of the primers) a millionth of a microliter of mouse blood is a potential source of a positive signal in a PCR assay for MLV. Indeed, there are anecdotal reports of false-positive MLV signals ultimately traced to the use of the same microtome blade for cutting mouse and PCa sections, and to the tiny amounts of mouse DNA contaminating the mouse anti-polymerase monoclonal antibody used in commercial "hot start" PCR kits.

The existence of endogenous MLVs may be pertinent to another recent set of observations. In an attempt to reproduce the detection of XMRV in cases of CFS, Lo et al. (18) performed PCR and reverse transcription-PCR on blood samples from CFS patients and healthy blood donors. They obtained positive signals from a high proportion of the CFS cases (and a much lower proportion of the healthy donors). However, when the PCR products were sequenced, they were found to differ from XMRV; thus these results are completely distinct from the reports of XMRV detection. In fact, the sequences match endogenous MLV sequences almost exactly. It should be emphasized that (unlike the studies reporting isolation of XMRV) this report does not include direct evidence for the presence of an infectious virus: the data consisted exclusively of amplification and detection of MLV-like sequences. Notably, the endogenous MLVs that they resemble most closely are defective MLV genomes which do not give rise to infectious MLV. While the authors provided strong experimental evidence arguing

against contamination of their clinical samples with mouse DNA, this remains a possible explanation for their results.

In conclusion, the fundamental question of whether XMRV is really an infectious agent circulating in the human population is still unresolved. This question will not be settled until reproducible assays for the virus are established and validated; in turn this will require exchange of samples and testing of well-characterized standards, followed by cross-comparison of results obtained in different laboratories. Efforts in this direction are now underway at the U.S. National Institutes of Health. However, based on the data presented here, as well as that from other investigators (8-11), we are doubtful that XMRV is commonly found in PCa. Over the years, many claims associating viruses with diseases have turned out to be mistaken (19, 20), and it is still possible that XMRV will fall into this category.

Finally, it is crucial to distinguish the question of the existence and prevalence of XMRV in the human population from the question of its causal role in PCa. In general, gammaretroviruses like XMRV induce malignant transformation by insertional mutagenesis, so that tumors induced by a gammaretrovirus are clones in which all the cells are infected (21). This mechanism of carcinogenesis has been observed not only in laboratory animals, but also in children exposed to gammaretrovirus-derived vectors in gene-therapy trials (22, 23). Although some exceptions to this insertional mutagenesis mechanism have been described (24), the viral genome is present in the transformed cells in all known cases. Thus, infection of an extremely minute fraction of the cells in some prostate tumors, even if confirmed, would seem to be incompatible with the possibility that XMRV plays a causal role in prostate tumorigenesis.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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Table 1. Tabulation of Tissues Assayed

PCR		
Microdissected prostate tumor	12	
Prostate tumor metastasis	10	
Prostate tumor	139	
IHC	**MLV30**	**MLV70**
TMA prostate tumor*	433/1524	433/1524
TMA prostate benign*	437/1890	437/1890
TMA prostate tumor metastasis*	52/121	52/121
Full sections prostate tumor	38	111
Full sections prostate benign	5	15

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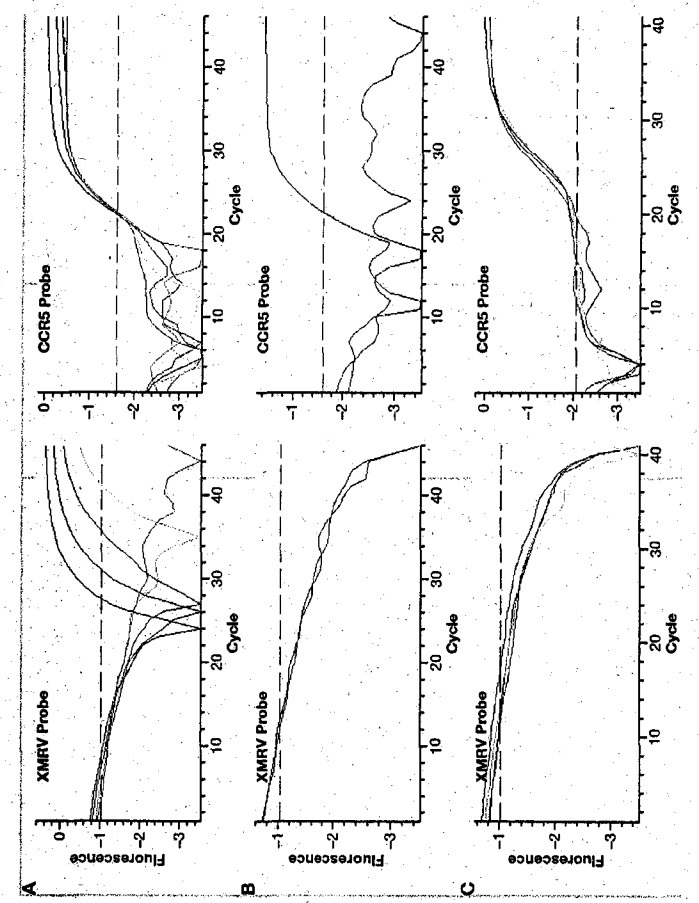
* Numbers shown are total number of cases / total number of TMA spots analyzed. Multiple TMA spots (typically at least 4) were analyzed per case.

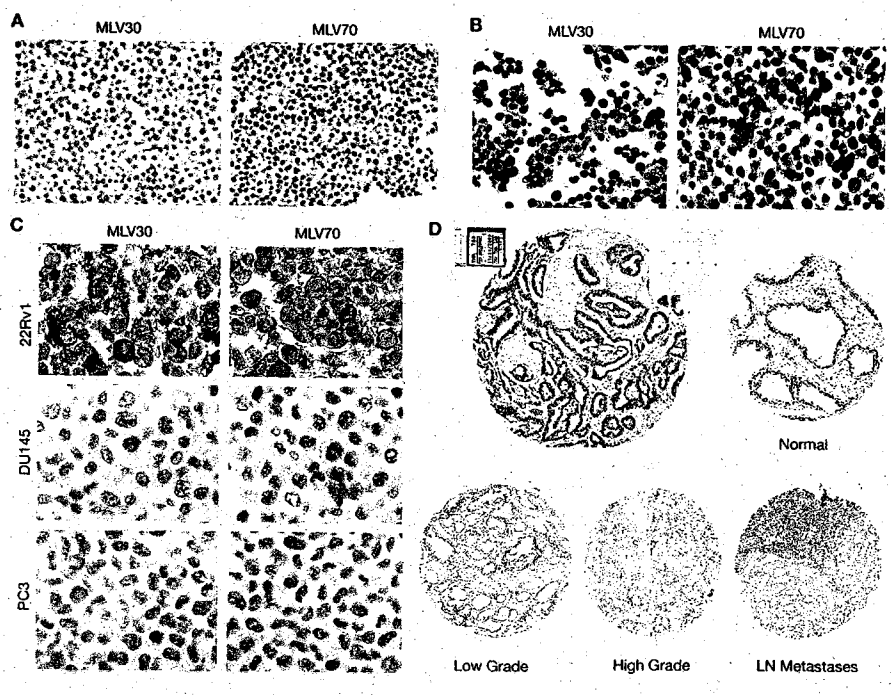
Table 1. Specification of prostate tumor samples tested. The Table shows the number of cases tested by either PCR or IHC.

Figure Legends

Figure 1. Duplex real time PCR. (A) XMRV positive control: wells contain 1ng (red), 0.1ng (green), 0.01ng (blue) or 0.001ng (orange/pink) of 22Rv1 gDNA in 100ng of HeLa gDNA. (B) XMRV and CCR5 negative controls: wells contain 100ng of HeLa gDNA (red) or 100ng of E. coli gDNA (green). (C) Typical sample data: wells contain 100ng of DNA from 4 different prostate tumors.

Figure 2. Immunohistochemistry with MLV30 and MLV70 antisera. (A) Antisera do not stain 293T cells transfected with pcDNA3.1. (B) Antisera stain 293T cells transfected with VP62 XMRV. (C) Antisera stain 22Rv1 cells, but do not stain DU145 and PC3 cells. (D) Typical sample data. Top left, example of TMA and typical TMA spot. No antisera staining was observed for normal prostate, low-grade PCa, high-grade PCa, or lymph node metastases. Examples shown were stained with MLV30 antisera.




A
MLV30
MLV70
B
MLV30
MLV70
C
MLV30
MLV70
22Rv1
DU145
PC3
D
Normal
Low Grade
High Grade
LN Metastases

資料 4－2

諸外国における慢性疲労症候群罹患者に対する献血制限について

平成 22 年 11 月現在

1．現時点において、XMRV 感染リスクに対する予防的措置として、既往歴も含め、慢性疲労症候群罹患者に対する献血制限の実施が確認されている国

カナダ（除くケベック州）・・別添１
オーストラリア・・別添２
ニュージーランド・・別添３

なお、イギリスは、現時点では慢性疲労症候群と XMRV との関係を示す疫学的エビデンスはないとした上で、ドナーの健康確保の観点から、既往歴も含めた献血制限を実施している（別添４）。

2．献血時に健康であることを前提とした上で、現時点において、慢性疲労症候群の既往歴まで含めた献血制限は勧告・実施していない国

米国（FDA）（注）・・別添５
カナダ・ケベック州・・別添６
日本

（注）なお、AABB（米国血液銀行協会）は、慢性疲労症候群の既往がある方の献血の辞退を促すよう、会員に対し自主的に勧告している。（別添７）

その他の欧州諸国については、現在調査中。

（血液対策課調べ）

別添１

Indefinite Deferral for History of Chronic Fatigue Syndrome

Canadian Blood Services is undertaking a deferral to protect blood product recipients from any potential risk that could come from a link between Xenotropic Murine Leukemia Virus-Related Virus (XMRV) and Chronic Fatigue Syndrome (CFS). XMRV is a type of retrovirus originating in mice ("murine" relates to mice).

Although the media is reporting that XMRV may be a threat to the blood supply, the deferral Canadian Blood Services is undertaking at this point relates to those patients with a history of CFS only. At this point there is no evidence that XMRV causes any disease in humans. This new information has reported association, but not causality.

Today, donors who have a history of CFS and who are well again are allowed to donate blood. Under the new deferral, it is this group that will no longer be able to donate blood at Canadian Blood Services' clinics. Blood donors with a history of CFS represent a very small segment of Canadian Blood Services' donor base, so the impact on the blood supply will be minimal.

Donors with active cases of CFS don't usually come in to donate blood because they are not feeling well. Historically, however, Canadian Blood Services has allowed people with a history of the illness to donate. This is what will change with the new deferral.

Health Canada, the body that regulates Canadian Blood Services, has approved this deferral. Implementation will occur in late April.

It is important to note that the available data related to the link between XMRV and CFS is conflicting. While it has been reported to have a strong association in American patients, the finding has not been substantiated in patients in the UK or the Netherlands, suggesting some geographic differences in the pattern of virus spread. Furthermore, there are as yet no data confirming that XMRV causes disease. So at this time, it is not possible to quantify the risk a donor with a history of CFS could pose to a blood recipient.

Once the scientific community understands more about the role of XMRV or other viruses in relation to chronic fatigue, Canadian Blood Services will revisit the deferral decision to determine whether the deferral is still warranted. Canadian Blood Services is part of an inter-agency North American task force led by the American Association of Blood Banks (AABB) that is investigating the XMRV issue.

How Canadian Blood Services currently handles potential threats to the blood supply system:

Canadian Blood Services operates one of the safest blood systems in the world. An essential element of our commitment to safety is our multilayered approach to ensuring that our blood products meet the highest level of safety available.

Before they donate, donors are asked an extensive list of questions about their behaviour and about their health status. People, who are unhealthy, including those with symptomatic diseases, are deferred from donation.

The organization then subjects each and every donation to a variety of blood screening tests for pathogens that are known to be transmissible by blood transfusion including HIV and the hepatitis B and C viruses.

Canadian Blood Services also maintains strong international networks with other blood systems to monitor the behaviour of possible pathogenic threats to the blood supply, so that if a new pathogen appears we can be ready to respond to the threat.

Canadian Blood Services
Société canadienne du sang

別添２

Australian Red Cross
BLOOD SERVICE

Published on *Australian Red Cross Blood Service* (http://www.donateblood.com.au)

Home > News & Events > Blood Service updates CFS donor policy

Blood Service updates CFS donor policy

23/04/2010
The Blood Service has decided to indefinitely defer donors with Chronic Fatigue Syndrome (CFS).

The Australian Red Cross Blood Service will indefinitely defer donors who have been diagnosed with Chronic Fatigue Syndrome (CFS).

This follows recent research, describing a possible link between chronic fatigue, and a retrovirus called Xenotropic Murine leukaemia virus-related Virus (XMRV).

As the Blood Service currently defers donors who have CFS, this change will delay their return to donating until there is more scientific literature on the possible viral link.

The number one priority of the Blood Service remains the safety of Australia's blood supply.

Blood Service specialist, Dr Tony Keller, said eligibility to donate is always a balance between risk and benefit.

"There is at present no test available for CFS or XMRV, but our donor questionnaire alerts us when someone has CFS. Very few donors will be affected by this decision," Dr Keller said.

"The science on this internationally is unclear. The recent North American research findings haven't been supported by research undertaken in Europe, and there is currently no Australian research on XMRV.

"We will review our decision in two years time, when further studies into the virus have been done."

The Blood Service currently has 570,000 donors a year. In the past two years, there have been only 70 donors deferred due to Chronic Fatigue Syndrome.

We are writing to a small number of donors to notify them of this change.

