医薬品
医薬部外品　研究報告　調査報告書
化粧品

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2010 年 2 月 17 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	A linked donor-recipient study to evaluate parvovirus B19 transmission by blood component transfusion. Steven H. Kleinman et al., BLOOD, 22 OCTOBER 2009 _ VOLUME 114, NUMBER 17	公表国 米国	
販売名（企業名）						

研究報告の概要

ヒトパルボウイルス B19（B19V）感染症は，溶血または赤血球生成不全症候群などのある血液病患者にとって重篤な感染症となり得る。血漿製剤とは対照的に，成分輸血による B19V 感染症例報告は稀であるが，いずれの研究においても，B19V DNA 陽性成分輸血の受血者への感染率は体系的に測定されていない。本研究では，供血者および受血者由来の保存血液検体中の B19V DNA 量を高感度のリアルタイム定量 PCR アッセイにより測定し，B19VDNA 陽性成分（赤血球製剤 77%，全血由来血小板製剤 13%，新鮮凍結血漿製剤 10%）の成分輸血による B19V 感受性（輸血前に B19V IgG 抗体陰性）受血者の B19V 感染率を評価した。実際には B19VDNA 陽性であった 105 例の供血者由来の B19VDNA 陽性成分 112 検体が輸血された，輸血前 B19VIgG 抗体保有率 78%の 112 人の患者群（２４名が感受性受血者）について調査を行い，IgG あるいは IgM への抗体転，もしくは B19V DNA の新規検出をもって，B19V 感染成立と定義した。その結果，B19V DNA 量が 10^6 IU/mL 以下の成分輸血を受けた感受性受血者 24 例への B19 感染伝播は見られなかった（95%信頼区間，11.7%）。B19V DNA 量が 10^{10} IU/mL 以上の成分輸血を受けた非感受性受血者（輸血前 B19V IgG 抗体陽性）1 例で既往反応が認められた。本研究では，B19V DNA 量 10^6 IU/mL 以下の成分輸血による感染伝播は起こらない，また，もし感染が起こったとしても，感染率が 50%以上を示す多くの輸血感染症（HIV，HCV など）と比較すると，B19V 感染はまれな事象であることが示された。

使用上の注意記載状況・その他参考事項等

BYL-2010-0397

報告企業の意見	今後の対応
本研究では，受血者の状態による評価はなされておらず，また調査の規模つまり，評価のターゲットである感受性受血者数が少ないためこれらを加味した研究がという問題が残されてはいるが，ヒトパルボウイルス B19 の DNA 量について，10E6IU/m l という安全域の目安が示された。なお，弊社のコージネイト FS の製造工程培地で使用されている血漿分画成分に使用されるミニプール血漿においては，ヒトパルボウイルス B19 に対する NAT を実施しており，10E5 IU/mL 以上が確認された場合は，そのミニプール血漿は製造工程から除去している。現在の科学水準では，ヒトパルボウイルス B19 を確実に不活化する方法は存在しないため，感染リスクを完全に排除することはできないが，伝播の可能性は非常に低いと考える。	現時点で新たな安全対策上の措置を講ずる必要はないと考えるが，今後ともウヒトパルボウイルス B19 の感染に関する情報収集に努める。

BYL-2010-0397

A linked donor-recipient study to evaluate parvovirus B19 transmission by blood component transfusion

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Parvovirus B19V infection can be a serious infection for hematology patients with underlying hemolysis or compromised erythropoiesis syndromes. Although case reports of B19V transmission by blood component transfusion (as contrasted to manufactured plasma derivatives) are rare, no studies have systematically determined a rate of transmission to recipients transfused with B19V DNA–positive components. We used a linked donor and recipient repository and a sensitive, quantitative B19V DNA polymerase chain reaction (PCR) assay to assess such transmission in B19V-susceptible (ie, anti-B19V immunoglobulin G [IgG] negative) recipients. We assessed 112 B19V DNA–positive components from 105 donors (of 12 529 tested donations) transfused into a population of surgical patients with a prefransfusion B19V IgG seroprevalence of 78%. We found no transmission to 24 susceptible recipients from transfusion of components with B19V DNA at concentrations less than 10^6 IU/mL (upper 95% confidence interval, 11.7%). We found an anamnestic IgG response in one pretransfusion seropositive recipient transfused with a component containing greater than 10^{10} IU/mL B19V DNA. These findings show either that transmission from components with less than 10^6 IU/mL does not occur, or, if it does, it is an uncommon event. These data do not support the need to routinely screen blood donations with a sensitive B19V DNA nucleic acid assay. (Blood. 2009;114:3677-3683)

Introduction

There have been multiple reports of parvovirus B19 (B19V) transmission by pooled plasma products, including factor VIII concentrate and solvent-detergent–treated pooled plasma, documented by recipient seroconversion in asymptomatic cases or, less frequently, by clinical diagnosis of B19V-related disease in association with positive B19V test results.[1-5] These cases, combined with the potential for very high B19V DNA concentrations (up to 10^{12} IU/mL) in plasma donations[4] and the relative resistance of B19V to inactivation methods,[4,6] have led to B19V DNA testing of plasma donations to ensure that manufacturing plasma pools destined for plasma derivatives have a B19V DNA concentration less than or equal to 10^4 IU/mL, a limit proposed by the Food and Drug Administration (FDA).[7-9] The same limit for this so-called "in process testing" is a European regulatory requirement for anti-D immunoglobulin (Ig) preparations and plasma treated for virus inactivation.[10] To achieve this B19V DNA concentration in the final plasma pool, B19V DNA screening of the plasma donations used to make the pool is performed using assays (applied in minipool format) with the ability to detect approximately 10^6 IU/mL in an input unit of plasma.[4]