National News & Events

別添３

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Detailed Eligibility Criteria and FAQ's

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Why should I donate blood?
The Donation Process
Different ways to donate
Corporate Blood Donors
Detailed Eligibility Criteria & FAQs
Make an appointment to donate
Information Leaflets for Donors
Information Videos for Donors

Antibiotics - I am taking antibiotics. Can I donate?
Accidents – I was involved in an accident and had stitches or other treatment. Can I donate?
Acne – I have active acne. Can I donate?
Acupuncture – I have just had acupuncture. Can I donate?
Addiction – Drugs. Can I donate if I have every injected or taken drugs?
Age - How does age affect my ability to donate?
Alcohol - I had several alcoholic drinks before going to give blood. Can I donate?
Allergy – I am allergic to one of the following: dust / a food / a medicine / an insect sting / other. Can I donate?
Anaemia - I have been anaemic. Can I donate?
Angioplasty – I have had an angioplasty. Can I donate?
Antibiotics – I am taking antibiotics. Can I donate?
Antidepressants - I take an antidepressant. Can I donate?
Arrhythmia – I have abnormal heart beats or I am being treated for an abnormal heart beat. Can I donate?
Arthritis – I have arthritis. Can I donate?
Asthma – I have asthma. Can I donate?
Bleeding disorder – I have been diagnosed with a bleeding condition/disorder. Can I donate?
Blood borne diseases - what is tested for?
Blood pressure - I take high blood pressure medicine. Can I donate?
Blood transfusion – I have had a blood transfusion. Can I donate?
Blood volume - What is the volume of blood in a person's body?
Body piercing - I have just had a part of my body pierced. Can I donate?
Breast-feeding - I am breast-feeding. Can I donate?
Cancer – I had cancer. Can I donate?
Chicken pox – I have chicken pox. Can I donate?
Childbirth - How long after the birth of my baby. Can I donate?
Cholecystectomy – I have had my gall bladder removed. Can I donate?
Cholecystitis – I have had cholecystitis recently. Can I donate?
Cholesterol - I take medication for cholesterol reduction. Can I donate?
Chronic fatigue syndrome - I have/had chronic fatigue syndrome. Can I donate?
People with a diagnosis of Chronic Fatigue Syndrome are permanently deferred from donating blood in New Zealand.
Coeliac Disease – I have Coeliac Disease. Can I donate?
Cold sores - Can I donate if I have a cold sore?
Colds - I have a cold. Can I donate?
Concussion – I was knocked unconscious. Can I donate?
Condoms What if I use Condoms Every Time?
Conjunctivitis – I have conjunctivitis. Can I donate?
Contraceptive pill – I take birth control pills. Can I donate?
Corneal Graft – Corneal transplant. I have had a corneal transplant. Can I donate?
Correctional Institutions - Why doesn't the NZ Blood Service collect blood from inmates of correctional institutions?
Crohn's Disease – I have Crohn's Disease. Can I donate?
Cystitis – I have had cystitis recently. Can I donate?
Cytomegalovirus (CMV) infection – I have been diagnosed with cytomegalovirus infection. Can I donate?
Deep vein thrombosis (DVT) – I have had a deep vein thrombosis in a leg. Can I donate?
Dengue fever - I had dengue fever. Can I donate?
Dental treatment - I have just been to the dentist. Can I donate?
Depression – I am being treated for depression. Can I donate?
Dermatitis – I have dermatitis. Can I donate?
Diabetes – I am diabetic. Can I donate?
Diarrhoea - I have diarrhoea. Can I donate?
Disability – I have a physical disability. Can I donate?
Diverticulitis/diverticulosis – I have diverticulitis or diverticulosis. Can I donate?
Drug use (recreational) - Can I still donate blood even if I have taken recreational drugs?
For men only - I have just had my ears pierced. Can I still donate blood?

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別添4

Media Statement

NHS Blood and Transplant

8 November 2010

MS033/10

ME/CFS sufferers permanently deferred from giving blood

From 1 November 2010, people with Myalgic Encephalitis/Chronic Fatigue Syndrome (ME) were permanently deferred from giving blood in the UK.

The change to donor selection guidelines, which applied across all four UK Blood Services, was as a result of recommendations by the UK Blood Services Standing Advisory Committee on the Care and Selection of Donors, and Joint Professional Advisory Committee (JPAC).

In the past, donors with a history of ME/CFS could give blood, provided they had completely recovered and were feeling well.

However, as ME/CFS is a condition where people can relapse and become ill again, donor selection guidelines were changed as a precaution to protect the donor's safety by ensuring the condition is not made worse by donating blood. There is no evidence that a donation from a donor with this condition could in any way harm a patient.

This change brought donor selection guidelines for ME/CFS into line with other conditions where individuals are permanently excluded from blood donation to protect their own health.

Ends

For further information, please contact the NHSBT press office on 0117 969 2444, at pressoffice@nhsbt.nhs.uk or out of hours on 07659 133583.

Notes to Editors

- Donor selection guidelines relating to donor safety are recommended by the UK Blood Services Standing Advisory Committee on the Care and Selection of Donors, and Joint Professional Advisory Committee (JPAC)
- The change to donor selection guidelines for ME/CFS applies across all four UK Blood Services – NHS Blood and Transplant (NHSBT) for England and North Wales; the Scottish National Blood Transfusion Service (SNBTS); the Welsh Blood Service (WBS); and the Northern Ireland Blood Service (NIBTS)

- NHS Blood and Transplant (NHSBT) is a Special Health Authority in the NHS. It is the organ donor organisation for the UK and is responsible for matching and allocating donated organs. Its remit also includes the provision of a reliable, efficient supply of blood and associated services to the NHS in England and North Wales
- In October 2009 a study from the United States suggested a link between the virus XMRV and Chronic Fatigue Syndrome. This was reviewed and discussed in the relevant advisory committees. Further studies by the Centres for Disease Control in the US and a number in Europe have failed to demonstrate a link between XMRV infection and CFS. Currently there is no epidemiological evidence of a link between XMRV and CFS in the UK. The research on XMRV has been considered by the relevant UK Blood Services/DH advisory committees; there is no current evidence of a threat to public health in the UK; and this will be kept under review by those committees in the light of any new evidence.

別添５

U.S. Department of Health & Human Services

FDA U.S. Food and Drug Administration

Home > Vaccines, Blood & Biologics > Safety & Availability (Biologics)

Vaccines, Blood & Biologics

New study on the detection of murine leukemia virus-related virus gene sequences in the blood of patients with chronic fatigue syndrome (CFS) and healthy blood donors - Questions and Answers

Questions and Answers

1. What are murine leukemia viruses?

Murine leukemia viruses (MLV) are retroviruses known to cause cancer in certain mice. In 2006, investigators found that a type of MLV, called xenotropic murine leukemia virus-related virus (XMRV), could potentially infect humans. XMRV is one of a number of MLVs that appear to be transmitted to humans.

2. What is CFS?

Chronic fatigue syndrome (CFS) is a debilitating disorder defined solely by clinical symptoms and the absence of other causes. It's unknown what causes CFS.

3. Has MLV or XMRV previously been associated with CFS or other disease?

A previous study, published in the journal [Lombardi et. al. *Science* October 23, 2009 326: 585], reported finding XMRV in a high percentage of CFS patients and a small percentage of healthy blood donors. However, other studies conducted in the U.S., Netherlands, and UK did not detect evidence of XMRV or other MLV-related viruses in CFS patients.

XMRV was first identified in tissue samples from some prostate cancer patients in 2006. However, one subsequent study failed to find XMRV in prostate cancer tissues, and another study found the virus only rarely in such tissues.

4. What did the new study evaluate?

Investigators from the Food and Drug Administration's (FDA) Center for Biologics Evaluation and Research, the National Institutes of Health (NIH) Clinical Center, and Harvard Medical School have published a study in the scientific journal *Proceedings of the National Academy of Sciences* that examines the presence of MLVs in blood collected from two groups -- patients diagnosed with CFS and healthy blood donors.

This study tested blood samples collected from the New England area in the mid-1990s from 37 patients diagnosed with CFS, as well as samples from 44 healthy blood donors collected in the Clinical Center Blood Bank, NIH, between 2003 and 2006. Investigators performed DNA sequencing on each sample that produced positive product for verification of MLV-like gene sequences. Diverse MLV gene sequences, similar to that of the recently discovered XMRV, were identified in samples from 32 of the 37 patients with CFS (86.5%) and 3 of the 44 (6.8%) healthy blood donors that were tested.

Follow-up samples were collected from 8 of the CFS patients in 2010, and 7 of these again tested positive for MLV-like gene sequences.

5. What did the new study conclude?

This study supports a previous investigation[Lombardi et al. *Science* October 23, 2009 326: 585]that showed XMRV, a genetic variant of MLV-like viruses, to be present in the blood of people with CFS. The study demonstrates a strong association between a diagnosis of CFS and the presence of MLV-like virus gene sequences in the blood. The study also showed that MLV-like viral gene sequences were detected in a small fraction of healthy blood donors. Although the statistical association with CFS is strong, this study does NOT prove that these retroviruses are the cause of CFS. Further studies are necessary to determine if XMRV or other MLV-related viruses can cause CFS.

6. Are there studies that support different conclusions?

Some previous studies from the United States (including a study by the Centers for Disease Control and Prevention), the United Kingdom and the Netherlands reported finding no evidence of XMRV or other MLV-related infections in people with CFS. These different findings could be caused by a variety of factors (for example, difference in study populations), and underscore the need for additional studies and standardized methods.

7. Can MLV or XMRV be transmitted by blood or tissue products?

Additional research is needed to investigate the possibility that these MLV-related viruses and XMRV may be transmitted by blood or human tissue and are capable of causing disease. Investigators at FDA, NIH, CDC and other scientific institutions are in the process of conducting studies to verify the capabilities of the tests used by the different laboratories for the detection of XMRV or MLV-related viruses in blood. These studies are intended to develop and standardize a highly sensitive and specific XMRV test to better study its association with disease, as well as the possibility that XMRV can be transmitted to blood or tissue recipients.

8. What are the implications for blood donors?

At present, FDA does not have a donor policy specific to XMRV or other MLVs. There is currently no evidence that XMRV or MLVs are transmitted by transfusion in humans or that XMRV or other MLVs cause human disease. FDA regulations require that donors be in good health at the time of donation.

9. Does FDA agree with the AABB recommendation to discourage donation by people with history of CFS?

FDA does not object to the AABB recommendation. The AABB recommendation is consistent with a long-standing position of the Chronic Fatigue and Immune Dysfunction Syndrome (CFIDS) Association of America that individuals with CFS voluntarily should not donate blood.

10. How are the differences between the CDC and FDA study results being evaluated?

Differences in the results could reflect differences in the patient populations that provided the samples. Alternatively, undefined differences in the method of sample preparation could be contributing to the discordant test results. All of the scientists involved are working collaboratively to design experiments to quickly answer this scientifically puzzling question. An independent investigator at the National Heart, Lung, and Blood Institute (NHLBI) set up a test set of 36 samples, including known positives and presumed negatives. Both the FDA/NIH and CDC labs participated in this test, and the results showed that both labs were able to detect XMRV present at low levels in blinded samples. Additionally, the CDC laboratory provided 82 samples from their published negative study to FDA, who tested the samples blindly. Initial analysis shows that the FDA test results are generally consistent with CDC, with no XMRV-positive results in the CFS samples CDC provided (34 samples were tested, 31 were negative, 3 were indeterminate).

11. What do these findings mean to CFS patients and clinicians who treat them?

Although this study found MLV-like viral gene sequences in a high percentage of CFS patients, this does not prove that these retroviruses are the cause of CFS or of any other disease. Moreover, other studies have not found evidence of such retroviruses in patients with CFS. Further studies are necessary to determine if XMRV or other MLV-like viruses are reproducibly associated with CFS, and if so whether the virus is a causative agent or a harmless co-traveler. The different findings from various studies reinforce the need for more research--including careful analysis of other cohorts of CFS patients from different geographic regions, studies of larger populations of healthy people, and testing of transmissibility of the agents through blood transfusions in animal models. FDA, NIH, and CDC have and will continue to collaborate with other agencies and groups involved in this research.

別添6

Hema-Quebec 86th Board meeting summary on XMRV

Tuesday, June 15, 2010

8.6.1 XMRV

The Vice-President, Medical Affairs presented the recommendation of the SAC and the RRAC. For many years now, Héma-Québec has accepted donors with a history of chronic fatigue syndrome (CFS) if they feel well on the day of the donation. As a result of the recent report of an association between CFS and XMRV (xenotropic murine leukemia virus-related virus), Héma-Québec management has decided to re-examine this criteria. The diagnostic criteria for CFS were described briefly. This syndrome is not new. Its manifestations have been reported for a long time. However, its etiology remains unknown. XMRV was also described. Its epidemiology and means of transmission remain unknown at present. A recent study identified a good proportion of people suffering from CFS as carriers of the XMRV. Subsequently, three other studies were unable to find positive subjects. In scientific circles, the first study is contested. Furthermore, the conflicting results of these studies cannot be clearly explained. These conflicting results were then discussed. It was also noted that there is no medical evidence demonstrating that CFS is transmitted by transfusion. However, some organizations have already taken measures in this respect. Specifically, the AABB recommends indefinitely prohibiting donors who have been diagnosed as infected with the XMRV. In the United States, the CFS Advisory Committee recommended prohibiting blood donors with CFS, although no measure has been announced by the FDA. As for the CBS, it has decided to prohibit donors with a history of CFS on a permanent basis (only if the information is provided spontaneously by the donor; no question is asked systematically). Australia and New Zealand have adopted the same measures as the CBS. The risk management options have been reviewed by the advisory committees and, for the reasons mentioned below, the option of the status quo is recommended by the SAC and the RRAC:

- **CFS is not an emerging disease.**
- **Although several micro-organisms have been studied, no etiological link has been established between them and CFS.**
- **Specifically in terms of XMRV, only one of the four studies found a link with CFS.**
- **Symptomatic donors (with an active illness) are already prohibited.**
- **There is no evidence that CFS is transmitted through transfusion.**

It was also mentioned that the Management Committee tracks XMRV at each meeting.
It was moved, duly seconded and unanimously resolved **to maintain the selection criteria for chronic fatigue syndrome (CFS), namely to accept donors with a history of CFS if they feel well on the day of the donation.**

http://www.hema-quebec.qc.ca/hema-quebec/profil/conseil-administration/compte-rendua/2010/juin10/index.en.html

別添７

AABB > Press Room > Recommendation on Chronic Fatigue Syndrome and Blood Donation

Recommendation on Chronic Fatigue Syndrome and Blood Donation

The AABB Interorganizational Task Force on Xenotropic Murine Leukemia Virus-Related Virus reviewed the risk of transfusion transmission of XMRV by individuals with chronic fatigue syndrome (CFS). The task force presented its recommendations to the AABB Board of Directors, which approved an interim measure intended to prevent patients with a current or past diagnosis of CFS from donating blood or blood components.

AABB released an Association Bulletin today recommending that, as an interim measure until further definitive data are available, its member blood collectors, through the use of donor information materials available at the donation site, actively discourage potential donors who have been diagnosed by a physician with CFS [also known as chronic fatigue and immune dysfunction syndrome (CFIDS) or myalgic encephalomyelitis (ME)] from donating blood or blood components.

The task force includes representatives from the blood community, patient advocacy representatives, XMRV subject matter experts and liaisons from several government agencies, including the Office of the Assistant Secretary for Health, the Centers for Disease Control and Prevention, the Food and Drug Administration and the National Institutes of Health.

AABB member institutions are required to follow all federal regulations regarding donor eligibility. At present, there are no specific regulations for deferral of individuals with diseases or syndromes that have been linked to XMRV.

AABB appreciates all individuals who want to donate blood but strongly urges that only those who are eligible and healthy do so.

Last updated: June 18, 2010

RESOURCES

- AABB XMRV Fact Sheet
- CDC XMRV Fact Sheet
- Association Bulletin #10-03 : Chronic Fatigue Syndrome and Blood Donation (member content)

資料 4−3

「慢性疲労症候群」（Chronic Fatigue Syndrome ; CFS）について

関西福祉科学大学　倉恒弘彦

【概要】

慢性疲労症候群（Chronic Fatigue Syndrome; CFS）とは、健康に生活していた人が風邪などに罹患したことがきっかけとなり、それ以降原因不明の強い全身倦怠感とともに、微熱、頭痛、筋肉痛、思考力の低下、抑うつ、不安などが長期に続いて健全な生活が送れなくなるという病態であり、CDC（米国疾病対策センター）により 1988 年に提唱された比較的新しい疾患概念である。

【患者数】

1999 年の厚生労働省研究班（班長：木谷照夫、大阪大学医学部）による疫学調査（名古屋地区 4000 名を対象，有効回答数 3015）では一般地域住民の約 0.3%がＣＦＳに該当していた。2004 年の文部科学省研究班（代表研究者：渡辺恭良、大阪市立大学）による疫学調査（大阪地区の一般地域住民を対象、有効回答数 2742）でも約 0.3%がＣＦＳに該当しており、日本における 15-65 歳のＣＦＳ患者数は約 24 万人と推定される。

【症状】

慢性的な疲労感とともに、発熱、リンパ節腫大、咽頭痛などの感染症様症状、頭痛、筋肉痛、関節痛、脱力感などの膠原病様症状、睡眠障害、思考力低下、抑うつ、不安などの精神・神経症様症状などの多彩な症状が認められる。

【原因】

種々の生活環境ストレスによって引き起こされた神経・内分泌・免疫系の変調に基づく病態であり、免疫力の低下に伴って種々のウイルスの再活性化が惹起され、これを制御するために産生されたインターフェロン（IFN）などのサイトカインが脳・神経系の機能障害を生じていると思われる。

【治療】

確実に有効な治療法は確立していないが、以下の治療法が試みられる。

抗酸化療法（ビタミンＣ大量、CoQ10 など）、免疫賦活療法（漢方薬など）、向精神薬（SSRI、抗うつ薬、抗不安薬など）、精神療法（認知行動療法）

日本における CFS と XMRV との関係について

関西福祉科学大学　倉恒弘彦

目的：昨年より米国で問題になってきた CFS と XMRV 感染症との関係を日本においても明らかにするため、以下の検討を行った。

対象：大阪市立大学医学部疲労クリニカルセンターに通院中の CFS 患者 100 名
（木谷研究班 CFS 診断基準、CDC の CFS 診断基準を満たす患者）

方法：

1. 抗体検査：XMRV のウイルス粒子（タンパク質）を抗原として、検体中の抗体の有無をイムノブロッティング法により解析した。
2. DNA 検査：末梢血単核球から DNA を抽出し、XMRV DNA の有無を genomic-PCR 法により解析した。
3. 上記解析は、京都大学ウイルス研究所の 2 カ所の研究部門（宮沢先生、小柳先生）、大阪府赤十字血液センター研究部（古田先生）の 3 カ所に血液検体を送付して実施した。

結果：

1. CFS 患者において XMRV の Gag カプシド蛋白に対する抗体が 100 例中 2 名に認められたが（陽性率　2.0%）、健常者 500 名の陽性率 1.6%と比較して有意な差は認めなかった。また、その他のウイルス蛋白に対する抗体は認められなかった。

2. XMRV DNA については、上記 PCR 解析で陽性例は認めなかった。

結論：

現時点の調査結果からは、日本においての CFS と XMRV 感染症との関係は認めなかった。しかし、今回用いた検査法の感度を高めると検出される可能性も否定できないため、引き続き調査研究を行う必要がある。

資料 5

研究開発等における血液製剤の使用に関する指針の策定について

1．背景

血液製剤は献血により得られる血液を原料とする貴重なものであり、「安全な血液製剤の安定供給に関する法律（昭和 31 年法律第 160 号）」においても、その適正な使用が求められている。血液製剤は主として患者の治療のために用いられるが、その他、輸血の有効性・安全性の向上のための研究、検査試薬製造及び品質管理試験等にも用いられている。「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（平成 20 年厚生労働省告示第 326 号）」においては、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ことが国に求められているところである[1]。

また、平成 21 年度第 1 回薬事・食品衛生審議会血液事業部会（平成 21 年 12 月 24 日開催）において、血液型判定試験に用いる試薬には現在輸入血液が用いられており、日本人特有の不規則抗体等を測定するには不十分であるため、血液安全の観点からも、国内血を用いた検査試薬の開発等を進めるべきとの意見も出されたところである。

かかる状況を踏まえ、今般、研究開発等における血液製剤の使用に関する指針の策定の作業を開始することとしたい。

2．現状

現在、献血で得られた血液のうち、検査等により血液製剤として不適合となった血液や、期限切れの血液製剤及び検体残余血液が、品質管理試験や研究開発等に用いられている。以下に平成 21 年度の使用実績を示す。

【本数】

	全血製剤	赤血球製剤	血漿製剤	血小板製剤	計
品質管理試験等	64	58,557	13,204	4,483	76,308
原料血漿	44	0	1,191	13,574	14,809
研究開発等	28	7,990	2,373	1,333	11,724
計	136	66,547	16,768	19,390	102,841

（本）

【容量】(*1)

	全血製剤	赤血球製剤 (*2)	血漿製剤	血小板製剤	検体残余血液	計
品質管理試験等	19.40	15965.74	3339.66	928.51	0.00	20253.31
原料血漿	16.20	0.00	294.90	1715.03	0.00	2026.13
研究開発等	10.80	2036.02	574.59	271.95	2282.62	5175.98
計	46.40	18001.76	4209.15	2915.49	2282.62	27455.42

(L)

*1：診療報酬の算定容量をもとに算出した。

*2：解凍赤血球製剤及び冷凍血は含まれていない。

使用者：日本赤十字社、大学、企業等

この他、血液センターで実施している ABO 血液型検査及び不規則抗体検査で使用する血球試薬については、血液型抗原を網羅する必要等もあることから、血液製剤としての規格に適合した血液を使用している。なお、これまで各血液センターで調製していた血球試薬については、本年度より試薬製造メーカーへ原料血液を提供し、製造委託している。

3．指針の骨格案及び今後の進め方について

別紙に指針の骨格案を示す。今後、本ペーパーを血液事業部会運営委員会でご審議いただいた上で、血液対策課において、必要に応じ省内関係部局と調整して指針案を作成し、再度血液事業部会運営委員会でご審議いただく。運営委員会で了承された後、血液事業部会でご審議いただき、了承が得られれば、通知として発出する。

[1] 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。

（別紙）

「研究開発等における血液製剤の使用に関する指針」骨格案

I. 基本的な考え方

II. 使用目的

a. 輸血の有効性・安全性の向上を目的とした使用について

① 研究開発
② 品質管理試験
③ 検査試薬
④ 疫学調査
⑤ その他

b. 広く国民の公衆衛生の向上を目的とした使用について

① 研究開発
② 検査試薬
③ 製薬
④ 疫学調査
⑤ その他

c. その他の目的のための使用について

III. 使用する血液

① 検査等により不適合となった血液
② 期限切れ血液
③ 検査用検体残余血液
④ 白血球除去フィルター内残余血液
⑤ 保管年限（11 年）を越えた保管検体
⑥ 血液製剤としての規格に適合する血液
⑦ 研究開発等を目的とした採血によって得られた血液

IV. 使用者について

① 日本赤十字社
② 公的機関（国立研究機関等）
③ 大学等の非営利機関
④ 民間企業
⑤ その他

V. 献血者への説明と同意について

VI. 血液の譲渡手続きについて

① 譲渡量に応じた手続きについて
② 有償・無償のあり方、費用負担等について

VII. 使用量について

① 需給との関係について

VIII. その他

① 個人情報の扱いについて
② 疫学調査の実施に係る指針について

平成 22 年 11 月 24 日
薬事・食品衛生審議会
血液事業部会運営委員会
提　出　用　資　料

資料６－１

日本赤十字社

血小板製剤に対する感染性因子低減化（不活化）技術の導入準備について

-① 10 単位血小板製剤の品質に及ぼすリボフラビン法処理の影響-

1. 目的

前回、リボフラビン法で処理した血小板の活性化による品質の低下について報告したが、我が国においては血小板製剤出荷本数の 8 割以上を 10 単位血小板製剤が占めていることから、感染性因子低減化血小板製剤を実用化するためには、低減化処理した 10 単位製剤の品質低下を出来る限り抑制する必要がある。そこで、リボフラビン法処理に最適な 10 単位製剤の条件について確認試験を実施した。

2. 実験方法

製剤容量、血小板濃度、血小板総数を 10 単位製剤の規格（血小板数:2×10^{11} 個以上、製剤容量:200±40mL）の範囲内で様々に変化させた検体（n=15)を調製し、リボフラビン法処理直後から 5 日目まで試験した。ただし、下限容量はリボフラビン法の下限である 170mL とした。

なお、リボフラビン法処理は、実際の製造を考慮して、採血の翌日（ただし、採血後 22 時間以内）に実施した。

3. 測定項目

pH（22℃）、二酸化炭素分圧（pCO_2）、酸素分圧（pO_2）、グルコース濃度、乳酸濃度、平均血小板容積（MPV)、低張液ショック応答（%HSR)、PAC1 結合率、スワリングスコア

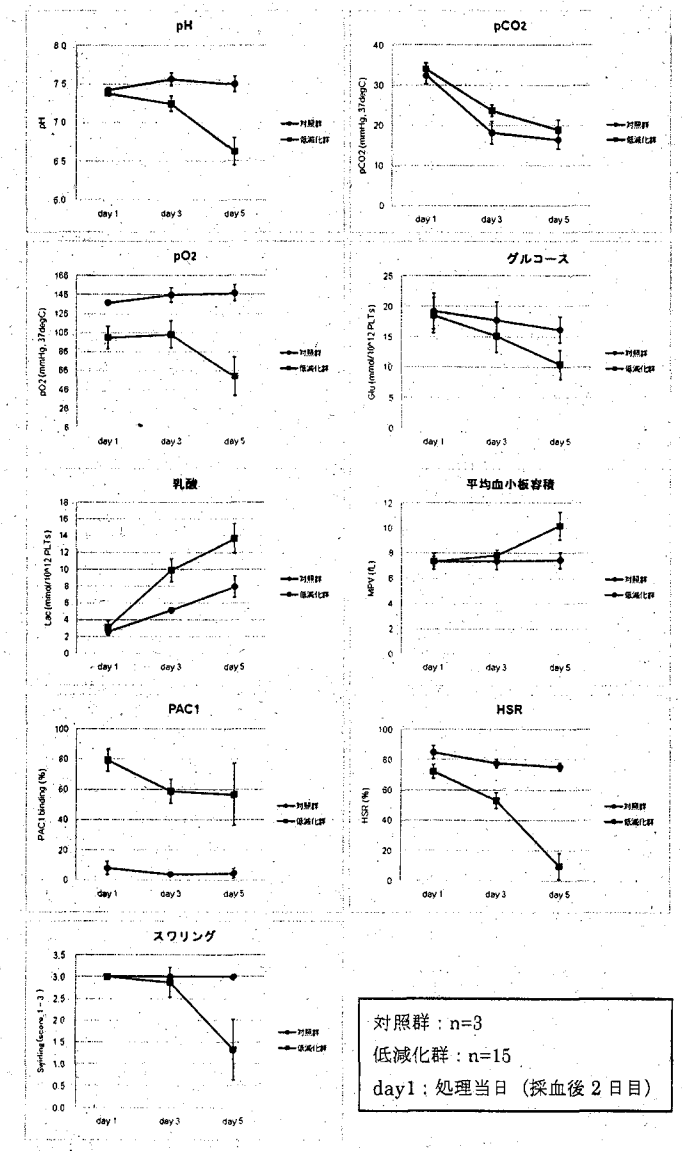
4. 結果及び考察

結果を図 1、図 2 に示す。

処理後 3 日目（採血後 4 日目）までは多くの測定項目で良好な値を示した。それ以降は品質が低下する傾向を示したが、処理後 5 日目（採血後 6 日目）の検体でも、血小板保存の指標とされるpH6.4 より高い pH が保持されていた（図 1)。