To date, no B19V transmissions from pooled plasma products have been documented when less than 10^3 to 10^4 IU/mL B19V DNA is present in an infused product.[3,4,11-13] The reason for this lack of infectivity is not completely understood. It may be due to an inadequate amount of infused infectious virions, a neutralization effect from B19V antibody present in other plasma units in the plasma pool, or a combination of these factors. Recipient factors may also play a role because it has been reported that B19V antibody is protective against B19V reinfection, and most of the adult population is B19V seropositive as a result of previous infection.[13]

Although concern for transmission of B19V from pooled plasma products has resulted in B19V DNA screening of input plasma donations, less is known about the potential for B19V transmission by transfusion of individual blood components (eg, red cells, platelets, plasma). There are only 4 published clinical cases of B19V transmissions from blood component transfusion (3 from red cells and 1 from platelets).[14-17] An additional asymptomatic case has been reported from a recent prospective study of transfusion-transmitted viral infections.[18] In contrast, 2 studies have reported a small number of negative results when patients transfused with B19V DNA–positive components were evaluated for laboratory markers of B19V infection.[19,20] Nevertheless, given the tropism for[21] and potential pathophysiologic effects of B19V infection on erythroid precursor cells,[22] concern remains for potential deleterious outcomes in frequently transfused hematology patients with underlying hemolysis or compromised erythropoiesis syndromes.[13]

Because the sensitivity of B19V DNA assays has improved, B19V DNA prevalence in blood donors has been shown to be

Submitted June 2, 2009; accepted July 29, 2009. Prepublished online as *Blood* First Edition paper, August 17, 2009; DOI 10.1182/blood-2009-06-225706.

An Inside *Blood* analysis of this article appears at the front of this issue.

The online version of this article contains a data supplement.

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higher than initially documented. B19V DNA is detectable in 0.5% to 0.9% of blood donations, with most showing relatively low DNA concentrations (< 100-1000 IU/mL).[23-25] In addition, it has become established that B19V infection is often persistent.[25-27] Thus, some donors may continue to donate for many years with B19V DNA (and potentially infectious virions) in their blood. These observations suggest that the potential for recipients to be exposed to low levels of B19V DNA from blood component transfusion is greater than previously thought.

To our knowledge, there have been no large-scale donor/recipient-linked transfusion-transmission studies to evaluate the rate of B19V transfusion transmission. Although it has been assumed by extrapolation from pooled plasma transfusions that single unit blood components with low-level B19V DNA should be noninfectious, this remains speculative because the mechanism of protection in the pooled plasma setting has not been established and may not apply to single unit transfusions.[12,13]

We undertook this present study to systematically evaluate whether transfusion of blood components with low or moderate levels of B19V DNA (defined as < 10^6 IU/mL) transmits infection to B19V-seronegative susceptible recipients.

Methods

Source of donor and recipient samples

Tested specimens were from the National Heart, Lung, and Blood Institute (NHLBI) Retrovirus Epidemiology Donor Study Allogeneic Donor and Recipient (RADAR) repository, which was established to investigate possible transfusion-transmitted infections and which has been described in detail in a previous publication.[28] Repository specimens were collected from 2000 through 2003 by blood centers and selected hospitals at 7 geographically dispersed US locations. Repository specimens consisted of 2 frozen 1.8-mL plasma aliquots and a 1.5-mL sample of frozen whole blood.

All enrolled donors and recipients gave informed consent for frozen specimen storage and for subsequent specimen testing for possible transfusion-transmissible infections, in accordance with the Declaration of Helsinki. The study protocol was approved by the institutional review board of each participating institution.

The linked portion of this donor-recipient repository contains pretransfusion and/or peritransfusion specimens and follow-up specimens, collected at a 6- to 12-month interval, from 3575 enrolled recipients. It also contains 13 201 donation specimens given by 12 408 distinct donors that were transfused to these recipients. The RADAR enrollment procedure targeted recipients with expected high 1-year survival rates; 88% were cardiac or vascular surgical patients, and the median recipient age was 68 years (range, 59-74 years). Recipients were not evaluated for coexisting immunosuppression, but this is considered unlikely given the primary diagnoses. The mean number of RADAR donation exposures per recipient was 3.9. The distribution of component types transfused was 77% red cells, 13% whole-blood–derived platelet concentrates, and 10% fresh-frozen plasma (FFP). In addition to receiving components with a stored donation specimen in the RADAR repository, these recipients also received a mean of 3.1 components not linked to stored RADAR donations.

The RADAR repository also contains 99 906 specimens from blood donations that were not transfused to enrolled RADAR recipients; this supplementary repository served as a sample source during the assay validation and donor prevalence phase of the study, which has previously been reported.[24]

Selection and testing of donations

All RADAR donations transfused to enrolled recipients were tested for B19V DNA, provided there was adequate specimen volume available.[24] Donations found reactive on the B19V DNA assay were subjected to DNA confirmatory and quantitative testing; confirmed positive donations were also tested for B19V IgG and IgM.

Selection and testing of recipients

Cases were recipients who were transfused with one or more B19V DNA–positive components. Control recipients were selected to measure the background rate of new infection as a result of factors other than transfusion of a B19V DNA–positive RADAR unit (ie, community-acquired infection in the 6- to 12-month follow-up interval or a transfusion-acquired infection from a B19V DNA–negative RADAR unit or a nontested, non-RADAR unit). A 1:2 case-control design was used to select control recipients fulfilling the following criteria: all RADAR units received by the recipient were B19V DNA negative, enrollment occurred at the same participating center in approximately the same time frame (to control for community-acquired infection), and age was within 10 years of the case recipient. Using this control selection algorithm, we established that all controls met preestablished age and center criteria, and 94.4% received their transfusion within 11 days of their matched recipient.