一方、低減化した血小板製剤の容量、血小板濃度、血小板総数を指標として品質の変化を比較したところ、血漿量の少ない検体ほど品質が低下する傾向がいくつかの測定項目で認められた（図 2)。この傾向は、血小板濃度や総血小板数では認められなかった。

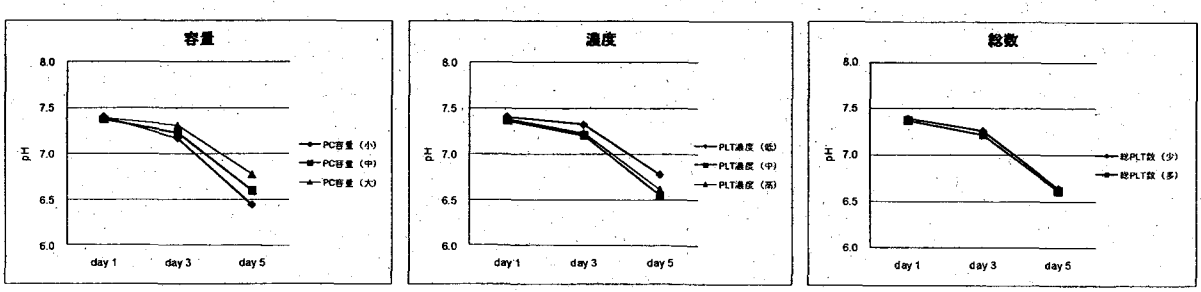
以上より、リボフラビン法を 10 単位製剤に導入する際は、血漿量を多めに設定することにより品質の低下が抑制され、現状の製剤と同じ有効期間（採血後 4 日間）を十分に確保できるものと考えられた。



対照群：n=3
低減化群：n=15
day1：処理当日（採血後 2 日目）

図 1　低減化処理後の血小板の品質

①pH



②平均血小板容積（MPV）

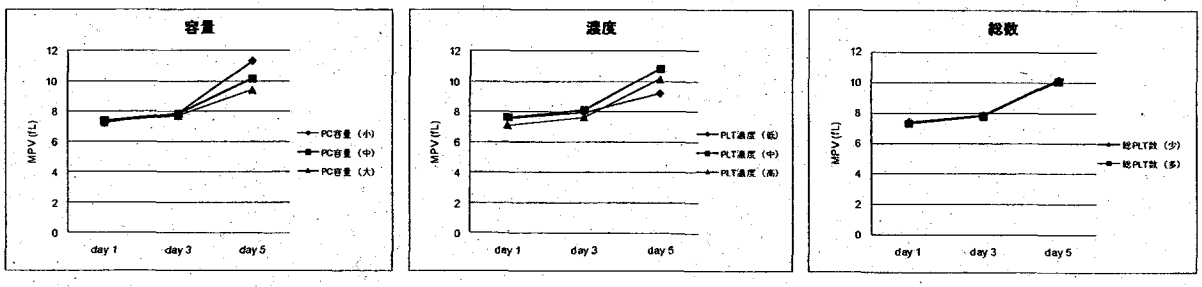


図２　低減化処理後の血小板の品質に及ぼす製剤容量、血小板濃度、総血小板数の影響
（図１の低減化群（n=15）を製剤容量、血小板濃度、血小板総数により 2-3 群に分けてプロット）

3

-② リボフラビン法処理後の白血球の増殖-

1. 目的

前回、リボフラビン法処理後の白血球の増殖について、PHA 及び抗 CD 抗体を用いて検討した結果を報告したところ、MLR（混合リンパ球培養反応）法でも検討すべきとの意見があった。そこで、PHA 及び抗 CD 抗体に加え MLR によりリボフラビン法処理後の白血球の増殖について検討した。

2. 実験方法

PHA、抗 CD 抗体及び同種白血球により刺激した白血球の増殖を、ブロモデオキシウリジン（BrdU）の取り込みで評価した。

3. 結果及び考察

結果を図 3-1～3 に示す。

いずれの系においても、リボフラビン法で処理（Mirasol 処理）した検体の BrdU の取り込みは、コントロールの X 線照射と同等以上に抑制されており、増殖能も同様に消失しているものと推察された。

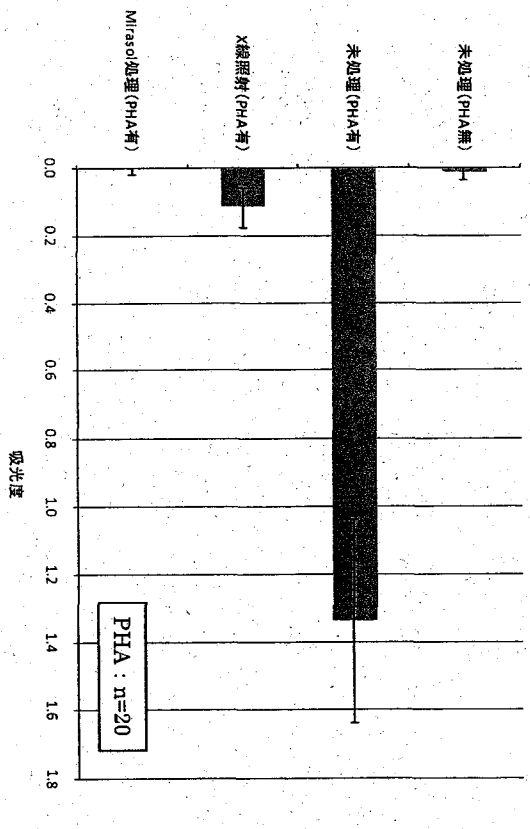


図 3-1　PHA 刺激による白血球の増殖

4

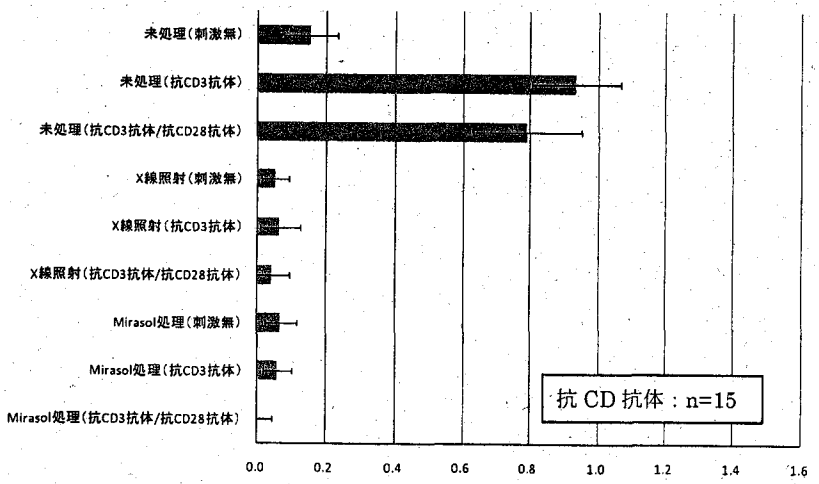


図 3-2 抗 CD 抗体刺激による白血球の増殖

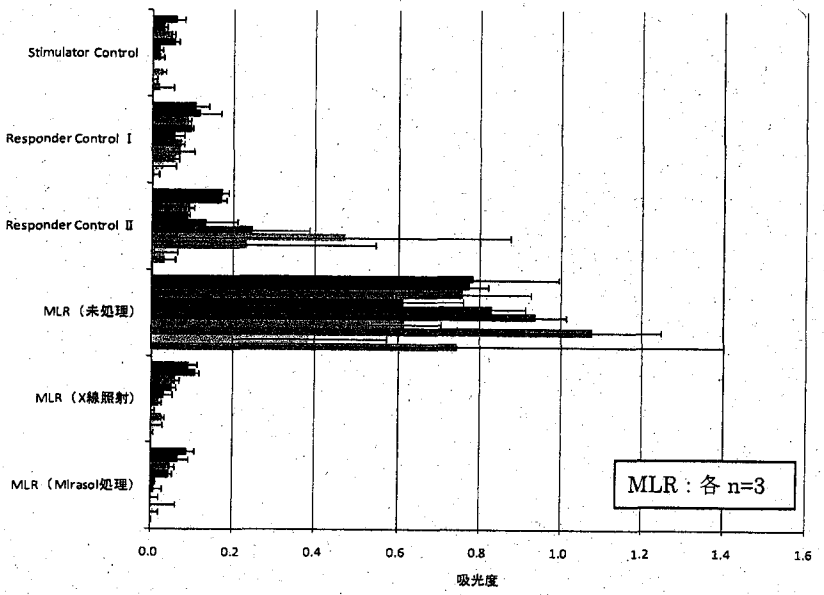


図 3-3 同種白血球刺激による白血球の増殖(MLR)

平成22年11月24日
薬事・食品衛生審議会
血液事業部会運営委員会
提出用資料

血小板製剤に対する感染性因子低減化血小板の臨床試験の概要

資料６－２

	リボフラビン法	アモトサレン法		
臨床試験名	MIRACLE	euro SPRITE	SPRINT	HOVON 82
試験実施国	フランス	オランダ・英国・フランス・スウェーデン	米国	オランダ
試験依頼者	Caridian BCT社	Cerus社	Cerus社	Sanquin Blood Bank
試験デザイン	非盲検、ランダム化、並行群間非劣性比較試験	二重盲検、ランダム化比較試験	二重盲検、ランダム化、非劣性並行群間試験	非盲検、ランダム化、非劣性比較試験
PC製造方法	成分採血/バフィーコート由来PC	バフィーコート由来PC	成分採血由来PC	バフィーコート由来PC
主要エンドポイント	CCI1hr	CCI1hr、CI1hr	WHOグレード2出血率	CCI1hr
副次エンドポイント	CCI24hr、出血、輸血間隔、血小板輸血回数(/患者) 赤血球輸血回数 不応状態の徴候	CCI24hr、CI24hr、止血状態、赤血球輸血単位数、PC輸血不応状態出現率及びPC輸血間隔	WHOグレード3,4出血率、 WHOグレード2出血日数、 CCI1hr、CCI24hr、 血小板輸血間隔、血小板輸血回数、赤血球輸血回数等	CCI24hr、出血、赤血球及びPC輸血の必要量、PC輸血間隔及び輸血副作用
エンドポイントの判定に使用した基準	WHO出血グレード *CTCAE Ver.3*	WHO出血グレード	WHO出血グレード	WHO出血グレード CTCAE
非劣性の確認条件	PRT処理PCのCCI_{1hr}平均値が未処理PCのCCI_{1hr}平均値からその20%を減じた値を下回らないこと		グレード2の出血をきたした全患者の割合に関して事前に設定した非劣性限界値を12.5%として検定し、$p<0.05$であること グレード3又は4の出血をきたした全患者の割合に関して事前に設定した非劣性限界値を7%として検定し、$p<0.05$であること	PC-PRT-PASⅢ群のCCI_{1hr}平均値がPC-血漿群のCCI_{1hr}より20%未満である場合
被験者数(人) 対照群	PC-血漿:58(解析対象:54)	PC-PAS:51 (対照群の一部は100%血漿)	PC-血漿:解析対象327	PC-血漿群 :99
被験者数(人) 被験群	PRT-PC:60(解析対象:56)	PRT-PC:52	PRT-PC:解析対象318	PC-PASⅢ群:94 PC-PRT-PASⅢ群:85
PC輸血期間	ランダム化時を0日とし、最長28日間	最長56日、これに28日の観察期間を加え、1サイクルとした	28日の輸血期間に、7日間の調査期間を加えた	最長42日間
PC輸血回数(回)	対象群:238 PRT処理群:303	対象群:286 PRT処理群:390	対象群:2041 PRT処理群:2678	PC-血漿群:357 PC-PASⅢ群:381 PC-PRT-PASⅢ群:391
PC保存条件	100%血漿	血漿:PAS=35:65 (対照群の一部は100%血漿)	血漿:PAS=35:65 (対照群は100%血漿)	血漿:PAS=35:65 (PC-血漿群を除く)
平均PC輸血間隔	対象群:2.30±1.48日 PRT処理群:2.16±1.69日 (p=0.2903)	対象群:3.4±1.21日 PRT処理群:3.0±1.23日	対象群:2.4日 PRT処理群:1.9日 (p <0.001)	PC-血漿群[1]:81±47 PC-PASⅢ群[1]:77±44 PC-PRT-PASⅢ群[1]:61±47
低減化処理PCのCCI(被検群vs対照群)				
CCI 1hr	11,005vs16,614	13,100±5,400vs14,900±6,200	11,100vs16,000	11,400±5,300vs17,100±7,300
CCI 24hr	7,162vs10,070	7,400±5,500vs10,600±7,100	6,700vs10,100	7,900±5,300vs12,800±7,800
出血状況の解析	グレード2～4の出血はPRT群で12例(グレード4は2例)、対照群で7例(グレード4は1例)を認めたが、本試験ではデータが不十分であったことから、出血リスクについての結論は示さないこととされた	血小板輸血後の出血性有害事象の発生率は、PRT群、対照群間で有意差を認めなかった	両群間でグレード2の出血比率に有意差を認めなかった。 両群間でグレード3,4の出血比率に有意差を認めなかった	PC-PRT-PASⅢ群は他の2群と比較するとき、出血発生件数及びグレード2以上の出血発生件数が有意に高かった($p<0.034$)
結 論	CCI1hr、CCI24hrとも非劣性であることは確認できなかった 血小板及び赤血球の使用量に有意な群間差は見られなかった	保存5日以内に輸血した場合、血小板減少症患者における支持療法の効果は、従来の血小板製剤と同等であった	PRT群では対照群と比較して、輸血後の血小板増加数が少なく、輸血間隔が短かったが、グレード2の出血発生率は等しかった	PC-血漿と比較するとき、PC-PRT-PASⅢは輸血効果に関連する全エンドポイントで劣性を示した
論 文	*Transfusion 2010; 50: 2362-2375*	Blood. 2003;101:2426-2433	Blood. 2004;104:1534-1541	Brit. J. Haemat. 2010;150:209-217

1):時間表示。日数に換算すると右の通り　PC-血漿 :3.4±2.0
PC-PASⅢ:3.2±1.8
PC-PRT-PASⅢ:2.5±2.0

【略号】

PC	血小板製剤
PRT-PC	感染性因子低減化処理済血小板製剤
PAS	血小板用添加液
CI	血小板増加数(Count increment)
CCI	補正血小板増加数(Corrected count increment)
CTCAE	有害事象共通用語基準(Common Terminology Criteria for Adverse Events)

Transfusion : Early View 2010 Nov.