Enrollment specimens from all case and control recipients were tested for B19V IgG. Before knowledge of B19V IgG enrollment results, posttransfusion follow-up specimens from all cases and controls were tested for B19V IgG, IgM, and DNA (see "Assay methods"). A positive B19V DNA or IgM result on the follow-up specimen triggered additional testing of the enrollment specimen for these analytes.

For analysis, case and control recipients with negative B19V IgG results before transfusion were subsequently classified as B19V susceptible, and those with positive results were classified as B19V nonsusceptible.

Protocol for evaluating transfusion-transmission

B19V transmission was defined as seroconversion to IgG or IgM or new detection of B19V DNA. Because our previous experience with B19V antibody testing has shown that specimens near the cutoff could show fluctuating results on different test runs, we required that seroconversion be independently shown by 2 laboratories.

Assay methods

B19V DNA PCR assay. The B19V DNA polymerase chain reaction (PCR) assay was originally developed by Chiron Corporation and subsequently refined through collaboration between Chiron and Blood Systems Research Institute (BSRI). We previously reported data on assay performance on 5020 plasma samples from the unlinked donor portion of the RADAR repository.[24] The assay had a 50% limit of detection (LOD) of 1.6 IU/mL (95% confidence interval [CI], 1.2-2.1 IU/mL) and a 95% LOD of 16.5 IU/mL (95% CI, 10.6-33.9 IU/mL). We determined that the assay could be used as a quantitative as well as a qualitative assay; because quantitation might not be precise at the lower LOD, we categorized all specimens with quantitative DNA values of greater than 0 but less than 20 IU/mL as having a value of less than 20 IU/mL.

The assay, performed at BSRI, included a magnetic-bead B19V DNA capture step followed by a TaqMan real-time PCR assay targeting the VP1 region of the genotype 1 B19V genome. The assay was subsequently validated as detecting genotype 2 but does not detect genotype 3, which has been identified in Africa but which is very rare outside that continent.[20] An internal control sharing homologous primer region sequences but with a different internal probe binding sequence as the viral target was included in each assay tube. All captured target DNA from 0.5 mL input plasma and the spiked internal control was amplified in a single PCR reaction by using the same primer pair. Amplification and detection occurred in a 96-well optical plate by using dual-plexed TaqMan PCR technology. B19V target and internal control DNA were detected and distinguished by fluorophore-tagged sequence-specific probes. Each plate contained 2 known positive, 2 blinded negative, and 2 blinded positive controls and up to 90 study specimens. A more detailed assay description is provided in the previous publication.[24]

Because the chosen assay cutoff of a cycle threshold (C_T) of less than 40 was designed to maximize assay sensitivity, an algorithm was developed

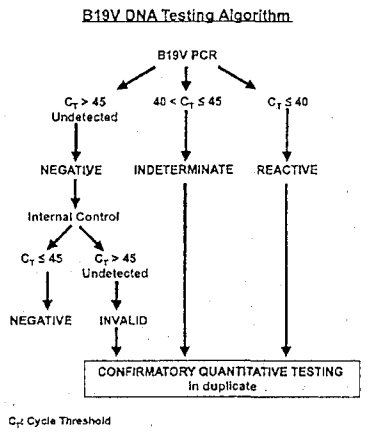

Figure 1. B19V DNA testing algorithm.

for final test interpretation so as to avoid classifying nonspecific reactivity on a single assay run as a confirmed positive result (Figure 1). All initially positive, indeterminate, and invalid specimens were retested in duplicate on plates that included quantitative run standards by using 2 separate 0.5-mL subaliquots subjected to the full extraction, amplification, and detection procedure. This testing served both as confirmation and quantitation. Final interpretation was based on the results of the 3 assays (ie, the initial screening assay and the duplicate repeat assays). Specimens were classified as B19V DNA positive if at least 2 of 3 tests showed reactivity at a C_T less than 40.

For determining DNA concentration, duplicate quantitative run standards (containing B19V DNA at 10^0 to 10^6 IU/mL) were placed on each plate, and quantitative results were determined by comparing the specimen C_T to the C_T of the known standards on the same test run.[24] The assigned quantitative value for each specimen was the average of the duplicate quantitative assays (including zero for a negative test result). Specimens with low C_T values (< 30) were diluted 1:10 and 1:100 and then run in triplicate at each dilution. The quantitative result was the average of the 3 test results at the most appropriate dilution adjusted by the dilution factor.

Serologic assays. Testing for B19V IgG and IgM was directed against a recombinant VP2 protein and was performed in duplicate by using FDA-cleared test kits (Biotrin) according to the manufacturer's instructions. Testing was conducted at BSRI and, for a large subset of samples, was repeated at a Center for Biologics Evaluation and Research/Food and Drug Administration (FDA) laboratory (Bethesda, MD). If results fell into the equivocal zone, the assay was repeated in duplicate on a new aliquot, and this repeat result was taken as the final result for the specimen.

Quantitative B19V IgG testing was performed by using a standard curve dilutional analysis method with the World Health Organization First International Standard for B19V serum IgG (93/724) obtained from the National Institute for Biological Standards and Control.[29] This testing was applied to enrollment and follow-up specimens of B19V IgG–positive ("nonsusceptible") recipients who had been transfused with the 5 highest titer B19V DNA components identified through donor testing.