Meta-analysis of the randomized controlled trials of the hemostatic efficacy and capacity of pathogen-reduced platelets

BACKGROUND: A recent independently funded randomized controlled trial (RCT; Br J Haematol 2010; 150: 209 - 17) questioned prevailing opinion concerning the hemostatic capacity of pathogen-reduced platelets (PLTs). Meta-analysis was used to calculate the effect of pathogen reduction (PR) of PLTs on hemostatic efficacy and capacity based on all available data and to investigate possible reasons for the variation in reported findings.

RESULTS: Studies were statistically homogeneous in all analyses. Pathogen-reduced PLTs were associated with a significant ($p < 0.05$) reduction in 1- and 24-hour posttransfusion corrected count increments (summary mean difference, 3260; 95% confidence interval [CI], 2450-4791; and summary mean difference, 3315; 95% CI, 2027-4603) as well as a significant increase in all and in clinically significant bleeding complications (summary odds ratio [OR], 1.58; 95% CI, 1.11-2.26; and summary OR, 1.54; 95% CI, 1.11-2.13). The frequency of severe bleeding complications did not differ.

CONCLUSION: The results of the recent RCT are not inconsistent with those of the earlier studies. Introduction of PR technologies in their current stage of development would result in an increase in mild and moderate (albeit not severe) bleeding complications, which the *transfusion-medicine community* must explicitly tolerate to reap the benefits from PR.

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感染性因子低減化血小板製剤の止血効果と能力に関する無作為化比較臨床試験のメタアナリシス（仮訳）

背景：最近の独自の資金による無作為化対照臨床試験（RCT：Br J Haematol 2010; 150:209-17）は、感染性因子低減化血小板（PLTs）の止血能力に関して広く受け入れられている意見に疑問を呈した。利用可能なすべてのデータに基づき、感染性因子低減化（PR）が及ぼす血小板（PLTs）の止血効果と能力への影響を評価し、論文間で結果が変動する要因について検討するため、メタアナリシスにより分析した。

結果：各報告の結果はすべての分析で統計的に一様であった。感染性因子低減化PLTsの輸血後1及び24時間の補正血小板増加数は有意（$p < 0.05$）な減少(summary mean difference, 3260; 95% confidence interval [CI], 2450-4791; and summary mean difference, 3315; 95% CI, 2027-4603)を示したのと同様に、全ての及び臨床的に意味のある（軽~中等度）出血性合併症も有意に増加(summary odds ratio [OR], 1.58; 95% CI, 1.11-2.26; and summary OR, 1.54; 95% CI, 1.11-2.13)した。重篤な出血性合併症の頻度に差は認められなかった。

結論：最近のRCTの結果は、以前の研究のものと矛盾していない。現在発展段階にあるPR技術の導入は、軽度および中等度（重篤ではないが）の出血性合併症の増加をもたらすため、輸血医療コミュニティはPRからの恩恵を享受するためには、このことを容認しなければならない。

血小板製剤への感染性因子低減化技術の適用に関する試験計画

試験名（国名）	IPTASP（イタリア）	PREPARES（オランダ）	PRESS（デンマーク）
低減化技術名	リボフラビン法 アモトサレン法	リボフラビン法	リボフラビン法
試験デザイン	ランダム化 単純盲検 非劣性試験	ランダム化 単純盲検 非劣性試験	ランダム化、 クロスオーバー試験
試験目的	リボフラビン・アモトサレン両法で処理したPCの品質比較試験	リボフラビン法処理PCが非劣性であることの確認	リボフラビン法処理PCの保存期間延長時の安全性、効果確認
実施期間	2010年11月開始 2012年終了予定	2010年11月開始 2014年終了予定	2010年9月開始 2011年終了予定
被験者数 対照	各210	309	40
PRT-PC	各210	309	40
PC保存 対照	両法による低減化処理PC共PAS保存	血漿	PAS
条件 PRT-PC		血漿	PAS
エンドポイント又は評価項目	Primary：≥WHOグレード2の出血発生率 Secondary：有害事象、CCI、HLA抗体等	Primary：≥WHOグレード2の出血合併症（5日保存内）Secondary：≥WHOグレード2の出血発生率、CCI1・24hr、輸血間隔等（7日保存内）	・トロンボエラストグラフィーのパラメーター変化・CCI・輸血間隔・SAEの発生率・出血率及び分類 PART Ⅰ：2-3日保存、PART Ⅱ：7日保存

血小板製剤への感染性因子低減化技術の適用に関する市販後調査及び観察研究の報告

試験名（国名）	イタリア・スペイン・ベルギー・ノルウェー・ドイツ	スペイン・ベルギー・フランス	フランス（レユニオン島）	ベルギー	フランス（アルザス）	ノルウェー
低減化技術名	アモトサレン法	アモトサレン法	アモトサレン法	アモトサレン法	アモトサレン法	アモトサレン法
試験デザイン	単一コホート研究（ヘモビジランス）	ヘモビジランス	後向き観察試験	後向き観察試験	後向き観察試験	前向き観察試験
試験目的	アモトサレン法で処理したPCの安全性確認	アモトサレン法で処理したPCの安全性確認	アモトサレン法で処理したPCの安全性確認	アモトサレン法で処理したPCの有効性確認	PAS保存アモトサレン法処理PCの有効性確認	10名の化学療法中の急性白血病患者における血小板輸血の治療効果確認
実施期間	2003.10-200512	2005.5-2007.1	2006.3-2007.3	対照：2001.1-2003.9 PRT-PC：2003.1-2006.10	Ⅰ期：2003.1-2004.2 Ⅱ期：2005.9-2006.6 Ⅲ期：2006.9-2007.8	
被験者数 対照				629	Ⅰ期：2,050 Ⅱ期：1,678	
PRT-PC	651	1,400	427	721	Ⅲ期：2,069	10
性別（M/F）	385/262[1]	858/542	262/165	M：F≒62：38（%）	Ⅰ期：M 59%, 3-97y	4/6
年齢	61.2±17.0	60.0±17.8	42.4±24.8[3]		Ⅱ期：M 60%, <1-99y	21-62y
					Ⅲ期：M 62%, <1-106y	
PC保存 対照				100%plasma	Ⅰ期：100% plasma	PAS[2]
条件 PRT-PC	PAS[2]	PAS[2]	PAS[2]	PAS[2]	Ⅱ期：PAS[2]	PAS
					Ⅲ期：PRT処理PAS[2]	
エンドポイント						$CCI_{1hr, 24hr}$ WHO 出血グレード TEGパラメーター
低減化処理PCのCCI PRT処理群 vs PRT未処理群						CCI_{1hr} 5,300±2,700 vs 9,200±4,100 CCI_{24hr} 1,800±4,400 vs 5,800±4,600 （PRT未処理PC：25Gyγ線照射）
結 論	PRT処理PC、5,106回の輸血のうち、99.2%にPCに起因する副作用を認めなかった	PRT処理PC、7,437回の輸血のうち、99.3%にPCに起因する副作用を認めなかった	PRT処理PC、1,950回の輸血のうち、99.5%にPCに起因する副作用を認めなかった	PRT処理PC、1,950回の輸血のうち、99.3%にPCに起因する副作用を認めなかった	PAS保存したPRT処理PCの有効性に特段の問題はない。有害事象の発生率低下を認めた	PRT未処理PCと比較するとき、PRT処理PCでは血小板数、品質の低下がみられ、CCI1hrでは有意な差を認めた
論 文	Transfusion 2008;48:1061-1071	Vox Sanguinis 2008;94:315-323	Transfusion 2009;49:1083-1091	Transfusion 2009;49:1412-1422	Transfusion 2010 first online publication	Transfusion 2010;50:766-775

1）：性別未登録者を含む
2）：40%前後のplasmaを含む
3）：小児51名、幼児（<1y）41名を含む。全員を対象とした年齢範囲は1-87歳
4）各期の輸血回数：Ⅰ期-10,629回、Ⅱ期-9,151回、Ⅲ期-13,241回

A randomized controlled clinical trial evaluating the performance and safety of platelets treated with MIRASOL pathogen reduction technology

*The Mirasol Clinical Evaluation Study Group**

BACKGROUND: Pathogen reduction of platelets (PRT-PLTs) using riboflavin and ultraviolet light treatment has undergone Phase 1 and 2 studies examining efficacy and safety. This randomized controlled clinical trial (RCT) assessed the efficacy and safety of PRT-PLTs using the 1-hour corrected count increment (CCI_{1hour}) as the primary outcome.

STUDY DESIGN AND METHODS: A noninferiority RCT was performed where patients with chemotherapy-induced thrombocytopenia (six centers) were randomly allocated to receive PRT-PLTs (Mirasol PRT, CaridianBCT Biotechnologies) or reference platelet (PLT) products. The treatment period was 28 days followed by a 28-day follow-up (safety) period. The primary outcome was the CCI_{1hour} determined using up to the first eight on-protocol PLT transfusions given during the treatment period.

RESULTS: A total of 118 patients were randomly assigned (60 to PRT-PLTs; 58 to reference). Four patients per group did not require PLT transfusions leaving 110 patients in the analysis (56 PRT-PLTs; 54 reference). A total of 541 on-protocol PLT transfusions were given (303 PRT-PLTs; 238 reference). The least square mean CCI was 11,725 (standard error [SE], 1,140) for PRT-PLTs and 16,939 (SE, 1,149) for the reference group (difference, −5214; 95% confidence interval, −7542 to −2887; $p < 0.0001$ for a test of the null hypothesis of no difference between the two groups).

CONCLUSION: The study failed to show noninferiority of PRT-PLTs based on predefined CCI criteria. PLT and red blood cell utilization in the two groups was not significantly different suggesting that the slightly lower CCIs (PRT-PLTs) did not increase blood product utilization. Safety data showed similar findings in the two groups. Further studies are required to determine if the lower CCI observed with PRT-PLTs translates into an increased risk of bleeding.

Over the past two decades significant progress has been made to prevent transmission of viruses and bacteria through blood transfusion including improved donor screening at the time of donation, introduction of nucleic acid testing for virus detection, screening for bacteria, and the diversion pouch used at the time of donation to reduce bacterial contamination.[1,2] In spite of these improvements, notable risks still remain for transmitting some blood-borne pathogens. Viral transmission can still occur during the window period when tests are unable to detect low pathogen load, because some tests lack optimal sensitivity, or due to the fact that practical and effective donor screening methods for certain known pathogens may not be available. Transfusion-associated sepsis due to bacteria in the blood product also occurs as bacterial testing is not performed universally, and current detection systems are only partially effective at identifying contaminated products. However, the greatest concern driving the development of new technologies to prevent pathogen transmission is the risk of blood supply contamination by new pathogens, or new strains of known pathogens, for which no tests currently exist.[1]

For more than a decade, research has focused on the development of safe and effective methods of pathogen reduction in the anticipation that these methods would be effective in preventing transmission of known pathogens and provide protection against emerging or mutant strains or viruses and bacteria.

Methods of pathogen reduction for red blood cells (RBCs), platelets (PLTs), and plasma are currently in development with some of these methods already in clinical use in Europe.[3] Several of these technologies use photochemical agents, which can be activated by ultraviolet (UV) light resulting in chemical modifications to DNA and RNA that prevent their replication.[4-12] This renders the pathogens present in the blood product incapable of replication during storage and also incapable of causing infectious complications in the patient after transfusion. One pathogen reduction process for PLTs (Mirasol pathogen reduction technology [PRT]; CaridianBCT, Lakewood, CO), utilizes exposure to UV light in the presence of riboflavin to introduce irreparable lesions to nucleic acids thereby inhibiting pathogens and white blood cell (WBC) replication.[13] Riboflavin is a nontoxic and nonmutagenic compound; hence, it does not have to be removed at the end of the process.[14] This technology has been shown to substantially reduce the active pathogen load[15-21] in PLT products, and effectively inactivate residual WBCs that may be present in blood components; hence, there is strong evidence that this technology prevents transfusion-associated graft-versus-host disease.[22-25] Laboratory studies have also documented acceptable metabolic and functional characteristics as measured by a battery of in vitro PLT function tests.[26,27] Hence, with Phase 1 and 2 studies suggesting that this technology appears safe and effective for reducing pathogen transmission, a larger clinical study was warranted.

We report on a randomized controlled trial (RCT) conducted to determine if pathogen-reduced PLTs (PRT-PLTs) are as effective as standard untreated PLT products when transfused to patients with chemotherapy-induced thrombocytopenia with respect to the corrected count increment 1 hour posttransfusion (CCI_{1hour}). The study was also designed to provide safety information of PRT-PLTs by documenting all adverse events.

ABBREVIATIONS: DSMB = Data Safety Monitoring Board; LS = least square; PRT-PLT(s) = pathogen reduction of platelet(s); RCT = randomized controlled trial; SAE(s) = serious adverse event(s).

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MATERIALS AND METHODS

Study design

This was a multicenter, open-label, parallel-group noninferiority RCT conducted in France by the Etablissement Francais du Sang and university hospitals (n = 6; see Acknowledgments), which compared PRT-PLTs and standard (reference) PLT products when transfused to thrombocytopenic hematology and/or oncology patients. The study was approved by the central research ethics committees for the participating centers, and was registered at http://www.clinicaltrials.gov (NCT00263809) and at the AFSSAPS official trial site.