Statistical methods

On the basis of a review of donor B19 viremia and recipient B19V serologic data from phase 1 of this study,[24] we determined that testing of the linked donor and recipient RADAR repository specimens would have sufficient statistical power such that a finding of zero documented transmissions to susceptible recipients would indicate with 95% confidence that the true B19 transfusion–transmission rate was between 0% and 25%. In this current study, StatXact (Cytel) was used to generate upper 95% confidence limits based on zero observed infections.[30] The upper confidence limit for transmission was calculated as a one-sided exact 95% confidence interval for the difference between the infection rate among susceptible cases and susceptible controls, using StatXact (Cytel).[30]

Results

Of the 13 201 linked blood donation repository specimens, 12 529 (95%) had adequate volume for testing. B19V DNA was detectable in 105 donations for a prevalence of 0.84% (95% CI, 0.68%-1.00%). As shown in Table 1, 53%, 71%, and 93% of these donations had B19V DNA concentrations below 20, 100, and 1000 IU/mL, respectively. The 2 donations with DNA concentrations greater than 10^6 IU/mL were negative for B19V-specific IgM and IgG, whereas B19V IgG was detectable in 96% and B19V IgM in 28% of the evaluable remaining B19V DNA–positive donations.

These 105 B19V DNA–positive donations came from 103 donors, 2 of whom gave positive donations on 2 occasions. The 105 positive donations resulted in the transfusion of 112 positive components to enrolled recipients. Four recipients received multiple DNA-positive components such that a total of 107 distinct recipients were transfused with one or more DNA-positive components. Table 2 provides a description of the DNA-positive components transfused to recipients, classified by the DNA concentration of the component, by whether the recipients were susceptible to B19V infection (ie, B19V IgG negative on their enrollment

Table 1. Quantitative B19V PCR and antibody results on confirmed positive donations

B19V DNA concentration, IU/mL, in donation	No. of B19V DNA–positive donations	No. (%) B19V IgM and IgG positive	No. (%) B19V IgM negative, IgG positive	No. (%) B19V IgM negative and IgG negative
Less than 20	56	2 (4%)	52 (93%)	2 (4%)
20 to less than 100	19*	5 (28%)	13 (72%)	0
10^2 to less than 10^3	23	18 (78%)†	2 (9%)	1 (4%)
10^3 to less than 10^4	4	4 (100%)	0	0
10^4 to less than 10^5	0	0	0	0
10^5 to less than 10^6	1	0	0	1 (100%)
Subtotal	103*	29 (28%)	67 (66%)	4 (4%)
More than 10^6	2	0	0	2
Total	105‡	29	67	6

The prevalence of B19V DNA–positive donations in 12 529 tested donations was 0.84%.
*One donor was not tested for B19V antibody; percentages have been calculated eliminating that donor from both the numerator and the denominator.
†Two donors were IgM equivocal and IgG positive.
‡The 105 B19V DNA–positive donations came from 103 donors, 2 of whom gave positive donations on 2 occasions.

Table 2. Transfusion of B19V DNA-positive components to recipients

B19V DNA concentration, IU/mL, in donation	No. of B19V DNA-positive donations	No. of B19V DNA-positive components transfused to susceptible recipients*				No. of B19V DNA-positive components transfused to nonsusceptible recipients				Total no. of B19V DNA-positive components transfused
		Red cells	Platelets	Plasma	Subtotal	Red cells	Platelets	Plasma	Subtotal	
Less than 20	56	15	0	1	16	33	6	5	44	60
20 to less than 100	19	3	0	0	3	9	5	3	17	20
10^2 to less than 10^3	23	3	1	0	4	16	3	2	21	25
10^3 to less than 10^4	4	0	0	1	1	2	0	1	3	4
10^4 to less than 10^5	0	0	0	0	0	0	0	0	0	0
10^5 to less than 10^6	1	0	0	0	0	1	0	0	1	1
Subtotal	103	21	1	2	24	61	14	11	86†	110†
More than 10^6	2	0	0	0	0	1	1	0	2	2
Total	105	21	1	2	24	62	15	11	88†	112†

*All B19V DNA-positive units transfused to susceptible recipients contained B19V-specific IgG.

†For 7 B19V DNA-positive donations, more than 1 component was transfused; also 4 nonsusceptible recipients received more than 1 positive component.

specimen), and the type of blood component. As per RADAR repository design, the majority (74%) of transfused DNA-positive components were red cell concentrates. Twenty-four of the 112 components (21%) were transfused into susceptible recipients. Among the 214 control recipients (2 controls selected per case), a very similar percentage (20%) were susceptible. Six of the 7 DNA-positive components with the highest concentrations were transfused to nonsusceptible recipients; these included all 3 components with DNA concentrations greater than 10^5 IU/mL.

The primary analysis of transfusion transmission was restricted to the 24 susceptible (B19V IgG negative) cases (21 transfused with red cells) and the 42 susceptible controls. There were no B19V infections observed in these 66 susceptible recipients based on the absence of B19V IgG, IgM, and DNA in the follow-up specimens. Thus, the transmission rate was 0% in both cases and controls, with an upper 95% CI of 11.7% in cases and 6.9% in controls. The transfusion-transmission rate was therefore estimated at 0.0% [0.0% (cases) − 0.0% (controls)], with an upper 95% CI of 11.7%.

Although IgG seroconversion could not be used as a criterion for establishing transfusion-transmission in nonsusceptible subjects (those with preexisting B19V IgG), the criteria of newly developed B19V DNA or IgM were still applicable. There were no such findings in case recipients. However, one IgM seroconversion was identified in a B19V IgG–positive (nonsusceptible) control recipient who remained DNA negative. Because this recipient was transfused with only 2 DNA-negative red cell units (and no non-RADAR units), it is likely that the IgM seroconversion represents a false-positive result or possibly a new community-acquired infection. Testing also identified B19V DNA in follow-up specimens of 3 other control recipients. However, testing of their enrollment specimens indicated that B19V DNA was present before transfusion at approximately the same concentration in all 3 cases. Furthermore, their enrollment and follow-up specimens were positive for B19V IgG antibodies. Thus, this pattern indicated persistent B19V infection (existing before receiving RADAR transfusions) rather than recent B19V acquisition.

To further evaluate whether transfusion with B19V DNA–containing units elicited an immune response in subjects with preexisting B19V IgG, we performed quantitative B19V IgG testing of enrollment and follow-up specimens of the 5 recipients who were B19V IgG positive at enrollment and who received the highest titer DNA-positive components, reasoning that these would provide the maximal stimulus for such an immune response. Pretransfusion B19V IgG levels were highly variable, ranging from 7 to 165 IU/mL. As seen in Table 3, 1 of the 5 recipients, who received the highest titer component (at a B19V DNA concentration of 2.9×10^{10} IU/mL or a total dose of $\sim 5.8 \times 10^{11}$ IU in the 20 mL plasma contained in the red blood cell component), showed a 4-fold increase in B19V IgG titer. This recipient had a relatively low pretransfusion titer of B19V IgG (15 IU/mL). Of the other 4 recipients, 1 showed a 2-fold increase, 2 had unchanged titers, and 1 showed an almost 2-fold decrease.

Discussion

In this study we identified donations that had a potential marker of B19V infectivity (ie, B19V DNA) through retrospective screening of blood donations and subsequently tested recipients of components from these donations for the development of new B19V infection. Our approach was designed to systematically determine a rate of transmission from all units with this potential infectivity marker and to establish either the presence or absence of transmission when it was known that a susceptible (ie, B19V IgG negative) recipient was transfused with a potentially infectious (ie, B19V DNA positive) unit. This study design is in contrast to most other B19V studies in which investigations were structured to prove that transmission occurred in a particular case.

On the basis of our finding of nontransmission in 24 evaluable susceptible (B19V seronegative) recipients of components with a B19V DNA concentration less than 10^6 IU/mL, we conclude that the rate of transmission from such components ranges from 0% to 11.7% (which is the upper 95% confidence bound); thus, either transmission from such components does not occur, or, if it does, it is a relatively uncommon event in comparison to most other transfusion-transmissible viruses in which infection rates exceed 50% (eg, HIV, HCV).[31]

Table 3. Antibody quantitation studies in recipients transfused with components with the highest B19V DNA concentrations

Transfused component results		Recipient results	
B19V DNA concentration, IU/mL, in donation	B19V IgM/IgG status	Enrollment B19V IgG titer, IU/mL	Follow-up B19V IgG titer, IU/mL
2.9×10^{10}	−/−	14.9	61.1
8.2×10^{7}	−/−	53.5	33.4
4.3×10^{5}	−/−	37.5	40.2
8.6×10^{3}	+/+	7.6	15.2
1.8×10^{3}	+/+	165.1	157.9

*One recipient who received a component with a DNA concentration of 3.1×10^3 IU/mL (which was also positive for B19V IgM and IgG) was not included in this table because the enrollment and follow-up specimens were both B19V IgG negative.

Our study is the first to evaluate transmission in multiple recipients who do not have preexisting B19V IgG and hence do not have this mechanism for potential protection against acquiring B19V infection. In a study from Africa, there was a single documented case of lack of B19V transmission to a susceptible pediatric recipient transfused with a red cell unit that had a B19V DNA concentration of 6×10^2 IU/mL in the presence of B19V IgG.[20] There are somewhat more data about the lack of transmission to recipients with preexisting B19V IgG. In a study conducted in an adult hematology service, 6 adult recipients with hematologic malignancies (5 of whom underwent stem cell transplantation) were identified as transfused with blood components that were retrospectively found to contain B19V DNA at less than 10^6 geq/mL; in 4 of 5 evaluated cases, the DNA-positive component also contained B19V IgG. Each recipient was B19V DNA negative when tested 3 to 18 days after transfusion,[19] and none showed clinical symptoms of B19V infection on retrospective chart review.[19]

The mechanism to explain lack of transmission to susceptible recipients by B19V DNA–containing units is unknown but could be related to the lack of a large enough inoculating dose of B19 virions to establish infection. This could be due to the ratio between infectious dose and virion number (which is not known), the low levels of transfused intact and/or replication competent virions in units with low DNA concentrations, or neutralization of otherwise infectious virions either by antibody in the transfused unit or by passively transfused antibody from other units.[12] In support of the latter explanations, we note that all DNA-positive units transfused to susceptible recipients in our study contained B19V-specific IgG. In addition,it is highly probable that all recipients of B19V DNA–containing components received some additional blood components with B19V IgG; this is based on our previous findings that 73% of donors who contributed to the RADAR repository had B19V IgG[24] and that RADAR recipients were transfused with an average of 7 blood components.[28]

Our negative transmission findings are consistent with previous publications that have shown that high plasma concentrations of B19V DNA are required for transmission in the setting of transfused pooled plasma products. The minimal infectious dose of B19V DNA documented to cause a symptomatic B19V infection in a recipient of factor VIII concentrate devoid of B19V IgG was 2×10^4 IU based on the infusion of 3 vials of a product with a DNA concentration of 6.5×10^3 IU/vial (ie, 1.3×10^3 IU/mL when each vial was reconstituted in a 5-mL volume).[3] Furthermore, we are aware of only one comprehensive quantitative transmission study of pooled plasma products manufactured from multiple donations.[11,32] That study, conducted approximately 10 years ago, was an open-label phase 4 trial of pooled plasma, solvent detergent-treated (PLAS + SD produced by Vitex, now defunct). One hundred B19V-seronegative volunteers were infused with product from 17 different manufacturing lots. Of 19 subjects who received the product from 3 lots that contained at least 2×10^9 geq B19V DNA (ie, 200 mL product infused at $> 10^7$ B19V DNA geq/mL), 18 seroconverted and 17 showed B19 viremia. Although the investigators expressed their results in geq/mL, it has subsequently become established that for B19V, an IU and a geq are approximately equivalent. In contrast, there were no seroconversions in 81 subjects who received product from 1 of 14 lots containing less than 10^4 geq/mL B19V DNA; however, the investigators did not more precisely quantitate the amount of B19V DNA in these nontransmitting lots.