Study population

There was a two-stage process for assessing patient eligibility. In Phase 1, patients were deemed eligible for further assessment if they met the following inclusion criteria: age 16 years or older; thrombocytopenia due to chemotherapy, malignant hemopathy, allogeneic or autologous hematopoietic stem cell transplantation, or diagnosis of a solid tumor with expectation to receive at least two PLT transfusions; and being treated as an inpatient. Eligible patients were excluded if one or more of the following criteria were satisfied: pregnancy, lactation, splenomegaly, and history or diagnosis of an autoimmune disease affecting hemostasis. Patients meeting the Phase 1 eligibility criteria were approached for informed consent. The rationale and objectives of the study were explained to patients by the site investigator or coinvestigator. Informed consent was required from all participants in accordance with the Declaration of Helsinki. Consenting patients underwent a Phase 2 screening process to confirm eligibility. Patients were excluded if any of the following criteria were present: positive serum or urine pregnancy test within 72 hours of randomization; history of hypersensitivity to riboflavin or metabolites; history of refractoriness to PLT transfusion (two successive $CCI_{1hour} < 5000$); presence of HLA antibodies, positive lymphocytotoxicity test, or previously documented alloimmunization to PLTs (as per individual site testing protocols); active bleeding requiring one or more RBC transfusions; acute or chronic disseminated intravascular coagulation; history or a diagnosis of immune/idiopathic thrombocytopenic purpura, thrombotic thrombocytopenic purpura, or hemolytic uremic syndrome; history of solid organ transplant; evidence of veno-occlusive disease; temperature of more than 39.5°C and/or signs of infection; enrollment in a pathogen reduction clinical trial within the previous 6 months; exposure to any other investigational product within 30 days of randomization; taking study-prohibited medications within 14 days of randomization (see Supporting Appendix S1, available as supporting information in the online version of this paper); evidence of chronic alcohol misuse;[28] and any other medical condition that could compromise participation.

Patients meeting the Phase 2 eligibility criteria were randomly assigned to receive reference PLTs or PRT-PLTs. The random treatment allocation scheme involved stratification by center and blocking and was computer generated by the coordinating center (MedPass International, Paris, France). Patient allocation was performed at each site using opaque envelopes containing the treatment assignment. Due to the slight yellow color of PRT-PLTs the study could not be conducted in a double-blind manner; however, those individuals assessing PLT counts and performing patient assessments were blinded to the patient's treatment allocation.

The following data were collected at the initial randomization visit: height, weight, vital signs, concomitant treatments, and laboratory test results (D-dimer, albumin, alkaline phosphatase, alanine aminotransferase [ALT], blood urea nitrogen, lactate dehydrogenase [LDH], potassium, total protein, fibrinogen, creatinine, urea, bilirubin, complete blood count, and lymphocytotoxicity testing). Blood samples were also collected for detection of photoproducts and neoantigen formation (results reported in a separate article).[28]

Interventions

Reference and PRT-PLT products were collected by apheresis (Trima Version 5.0, CaridianBCT) or prepared from buffy coats using pools from six whole blood collections using the OptiPress (Fenwal, Inc., Round Lake, IL) device with a top-and-bottom separation process and conventional PLT pooling methods. All PLTs were leukoreduced in accordance with French requirements (residual WBC content below 10^6/product in >97% of production). Product requirements included: volume of 170 to 360 mL, concentration of 1180×10^9 to 2100×10^9 PLTs/L plasma, and minimum-maximum PLT yield of 3.0×10^{11} and 5.1×10^{11} PLTs, respectively. All products were suspended in plasma and stored at 22°C with agitation for a maximum of 5 days. Products that failed requirements (see Supporting Appendix S2, available as supporting information in the online version of this paper) were not used in the study.

The PRT-PLTs were prepared using MIRASOL PRT. After the rest period (2 hr postcollection of apheresis PLTs and 1 hr postpreparation of buffy coat PLTs), the PLTs were transferred into an illumination/storage bag and riboflavin solution was added (500 µmol/L, 35 ± 5 mL). The bag was sealed using the MIRASOL PRT Welder. The product was placed in the illuminator and exposed to light at 6.24 J/mL[26] and then labeled "Exclusively for Clinical Investigation."

The recommended transfusion trigger was 10×10^9/L when clinical risk factors were absent; 20×10^9/L when there was fever, hypertension, evidence of Grade 2 mucositis, lesions with bleeding potential and/or a rapid decrease in PLT count occurred within 72 hours; and 50×10^9/L if antithrombotics were administered, if there was evidence of fibrinolysis or coagulopathy, or invasive surgery was required.[29] Patients could withdraw from the study at any time or could be withdrawn at their physician's discretion based on clinical or laboratory findings that suggested that participation may not be in the patient's best interest.

The treatment period started at the time of randomization (Day 0) and continued for a maximum of 28 days. The following reasons accounted for patient termination before Day 28: no need for additional on-protocol PLT transfusions, withdrawal due to an adverse event, withdrawal of consent, lost to follow-up, transfer to another hospital service (e.g., intensive care unit), or death. After the treatment period, a safety follow-up period began with Day 1 being the day after the last on-protocol transfusion in the treatment period continuing for 28 days (range, 23-42 days considered acceptable), day of withdrawal (adverse event/withdrawal of consent), lost to follow-up, or death, whichever occurred first. A transfusion was defined as off-protocol if 1) the product did not meet the prespecified criteria (defined above), 2) a patient randomized to PRT-PLTs received a non–PRT-PLT product, or 3) a PLT transfusion was given outside of the 28-day treatment period.

Product information collected with each on-protocol transfusion included weight (g), PLT count, ABO group, collection and/or manufacturing method, whether the product was gamma irradiated, transfused volume, and date and time of transfusion. Patient information for each on-protocol PLT transfusion was collected before and 24 hours posttransfusion and included weight, vital signs, evidence of bleeding, concomitant treatments, creatinine, urea, bilirubin, and complete blood count. Similar documentation occurred at 1 hour posttransfusion with the exception of creatinine, urea, and bilirubin. At the end of the safety follow-up period the same assessment was performed as the pretransfusion assessment. Bleeding assessments for on-protocol PLT transfusion were performed by hospital staff (physicians or nursing staff) who were appropriately trained to score according to WHO bleeding assessment criteria.[30] This included a physical examination for signs and symptoms of bleeding and a review of the patient's chart for documentation of bleeding. A bleeding assessment was also performed at the last study follow-up visit.

Study outcomes

The primary efficacy outcome was the CCI_{1hour} measured 30 to 90 minutes posttransfusion for each of a maximum of eight on-protocol PLT transfusions per patient occurring within the 28-day treatment period. The patient's pretransfusion PLT count for this calculation had to be measured within 12 hours of the transfusion. Transfusions where the 1-hour measurement was taken 30 to 90 minutes posttransfusion were considered time compliant. Measurements taken within 0 to 120 minutes posttransfusion were also analyzed as an extended time period. Transfusions with measurements taken after 120 minutes were not included in these analyses. CCI was calculated using the formula

$$CCI = \frac{Post - pre\,count\ (\times 10^9/L)}{Platelet\ dose\ transfused\ (\times 10^{11})} \times BSA^*$$

$$^*BSA\ (Body\ Surface\ Area) = 0.0202457 \times Height_m^{0.725} \times Weight_{kg}^{0.425}.$$

Secondary outcomes included CCI_{24hour} (specimens collected 18-26 hr posttransfusion were considered time compliant and 15-30 hr posttransfusion defined the extended time period), interval between transfusions, number of PLT and RBC transfusions per subject during the treatment period, number of PLTs transfused normalized by body surface area and for the number of days in the treatment period, evidence of refractoriness (two consecutive transfusions with a $CCI_{1hour} < 5000$), frequency of transfusion associated infections, and bleeding (WHO Grades 1-4).

Safety outcomes were captured during the treatment and follow-up periods including adverse events, serious adverse events (SAEs), bleeding status on days of PLT transfusion, transfusion-associated infections, and death. Adverse events were categorized as mild, moderate, or severe. The causal relationship was classified as unrelated, unlikely, possible, probable, or highly probable (see Supporting Appendix S3, available as supporting information in the online version of this paper). Adverse events were coded according to the Common Toxicity Criteria Scale (CTCAE Version 3.0/MedDRA Version 6.0, MedDRA MSSO, Chantilly, VA). All SAEs were reported to the coordinating center within 24 hours of the event being identified and to other relevant authorities. Alloimmunization to neoantigens was also assessed with results reported in a separate publication.[28]

Sample size

It was estimated that the mean CCI_{1hour} in the reference group would be 14,700 (standard deviation [SD], 5200; based on the results of the TRAP study).[31] With a Type 1 error of 2.5% and power of 80%, it was determined that 50 patients would be required per group to claim noninferiority of PRT-PLTs compared to standard practice with a noninferiority margin of 20% (CCI difference of 2940). This sample size was increased to 118 to accommodate some loss to follow-up. If the lower limit of a two-sided 95% confidence interval (CI) for the difference (PRT-PLTs—reference) in mean CCI_{1hour} is above –2940, noninferiority would be demonstrated.

Data Safety Monitoring Board

The Data Safety Monitoring Board (DSMB) was composed of two transfusion medicine experts, one biostatistician, and one physician, all independent of the study sponsor. The DSMB monitored unblinded safety and performance data, made recommendations related to protocol changes and continuing/stopping the study, and reviewed all SAEs providing their final adjudication. An interim analysis was planned a priori and performed by an independent group after 54 randomized patients completed follow-up; however, formal stopping rules were not specified a priori.

Statistical analysis

Descriptive analyses were conducted for the demographic and clinical variables. Continuous variables were summarized by their means and SDs and categorical variables by frequencies and percentages. The frequency of on-and off-protocol transfusions was tabulated.

The primary and secondary outcomes (CCI_{1hour} and CCI_{24hour}, respectively) were analyzed using a mixed-effects analysis of covariance model with a random patient effect to accommodate the association in the responses within patients over multiple transfusions and controlling for pretransfusion PLT count and treatment group.[32] For each treatment group, least square (LS) means and standard errors (SE) were reported based on fits using computer software (PROC MIXED, SAS 9.1.3, SAS Institute, Inc., Cary, NC) and compared between treatment arms. By recognizing that responses to serial transfusions may not be independent within patients, this approach recognizes all sources of variability and ensures valid inferences. Analysis included up to the first eight time-compliant on-protocol PLT transfusions received during the treatment period for all randomized patients who received at least one transfusion. A secondary analysis also included transfusions where posttransfusion measurements occurred within the extended time period.

Interactions between treatment group and pretransfusion PLT count were tested to examine whether there was evidence that the effect of PRT-PLTs varied for different pretransfusion PLT counts. Similar tests were carried out for interactions between response and site to test for the poolability of data across sites.

A mixed longitudinal logistic regression model[33] was also fit to assess the effect of PRT-PLTs versus reference PLT products on achieving a 7500 CCI at 1 hour and 4500 CCI at 24 hours posttransfusion.[34] Pretransfusion PLT count and a random patient effect were included in this model with the latter accounting for an association in the responses over time. Frailty models were fit to estimate the distribution of times between transfusions while accounting for the within-patient dependence in the gap times.[35] All p values for secondary outcome comparisons were two-tailed tests. Adverse event data were summarized in tabular form and analyzed descriptively.

The primary and secondary analyses were repeated in a post hoc subgroup analysis of 95 patients. This subgroup was obtained by excluding 15 patients with incomplete data (eight receiving reference PLTs and seven receiving PRT-PLTs) after discussion with the DSMB.

RESULTS

Six centers enrolled 118 patients into the study between December 2005 and September 2007: 60 patients received PRT-PLTs and 58 received reference PLTs. Four patients in each treatment group did not receive PLT transfusions leaving 110 patients that could be included in the *intention-to-treat* analysis. There were 10 of 110 patients who withdrew from the study before Day 28 in the treatment period (six in the PRT-PLT arm; four in the reference arm); hence, the proportion of patients completing the treatment period in the PRT-PLT group was 91.1% (51/56) and 98.1% (53/54) in the reference group. Data from these 10 patients were included in the analyses up until the time of their withdrawal. The proportion of patients completing the safety follow-up period was 73.2% (41/56) for PRT-PLTs and 81.5% (44/54) for the reference arm (median durations both study periods being 45 and 44 days, respectively). Patient flow through the study is summarized in Fig. 1.

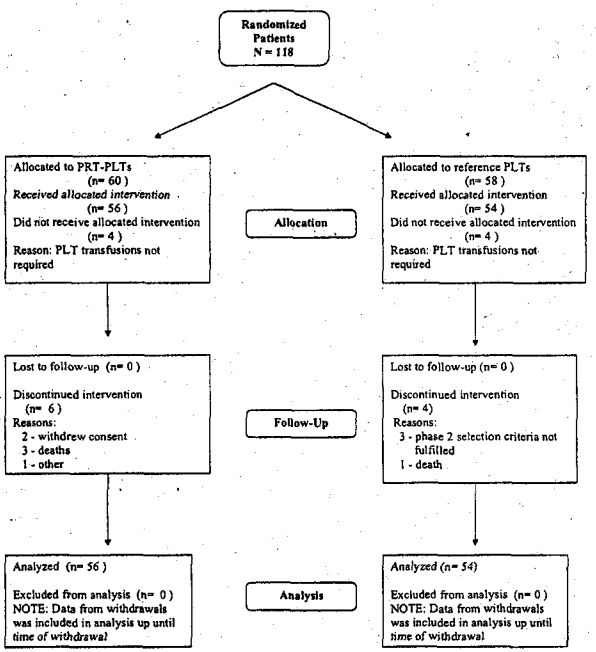

Fig. 1. CONSORT flow diagram showing the flow of patients through the study from the time of randomization to analysis.