In our study, which was designed to systematically study transmissibility from B19V DNA–positive units with less than 10^6 IU/mL, we transfused only 2 components with high B19V DNA concentrations ($> 10^7$ IU/mL) but were unable to directly evaluate their transmissibility in susceptible recipients, because both were transfused to recipients with preexisting B19V IgG. We used quantitative B19V antibody testing to investigate whether exposure to this very high B19V DNA concentration could stimulate the recipient's immune system to respond. Although not definitive, a 4-fold boost in B19V IgG in the follow-up specimen from one of these recipients suggests that a component with very high B19V DNA concentration ($\sim 5.8 \times 10^{11}$ IU B19V DNA infused) can result in an anamnestic response (implying transient active viral replication) in a previously exposed recipient when the pretransfusion antibody titer is relatively low (15 IU/mL in this recipient). Our results are consistent with similar 4-fold B19V IgG increases which were reported 1 month after transfusion in 2 of 2 B19V IgG–positive volunteers who remained asymptomatic after transfusion of 200 mL PLAS + SD at a B19V DNA concentration of 1.6×10^8 IU/mL.[32] In addition, in the previously described study of adult hematology patients, there was also one B19V IgG–positive recipient of a red blood cell unit containing 2.2×10^6 geq/mL of B19V DNA; this recipient was positive for B19V DNA at posttransfusion day 5, negative when retested on day 35, and asymptomatic for B19V infection on chart review; B19V IgG titer was not reported.[19]

Despite the large size of our linked donor–recipient repository, the use of a very sensitive B19V DNA assay, and a rigorous testing algorithm, this study was subject to several limitations. The collection of recipient follow-up specimens 6 to 12 months after transfusion limited the laboratory techniques that we could use to diagnose new B19V infection. In addition to our primary assessment of the development of new B19V IgG formation, we also tested for new appearance of B19V IgM and B19V DNA. However, the natural history of acute B19V infection predicts that both of these markers would probably no longer be detectable at the time our follow-up specimens were collected, unless the recipient had developed a persistent infection.[13,33] Our study was also limited because most recipients (78%) of B19V DNA–positive units were B19V IgG positive before transfusion and thus presumably were partially or totally protected against B19V reinfection. This limited the statistical power of our negative result such that the upper 95% CI could not rule out a transmission rate as high as 11.7%. Furthermore, most of the 24 susceptible recipients received components with very low B19V DNA concentrations (< 20 IU/mL). We identified only 5 transfused components with DNA concentrations between 10^3 and 10^6 IU/mL; 4 of these were B19V IgM and IgG positive, and one of these (DNA level of 4.3×10^5 IU/mL) lacked B19V antibody. Furthermore, only one of these components, a plasma unit containing a total infused dose of approximately 7×10^5 IU in the presence of B19V IgG, was transfused to a susceptible recipient. Similarly, although we identified 45 transfused components with B19V DNA concentrations between 20 and 1000 IU/mL, only 7 were transfused to susceptible recipients. Finally, although we obtained questionnaires from recipients at the time of follow-up (6-12 months after transfusion) and none of the recipients had been diagnosed with B19V disease, we were unable to definitively assess nonspecific symptoms that can occur with B19V infection at such a long interval after transfusion.

We expressed our findings as the rate of transmission in susceptible recipients because this allowed us to extrapolate our findings to other transfused recipient populations; ie, it allowed us to calculate a per unit risk. This per unit risk in our older surgical recipients can then be applied to populations with a higher susceptibility rate (eg, fetuses undergoing intrauterine transfusion, young patients with sickle cell anemia or thalassemia, patients with

congenital or acquired hypogammaglobulinemia), based on the assumption that the equivalent dose of B19V transfused into a B19V IgG–negative hematology or surgical patient will result in productive infection (ie, viral replication) at the same rate. In our opinion, it is unlikely that the infectivity of a B19V DNA–positive transfused unit will be related either to the underlying disease or to the overall immune status of a B19V seronegative recipient, even though it is well accepted that the clinical manifestations of a B19V infection will be influenced by such host factors (ie, if infected with B19V, an immunosuppressed patient or one with an underlying hemolytic syndrome might have a worse clinical outcome).[7]

We can also analyze our data on a population-wide basis; looked at in this way, we did not detect any cases of definite B19V transmission (with the exception of the one possible case of an anamnestic immune response) after the transfusion of blood components from 12 529 B19V DNA–tested donations into a recipient population with a pretransfusion B19V IgG prevalence of 78%.

As part of this study, we also generated a large body of blood donor data. We found that B19V DNA prevalence in 12 529 tested donations was 0.84%, consistent with our previous report of 0.88% in 5020 donation samples from the same RADAR repository and with higher end estimates in literature.[23-25] The large majority of our DNA-positive donations had low or very low DNA concentrations (53%, 71%, and 93% below 20, 100, and 1000 IU/mL, respectively), consistent with the interpretation that the increased DNA prevalence found in recent donor studies is due to the use of more sensitive nucleic acid testing assays. In contrast to the high rate of overall DNA detection, our rate of detection of high-titer DNA positives ($> 10^6$ IU/mL) was approximately 1 in 6000, consistent with both the newer and older literature.[7,34,35] These high-titer units are known to occur in the acute phase of B19V infection; thus, they lack both B19V IgG and IgM antibody as was the case in this study.[31] In contrast, 96% of the remaining DNA-positive donations were B19V IgG positive, which is the expected result in resolved or persistent infection.[35,36]

Current practices for blood donor screening for B19V in developed countries are almost exclusively confined to testing plasma designated for fractionation for the presence of high B19V DNA concentrations.[8,13] There has been recent debate about whether such screening should also be applied to transfused blood components; this is currently not done because of the lack of demonstrated adverse clinical outcomes from B19V infection in blood component recipients and the considerable expense of such testing. We are aware of only one country, Germany (which also performs blood testing for Austria), in which some blood banks currently conduct B19V DNA screening of blood donations and use the results to release blood components for transfusion.[35] Their testing is conducted in pools of 96 samples with an assay that can reliably detect units with B19V DNA greater than 10^5 IU/mL. Other German blood banks conduct B19V DNA testing retrospectively after the red cell component has been transfused.[35] In a recent abstract, preliminary data indicate that B19V transmission (documented by a positive B19V DNA test in the transfused recipient) from retrospectively tested red cell components occurred when the B19V DNA concentration was greater than 10^5 IU/mL but not when the concentration was below this threshold.[37]

Our study results confirm that, if prospective, real-time B19V DNA blood donor screening were to be performed, the assay sensitivity used in Germany (ie, detection limit $< 10^5$ IU/mL) is reasonable in that it ensures recipient safety while preventing unnecessary discard of a much larger number of blood components. Our findings do not support the need to use more-sensitive B19V DNA nucleic acid screening assays. In conclusion, our data indicate that blood components with B19V DNA less than 10^6 IU/mL (almost all of which contain B19V-specific antibody) are unlikely to transmit B19V infection.

Acknowledgments

We thank Dr Jim Bethel of Westat for statistical support, Leilani Montalvo of BSRI for performing B19V DNA assay validation work and for performing the B19V DNA assays reported in the study, Hailing Yan of CBER/FDA for performing some of the anti-B19V assays, and Dr Venkatakrishna Shyamala, formerly of Chiron Corporation, for research and development on the prototype assay and for further assay validation work.

This work was supported by NHLBI REDS-II (contracts N01-HB-47175 and N01-HB-57181).

The findings and conclusions in this article have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any agency determination or policy.

Authorship

Contribution: S.H.K., S.A.G., and M.P.B. designed the study; T.-H.L., L.H.T., D.S.T., and M.-y.W.Y. supervised laboratory testing; S.H.K., S.A.G., M.P.B., K.S.S., D.S.T., and H.Q. analyzed data; and S.H.K., S.A.G., M.P.B., and M.-y.W.Y. wrote the manuscript.

Conflict of interest disclosure: The authors declare no competing financial interests.

A complete list of the members of the NHLBI REDS-II appears in the supplemental Appendix (available on the *Blood* website; see the Supplemental Materials link at the top of the online article).

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医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2010. 1. 19	該当なし	
一般的名称	人血清アルブミン		研究報告の公表状況	Erlwein O, Kaye S, McClure MO, Weber J, Wills G, et al. PLoS ONE 5(1): e8519. doi:10.1371/journal.pone.0008519.	公表国 英国	
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)					

研究報告の概要

○慢性疲労症候群において新規レトロウイルスXMRVは検出されなかった

背景:2009年10月、米国の慢性疲労症候群(CFS)患者101名のうち68名が、異種指向性ネズミ白血病ウイルス関連ウイルス(XMRV;以前に前立腺がんとの関連性が示された新規ガンマレトロウイルス)に感染していることが報告された。本知見が確認された場合、世界中で数百万人が罹患し、身体機能を奪う当該疾患の理解と治療に多大な影響を及ぼすであろう。我々は、英国のCFS患者がXMRVキャリアであるかどうかを調べた。

方法:本試験のCFSコホート患者は、検査により他の器質性疾患を除外されており、CFSのCDC基準を満たしていた。CFS患者186名の血液検体から抽出したDNAについて、特異的オリゴヌクレオチド・プライマーを用いたnested PCRによる、XMRVプロウイルスおよび関連性の高いネズミ白血病ウイルス(MLV)のスクリーニングを行った。DNAの内部コントロールのため、細胞βグロビン遺伝子を増幅した。陰性対照(水)と陽性対照(XMRV感染分子クローンDNA)を含めた。βグロビン遺伝子を186名全員の検体で増幅したが、XMRVもMLV配列も検出されなかった。

結論:英国のCFS患者由来DNAからは、XMRVまたはMLV配列は増幅されなかった。本試験では英国のXMRVがCFSに関連する証拠を見つけなかったが、北アメリカとヨーロッパ間でのXMRV感染の一般有病率に集団差がある可能性があり、米国の2グループが前立腺がん組織にXMRVを発見したにもかかわらずヨーロッパの2試験で発見されなかったのは、このためであるかもしれない。

使用上の注意記載状況・その他参考事項等

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

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
英国の慢性疲労症候群患者186名の血液検体から、新規レトロウイルスXMRVのDNAは検出されなかったとの報告である。 XMRVはマウス白血病ウイルスと類縁な脂質膜を持つ大型RNAウイルスである。この性状からは本製剤の製造工程でウイルス不活化・除去されると期待されることから、本製剤の安全性は確保されていると考える。	注目すべきウイルスとして今後も引き続き、新たなウイルス等に関する情報の収集に努める。

(16)

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OPEN ACCESS Freely available online

PLoS one

JRC2010T-004

Failure to Detect the Novel Retrovirus XMRV in Chronic Fatigue Syndrome

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Abstract

Background: In October 2009 it was reported that 68 of 101 patients with chronic fatigue syndrome (CFS) in the US were infected with a novel gamma retrovirus, xenotropic murine leukaemia virus-related virus (XMRV), a virus previously linked to prostate cancer. This finding, if confirmed, would have a profound effect on the understanding and treatment of an incapacitating disease affecting millions worldwide. We have investigated CFS sufferers in the UK to determine if they are carriers of XMRV.