Baseline demographics for the study patients were similar between the two groups and are summarized in Table 1. Other baseline characteristics were documented (data not shown) and showed a similar distribution in both groups (physical findings, vital signs, complete blood count, fibrinogen, albumin, alkaline phosphatase, ALT, creatinine, urea, direct and total bilirubin, blood urea nitrogen, LDH, potassium, and total protein).

There were a total of 678 PLT transfusions given to patients during the study period: 368 PRT-PLT transfusions (303 on-protocol; 65 off-protocol) and 310 reference group transfusions (238 on-protocol; 72 off-protocol). The frequency of off-protocol PLT transfusions was 17.7% for PRT-PLTs and 23.2% in the reference group. Criteria for off-protocol transfusions were prespecified in the protocol; however, the data collection process did not capture the reason.

The prespecified primary outcome analysis for the CCI_{1hour} was based on a maximum of eight PLT transfusions per patient occurring in the 28-day treatment period: 258 for PRT-PLTs and 209 for the reference group (total 467). The test for homogeneity of treatment effects between sites for the CCI_{1hour} was not significant ($p = 0.1728$), indicating that data from all sites could be pooled to estimate the treatment effect. The LS mean CCI_{1hour} in the PRT-PLT group was 11,725 (SE, 1140) and in the reference group 16,939 (SE, 1149), a difference of −5214 (95% CI, −7542 to −2887; $p < 0.0001$). The CI for the difference includes the prespecified upper limit of the zone of noninferiority (set at 20% of the mean CCI anticipated in the reference group, which was 2940); hence, noninferiority could not be claimed since to do so would have required the lower limit of this CI to be above −2940. The CCI_{1hour} was also calculated for the extended time period, adjusted for pretransfusion PLT count (continuous variable) and site (Table 2). The CCI_{1hour} data for time-compliant and extended time period transfusions are illustrated in Fig. 2 using box plots.

Secondary outcomes

The CCI_{24hour} was analyzed according to the time-compliant and extended time periods and adjusted for pretransfusion PLT count as a continuous variable and site. The test for homogeneity of the effect of treatment between sites for the CCI_{24hour} was not significant ($p = 0.1336$) allowing for data to be pooled. The LS mean for time-compliant CCI_{24hour} was 6676 (SE, 883) for the PRT-PLTs and 9886 (SE, 915) in the reference group (difference, −3210; 95% CI, −5160 to −1260). The CCI_{24hour} results are summarized in Table 2 and Fig. 2. Table 2 also contains the results of the mixed logistic regression models and reports the odds ratios (ORs) for achieving the desired CCI increment (7500 and 4500 for CCI_{1hour} and CCI_{24hour}, respectively). The odds of achieving a successful response is significantly lower in the PRT-PLTs arm for the CCI_{1hour} among time-compliant transfusions (OR, 0.284; 95% CI, 0.105 to 0.767; $p = 0.0130$) but not significantly lower for the CCI_{24hour} among time-compliant transfusions (OR, 0.481; 95% CI, 0.211 to 1.098; $p = 0.0822$). Similar results were found when considering transfusions within the extended time period although the 24-hour CCI result becomes significant in this analysis.

A meaningful interval between transfusions was difficult to calculate as patients in both treatment groups had off-protocol transfusions within the treatment period.

TABLE 1. Baseline characteristics for the patients in the PRT-PLTs and reference group

Demographic characteristic	Treatment arm: PRT-PLTs (n = 56)	Reference (n = 54)
Median age, years (range)	58 (20-73)	53 (20-74)
Sex (male/female)	32/24	34/20
Median height, m (range)	1.7 (1.5-1.86)	1.7 (1.51-1.93)
Median weight, kg (range)	71.5 (46.3-121.0)	73.6 (45.0-110.3)
ABO blood group, number (%)		
A	30 (53.6)	32 (59.3)
B	5 (8.9)	6 (11.1)
O	21 (37.5)	16 (29.6)
AB	0 (0.0)	0 (0.0)
Diagnosis, number (%)		
Acute lymphocytic leukemia	2 (3.6)	2 (3.7)
Acute myelogenous leukemia	26 (46.4)	27 (50.0)
Multiple myeloma	4 (7.1)	2 (3.7)
Non-Hodgkin's lymphoma	19 (33.9)	18 (33.3)
Hodgkin's lymphoma	1 (1.8)	3 (5.6)
Other*	4 (7.1)	2 (3.7)
Mean (SD duration of thrombocytopenia), days†	16.3 (7.2)	14.8 (7.0)
Median (range) baseline test results		
Hemoglobin (g/dL)	9.5 (8-14)	9.5 (7-15)
PLT count (×10⁹/L)	42.5 (8-479)	43.0 (6-206)
WBC count (×10⁹/L)	1.05 (0-14)	1.30 (0-51)

* Other includes severe idiopathic medullary aplasia (1), biphenotypic acute leukemia (1), chronic lymphocytic leukemia (1), myelodysplasia-refractive anemia with excess blasts (2), and mediastinal teratocarcinoma (1).
† During treatment period.

TABLE 2. Summary CCI values by treatment group based on the first eight on-protocol transfusions (primary outcome) and all on protocol PLT transfusions within the treatment period*

Outcome	PRT-PLTs		Reference		PRT-PLTs minus Reference		
CCI—continuous outcome	Number	LS mean (SE)	Number	LS mean (SE)	Difference	95% CI	p value
Analysis based on the first eight on-protocol transfusions within the 28-day treatment period							
CCI_{1hour}							
Time compliant	195	11,725 (1,140)	164	16,939 (1,149)	−5214	(−7542 to −2887)	<0.0001
Extended time	216	11,766 (1,072)	174	17,170 (1,057)	−5404	(−7721 to −3088)	<0.0001
CCI_{24hour}							
Time compliant	175	6,676 (883)	160	9,886 (915)	−3210	(−5160 to −1260)	0.0014
Extended time	209	6,998 (811)	179	10,385 (811)	−3387	(−5232 to −1542)	0.0004
Analysis based on all on-protocol transfusions within the 28-day treatment period							
CCI_{1hour}	273	11,005 (962)	220	16,614 (977)	−5609	(−7791 to −3427)	<0.0001
CCI_{24hour}	267	7,162 (831)	211	10,070 (839)	−2907	(−4802 to −1013)	0.0027
CCI—dichotomous outcome	Number	Number (%)	Number	Number (%)	OR	95% CI	p value
Analysis based on the first eight on-protocol transfusions within the 28-day treatment period							
Time compliant							
$CCI_{1hour} > 7500$	195	139 (71.3)	164	138 (84.1)	0.284	(0.105 to 0.767)	0.0130
$CCI_{24hour} > 4500$	175	103 (58.9)	160	109 (68.1)	0.481	(0.211 to 1.098)	0.0822
Extended time							
$CCI_{1hour} > 7500$	216	151 (69.9)	174	147 (84.5)	0.233	(0.081 to 0.667)	0.0067
$CCI_{24hour} > 4500$	209	118 (56.5)	179	120 (67.0)	0.423	(0.189 to 0.945)	0.0360

* Results for the first eight on-protocol transfusions are also presented using CCI as a dichotomous outcome.

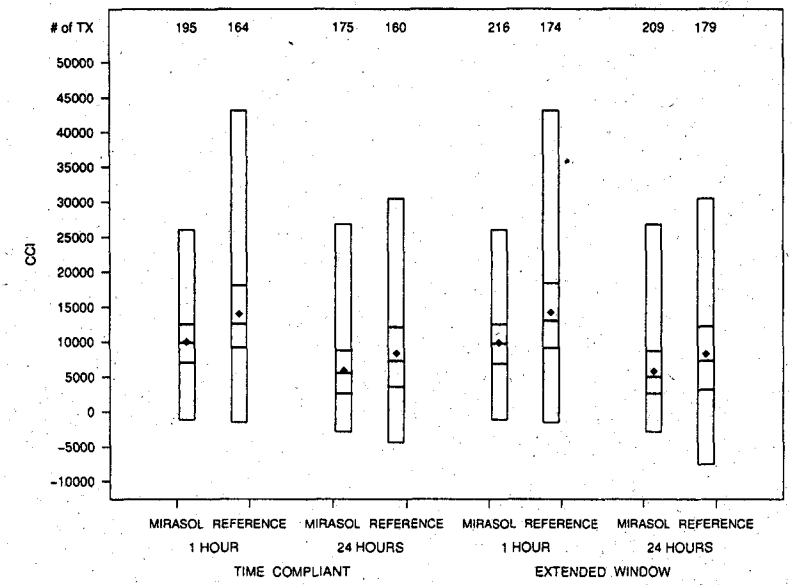

Fig. 2. Box plots of 1- and 24-hour CCIs for transfusions in the time-compliant and extended time periods by treatment group. The lines within the boxes represent the Q_{75} (upper line), median (middle line) and Q_{25} (lower line). The diamond indicates the raw means (the mean of the raw CCI values). The height of the rectangular box indicates the minimum and maximum values.

When both on- and off-protocol transfusions within the 28-day treatment period are included in this analysis the mean number of days between transfusions was 2.16 (SD, 1.69) for PRT-PLTs and 2.30 (SD, 1.48) for the reference arm (p = 0.2903). The mean number of PLT transfusions per patient-day during the treatment period (includes on- and off-protocol transfusions) was not significantly different: PRT-PLTs 0.24 (SD, 0.16) and reference group 0.20 (SD, 0.19; p = 0.2046). Summaries of secondary outcomes are given in Table 3. None of the differences observed were significant.

RBC requirements were similar in the two groups. In the PRT-PLT group 183 RBC units were transfused in the treatment and follow-up periods: 155 were given in the treatment period with a mean (SD) per patient of 2.8 (1.7). In the reference group 142 of 160 RBC units were given in the treatment group with a mean per patient of 2.6 (2.4; p = 0.7257).

Alloimmunization and refractoriness

Two patients in the PRT-PLT group (3.6%) became alloimmunized and four patients in the reference group (7.45%) developed HLA antibodies (p = 0.4336; Fisher's exact). Only 5 of 110 patients (4.5%) became refractory during the study: three (5.4%) in the PRT-PLT group and two (3.7%) in the reference group (p = 1.0000; Fisher's exact).

Infections

There were a total of 88 infectious adverse events reported in 58 study patients. In the PRT-PLT group 45 infections were reported in 28 patients (1.61 infections/patient). Six infections were categorized as severe adverse events: cytomegalovirus (CMV; 1), *Klebsiella* (2), *Escherichia* urinary tract (1), infection (1), and sepsis (1). The one patient who developed CMV infection had positive CMV serology before stem cell transplantation and transfusion. In the reference group there were 43 infections in 30 patients (1.42 infections/patient): nine of these were categorized as severe adverse events: bacterial infection (1), bacterial sepsis (1), bronchopulmonary aspergillosis (1), *Clostridium* colitis (1), *Clostridium difficile* colitis (1), herpes virus infection (1), urinary tract infection enterococcal (1), sepsis (1), and septic shock (1), but none were considered transfusion related. There were no significant differences in the proportion of patients with one or more infections (p = 0.5731; Fisher's exact test), and the mean number of infections/patients/group (p = 0.4571). Table 4 summarizes rates of adverse events and SAEs.

TABLE 3. Summary of the characteristics of the PLT transfusions and the secondary outcomes related to PLT transfusion

	PLT transfusions					
	On-protocol, limited to first eight transfusions within the 28-day treatment period			On protocol, within the 28-day treatment period		
Outcome/characteristic	PRT-PLTs	Reference	p value	PRP-PLTs	Reference	p value
Total number of PLT transfusions	258	209		303	238	
Number of apheresis PLT transfusions (%)	180 (69.8)	149 (71.3)		224 (73.9)	178 (74.8)	
Number of buffy coat PLT transfusions (%)	78 (30.2)	60 (28.7)		79 (26.1)	60 (25.2)	
Median number of PLT transfusions/patient (range)	4.0 (1-8)	3.0 (1-8)		4.5 (1-21)	3.0 (1-19)	
Mean (SD) PLT dose transfused ($\times 10^{11}$)	5.37 (2.14)	5.38 (2.10)	0.9615	5.23 (2.09)	5.22 (2.02)	0.9867
Mean age of product at transfusion (days)	2.8 (1.1)	2.6 (1.1)	0.0891	2.7 (1.1)	2.6 (1.1)	0.2210
Total number of ABO-mismatched transfusions (%)	41 (15.9)	37 (17.7)		50 (16.5)	40 (16.8)	
Major mismatch	39 (15.1)	32 (15.3)		46 (15.2)	35 (14.7)	
Minor mismatch	2 (0.8)	5 (2.4)		4 (1.3)	5 (2.1)	
Number (%) of transfusions CCI_{1hour} compliant	195 (81.9)	164 (85.4)		222 (81.3)	185 (84.1)	
Number (%) of transfusions CCI_{24hour} compliant	175 (75.8)	160 (83.3)		196 (73.4)	173 (82.0)	
Mean (SD) interval between PLT transfusions*	2.32 (1.79)	2.72 (1.44)	0.0107	2.16 (1.69)	2.30 (1.48)	0.2903

* The mean (SD) interval between PLT transfusions is calculated based on both on- and off-protocol transfusions.

TABLE 4. Summary of the number of patients with different grades of WHO bleeding and mean rates of all and severe adverse events

	All analyzable patients (n = 110)		
Bleeding by WHO grade and adverse event rates	PRT-PLTs (n = 56)	Reference (n = 54)	p value
Number of subjects with bleeding episodes			
Grade 1	28	19	0.128
Grade 2	11	5	0.176
Grade 3	4	2	0.679
Grade 4	2	1	1.000
Grade 1-4	33	23	0.127
Grades 2-4	12	7	0.315
Grades 3 and 4	6	3	0.490
Mean (SD) rate*			
All adverse events	0.28 (0.19)	0.23 (0.16)	0.144
Severe adverse events	0.07 (0.17)	0.04 (0.06)	0.171

* Total number of events/duration of treatment period and follow-up period.

Bleeding outcomes

Bleeding assessments were only performed for on-protocol PLT transfusions with assessments done before transfusion, after transfusion at 1 and 24 hours, and on the final follow-up visit. There were 19 patients with WHO bleeding of Grade 2 or higher: 12 patients in the PRT-PLT arm (21.4%) and seven patients (13.0%) in the reference group. Eleven subjects receiving PRT-PLTs had Grade 2 bleeding, four had Grade 3, and two had Grade 4 (both central nervous system bleeding: one patient died on Day 17 and one patient completed the study). In the reference group there were five patients with Grade 2 bleeding, two had Grade 3, and one had Grade 4. The Grade 4 bleed was genitourinary and the patient completed 41 study days. The numbers of bleeding events by grade are summarized in Table 4.

The results of the primary and secondary endpoints are also summarized for the 95 patients that were included in the post hoc subgroup analysis (see Supporting Appendix S4, available as supporting information in the online version of this paper). For the primary endpoint (CCI_{1hour}) noninferiority was not demonstrated. For all secondary endpoints the results were very similar to the analysis including all 110 patients.

Safety outcomes

All patients receiving PRT-PLTs and 98.1% (53/54) of patients in the reference group had at least one reported adverse event; however, the majority of adverse events were not related to the PLT products transfused (Table 5). There were five adverse events (five patients) in the PRT-PLT group that were categorized as "possible, likely, or very likely" and eight adverse events (five patients) in the reference group that fell into these categories. For the severe adverse events two patients in the PRT-PLT group (1.8%) had events that were "very likely" related to a transfusion and two patients in the reference arm had events categorized as "very likely" related. These patients developed anaphylactic shock (one reference patient), hypersensitivity plus eyelid edema (one reference patient), and refractoriness to PLT transfusions (one PRT-PLT patient). There

TABLE 5. The number and frequency of adverse events, severe adverse events, and SAEs by relationship to transfusion*

Adverse events categorized by relationship to transfusion	PRT-PLTs (n = 56)	Reference (n = 54)
Adverse events		
Subjects with at least one adverse event	56 (100)	53 (98.1)
Total number of adverse events	*654*	*507*
Relationship of adverse event to study transfusion†		
None	596 (91.1)	477 (94.1)
Unlikely	53 (8.1)	22 (4.3)
Possible	3 (0.5)	2 (0.4)
Likely	0 (0.0)	3 (0.6)
Very likely	*2 (0.3)*	*3 (0.6)*
Severe adverse events		
Subjects with at least one adverse event	38 (67.8)	30 (55.6)
Total number of adverse events	110	90
Relationship of adverse event to study transfusion‡		
None	100 (90.9)	86 (95.6)
Unlikely	*7 (6.4)*	*1 (1.1)*
Possible	1 (0.9)	0 (0.0)
Likely	0 (0.0)	0 (0.0)
Very likely	2 (1.8)§	3 (3.3)‖
SAEs		
Subjects with at least one adverse event	13 (23.2)	11 (20.4)
Total number of adverse events	*17*	*14*
Relationship of adverse event to study transfusion¶		
None	12 (70.6)	12 (85.7)
Unlikely	5 (29.4)	1 (7.1)
Possible	0 (0.0)	0 (0.0)
Likely	0 (0.0)	0 (0.0)
Very likely	*0 (0.0)*	*1 (7.1)***

* Data are reported as number (%). A severe adverse event was defined as any untoward medical occurrence in a subject causing severe discomfort and significant impact on the patient's usual activities and requiring treatment. A SAE included one or more of the following: death; serious deterioration in the subject's health resulting in life-threatening illness or injury, permanent impairment of body structure or function, prolonged hospitalization, or medical/surgical intervention; and failure to complete the transfusion.
† As reported by the investigator; percentage based on the number of adverse events reported in each treatment arm.
‡ As reported by the investigator; percentage based on the number of severe adverse events reported in each treatment arm.
§ Refractoriness to PLT transfusion.
‖ One patient developed anaphylactic shock during the transfusion; one patient developed hypersensitivity during one transfusion and eyelid edema during another transfusion.
¶ As reported by the investigator; percentage based on the number of SAEs reported in each treatment arm.
** One patient developed anaphylactic shock during the transfusion.

were five thrombotic events reported; however, none was related to study transfusions: one event occurred in the PRT-PLT arm (pulmonary embolism) and four occurred in patients receiving reference PLTs (cerebral vascular thrombosis [1], myocardial infarct [1], jugular vein thrombosis [1], and veno-occlusive disease [1]). A summary of all adverse events and severe adverse events categorized by organ system are summarized in Table 6. The frequencies of all adverse events and SAEs were similar between both treatment arms. Most adverse events were categorized according to the following organ systems: gastrointestinal, general disorders and administrative site conditions, and blood and lymphatic disorders.

DISCUSSION

This study was designed to determine whether the CCI_{1hour} for PRT-PLTs was noninferior to untreated PLT products. CCI was selected as the primary outcome because this has been the outcome historically used for licensing of new PLT products treated with PRT methods in Europe.[11] When planning the study, noninferiority would be claimed if the mean CCI_{1hour} of the pathogen-inactivated product did not exceed a reduction in mean CCI of more than 20% of the value observed with untreated PLTs. The study failed to demonstrate noninferiority for either the CCI_{1hour} (primary outcome) or the CCI_{24hour} (secondary outcome). Why pathogen inactivation of PLTs results in a lower CCI is not clear; however, this has been a consistent finding in several other studies. In a crossover RCT enrolling normal subjects, Aubuchon and colleagues[26] found that PRT-PLTs had a reduced mean survival (16.5% lower) and recovery (38 hr less) compared to untreated PLT product. The SPRINT study using amotosalen HCL (S-59) and UVA light to pathogen inactivate also reported lower CCIs at both 1 and 24 hours with the pathogen-inactivated PLT products. The mean CCIs per treatment group reported in the SPRINT study were almost identical to the values observed in this study.[12]

Metabolic activity and expression of activation markers increase in PRT-PLTs during storage;[26] hence, one could hypothesize more rapid utilization of these cells at sites of injury or damage, due to their increased activation status. Similar effects have been seen with dimethyl sulfoxide–cryopreserved PLTs; however, despite demonstrating highly elevated levels of P-selectin expression and other activation markers,[36-40] significantly increased degranulation,[41] and significantly lower levels of recovery in circulation,[42] the cryopreserved PLTs were associated with less bleeding, fewer transfusion support needs, and fewer complications compared to conventional, liquid-stored PLTs.[36,38,43,44] These findings emphasize the need for studies assessing the clinical impact of pathogen-inactivated PLTs that can clearly elucidate the relevance of the in vitro findings.

TABLE 6. Summary of the number of adverse events by organ system/disorder for the PRT-PLT and reference groups by treatment and follow-up period

	Treatment period*				Follow-up period†				Overall			
	Reference		PRT-PLTs		Reference		PRT-PLTs		Reference		PRT-PLTs	
Adverse event by organ system/disorders	Number of AEs	Number of patients	Number of AEs	Number of patients	Number of AEs	Number of patients	Number of AEs	Number of patients	Number of AEs	Number of patients	Number of AEs	Number of patients
Any organ system	384	51	506	56	123	41	148	37	507	53	654	56
Blood and lymphatic	46	25	59	33	8	6	7	6	54	30	66	36
Cardiac	6	4	15	13	6	6	1	1	12	9	16	14
Congenital/familial/genetic	0	0	1	1	1	1	1	1	1	1	2	2
Ear and labyrinth	3	3	0	0	0	0	0	0	3	3	0	0
Endocrine	0	0	0	0	1	1	0	0	1	1	0	0
Eye	5	5	4	3	2	2	3	3	7	7	7	6
Gastrointestinal	88	40	102	38	27	15	26	12	115	45	128	41
General/administrative site	77	43	91	43	18	15	22	12	95	46	113	46
Hepatobiliary	3	3	2	2	0	0	1	1	3	3	3	3
Immune system	5	5§	0	0§	1	1	1	1	6	6	1	1
Infections and infestations	28	21	32	22	15	14	13	9	43	30	45	28
Injury/poisoning/procedural	2	1§	9	8§	0	0	1	1	2	1§	10	9§
Investigations‡	0	0	5	5	0	0	0	0	0	0	5	5
Metabolism and nutrition	19	15	31	21	5	3	13	10	24	18	44	29
Musculoskeletal and connective tissue	6	4	6	6	7	6	4	3	13	8	10	9
Nervous system	11	8	15	13	7	5	5	4	18	12	20	14
Psychiatric	13	9	26	17	3	3	8	7	16	12	34	21
Renal and urinary	9	8	6	5	2	2	1	1	11	10	7	6
Reproductive and breast	1	1	1	1	1	1	3	2	2	2	4	3
Respiratory, thoracic, and mediastinal	19	14	39	23	5	5	12	6	24	16	51	26
Skin and subcutaneous tissue	29	22	36	26	10	7	12	9	39	27	48	31
Vascular	14	11	26	13	4	4	14	12	18	13	40	19

* Treatment period = randomization to day of last on-protocol PLT transfusions.
† Follow-up period = day after the last on-protocol PLT transfusions and up to study discontinuation/completion date.
‡ Investigations = bleeding time prolonged; blood creatine increase; weight increase.
§ Comparisons between reference and PRT-PLTs reached significance.
AE = adverse event.

Although the mean CCI values for both 1 and 24 hours were lower with PRT-PLTs, the mean values for both determinations were above the 7500 and 4500 thresholds, respectively, that have been used to define successful transfusions.[45] At 1 hour posttransfusion, 71.3% of the pathogen-inactivated products resulted in successful transfusion increments compared to 84.1% in the reference group. At 24 hours posttransfusion the proportions successful were 58.9% for PRT-PLTs and 68.1% for reference PLTs. Although the percentages of successful transfusions are lower than desired (both groups), they are within the ranges reported in other PLT transfusion studies raising questions as to why 30% to 40% of PLT transfusions are not considered successful based on current established thresholds.[46,47] Patient factors that affect increments and product variability may explain part of this failure; however, our understanding of these poor responses is still limited. Because of this observation, the sensitivity of the CCI as a clinical outcome measure could be questioned and indeed many studies have now used bleeding as their primary outcome.[12,48,49]

The time to next transfusion and overall blood product utilization analyses provided information about the resource implications of using PRT-PLTs. The time to next transfusion was determined for both study groups; however, there were limitations with this analysis as on-protocol transfusions during the treatment period were not always consecutive; hence, the interpretation was problematic. The overall PLT and RBC utilization in the two study groups was not significantly different, suggesting that the lower CCIs with the PRT-PLTs did not translate into significantly higher blood product use.

Safety information using PRT-PLTs was also obtained from this study. The study was designed to capture all adverse events regardless of whether they were related or unrelated to the transfusion of PLTs. Over 1100 adverse events occurred during the treatment and follow-up phases of the study, indicating the severe degree of illness and complications that occur in this patient population. However, only four patients had adverse events (two with PRT-PLTs and two with reference) that were categorized as having a very likely relationship to PLT transfusion. The two events in the PRT-PLT group were refractoriness to PLT transfusions. The events in the reference group included anaphylactic shock during a transfusion, hypersensitivity, and eyelid edema. All adverse events were categorized by organ system and/or disorder. The most frequently reported events in both treatment arms were gastrointestinal, general disorders and administrative site problems, blood and lymphatic disorders, and infections and infestations. These events occurred with similar frequency in both treatment groups suggesting an acceptable safety profile with PRT; however, additional safety data would be useful collected either as postmarketing surveillance or as part of a larger clinical trial where bleeding could be used as the primary outcome. Bleeding data were collected as a secondary outcome during this study but they were only actively assessed during the 24-hour time period around on-protocol transfusions. Each treatment group had Grade 4 bleeding events (two in the PRT-PLT group and one in the reference group). The study was not powered to show difference in bleeding and given the paucity of data we do not attempt to make conclusions related to risk of bleeding.

There were a number of additional limitations to this study. The frequent use of off-protocol transfusions made it difficult to analyze some of the secondary outcomes that involved measures over time. The reasons for the off-protocol transfusions were not documented. This information would have been useful to understand some of the logistical considerations when using PRT-PLTs and to provide further insight into the challenges with producing a standardized product volume and dose. The responses to off-protocol transfusions were not available, which also precluded traditional intention-to-treat analyses. These data would have been helpful to provide a more complete representation of the full transfusion history. There were also a number of protocol violations where posttransfusion samples for CCI determination were collected outside of the time-compliant period: 17.4% (86/493) for the CCI_{1hour} and 22.8% (109/478) for $CCI_{24hours}$. To avoid excluding these data, we prespecified an extended time period in addition to the time-compliant period and analyzed the data both ways; however, this compliance issue illustrates the challenges with getting CCI measurements posttransfusion in this complex patient population.

In conclusion, the noninferiority of PRT-PLTs compared to reference PLTs using the surrogate outcome measure of CCI_{1hour} was not demonstrated in this controlled clinical trial in 110 patients. Safety data did not identify any major adverse effects associated with the transfusion of PRT-PLTs. Overall PLT and RBC utilization in the two study groups was not significantly different, suggesting that the lower CCIs with the PRT-PLTs did not translate into significantly higher blood product use. Further studies are needed to show whether the lower CCI observed with PRT-PLTs is associated with any change in the risk of bleeding.

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CONFLICT OF INTEREST

Raymond P. Goodrich is an employee of CaridianBCT Biotechnologies, LLC. Dr Goodrich assumes full responsibility for the overall content and integrity of the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Patients were not eligible for the study if the following medications had been taken within 14 days of randomization.
Appendix S2. Product withdrawal criteria.
Appendix S3. Categories of adverse event severity used.
Appendix S4. Ninety-five patient post hoc subgroup analysis.

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