Methodology: Patients in our CFS cohort had undergone medical screening to exclude detectable organic illness and met the CDC criteria for CFS. DNA extracted from blood samples of 186 CFS patients were screened for XMRV provirus and for the closely related murine leukaemia virus by nested PCR using specific oligonucleotide primers. To control for the integrity of the DNA, the cellular beta-globin gene was amplified. Negative controls (water) and a positive control (XMRV infectious molecular clone DNA) were included. While the beta-globin gene was amplified in all 186 samples, neither XMRV nor MLV sequences were detected.

Conclusion: XMRV or MLV sequences were not amplified from DNA originating from CFS patients in the UK. Although we found no evidence that XMRV is associated with CFS in the UK, this may be a result of population differences between North America and Europe regarding the general prevalence of XMRV infection, and might also explain the fact that two US groups found XMRV in prostate cancer tissue, while two European studies did not.

Citation: Erlwein O, Kaye S, McClure MO, Weber J, Wills G, et al. (2010) Failure to Detect the Novel Retrovirus XMRV in Chronic Fatigue Syndrome. PLoS ONE 5(1): e8519. doi:10.1371/journal.pone.0008519

Editor: Douglas F. Nixon, University of California San Francisco, United States of America

Received December 1, 2009; **Accepted** December 4, 2009; **Published** January 6, 2010

Funding: AC, DC and SW are partially funded by the South London and Maudsley NHS Foundation Trust/Institute of Psychiatry National Institute of Health Biomedical Research Centre. The team from Imperial College is grateful for support from the NIHR Biomedical Research Centre Funding Scheme. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

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Introduction

A recent study by Lombardi *et al.* [1] describing a gammaretrovirus infection in 68 of 101 chronic fatigue syndrome (CFS) patients was notable not only for its claim of a new viral aetiology of a hitherto controversial disease, but also for the fact that proviral DNA could be amplified from the peripheral blood mononuclear cells (PBMC) of 3.75% (8/218) of the healthy controls. This follows an earlier claim that 1.7% (5/300) of healthy Japanese blood donors carried antibodies to the same virus [2]. The virus in question is a recently discovered retrovirus, Xenotropic Murine Leukaemia Virus (MLV)-Related Virus (XMRV).

In the original identification of XMRV in prostatic cancer stromal cells, Urisman *et al.* [3] confirmed by sequence analysis that XMRV is not a laboratory contaminant, as is often the case with claims of new retroviral associations with disease. It shares >90% sequence identity in *gag* and *env* (two of the three viral structural genes) with other xenotropic MLVs.

An association between XMRV and prostate cancer was strengthened with the demonstration of XMRV protein expression in malignant epithelial cells [4]. However, these results have not been duplicated in studies conducted in Europe [5–7]. Both prostate cancer and CFS have been linked to an Arg to Gln mutation at codon 462 (R462Q) in the RNaseL gene, an interferon-induced ribonuclease [8]. On activation, RNaseL destroys single stranded cellular and viral RNA, thereby preventing viral replication, blocking protein synthesis, triggering cellular apoptosis and providing an innate anti-viral response. The two US studies are of interest, not only because this would be a further example of a virus association with cancer, but because they represent the first demonstration of a gamma-retrovirus able to infect human cells, over-riding the intrinsic immune mechanisms that were believed to protect humans from MLV infection.

The XMRV sequences derived from prostate cancer tissue are identical to those from CFS patients, but differ from xenotropic MLV sequences, endorsing a genuine cross-species transmission. However, the claim that XMRV is preferentially found in prostate tumours from patients homozygous for the R462Q variant [3] is not borne out by the second prostate cancer study to find XMRV in patients [4], nor was the genetic variant detected in CFS patients carrying XMRV [5].

The finding of Lombardi *et al.* of a 67% XMRV infection rate among CFS patients, if confirmed, would have a serious impact on understanding the pathogenesis of this complex and debilitating disease and its treatment. Therefore, it was important to determine if CFS sufferers in the UK were carriers of XMRV. We have screened DNA extracts from the blood of CFS sufferers by PCRs targeted at an XMRV-specific sequence and at a sequence conserved amongst most murine retroviruses (MRV).

Methods

Patients

All patients gave written informed consent for the use of their DNA to test aetiological theories of CFS, and the study was approved by the South London and Maudsley NHS Trust Ethics Committee. The study recruited 186 patients (62% female, age range 19–70, mean 39.6±11.3years) from consecutive referrals to the CFS clinic at King's College Hospital, London. All patients had undergone medical screening to exclude detectable organic illness, including a minimum of physical examination, urinalysis, full blood count, urea and electrolytes, thyroid function tests, liver function tests, 9 a.m. cortisol and ESR. Patients were interviewed using a semi-structured interview for CFS [9] to determine whether they met international consensus criteria for CFS. All subjects met the CDC criteria [10]; patients with the Fukuda-specified exclusionary psychiatric disorders, or somatisation disorder (as per DSM-IV), were not included. The patient set studied is a well-characterised and representative sample of CFS patients who have been described previously: all were routine clinic attendees, referred within the UK National Health Service, who had taken part in prior studies of neuroendocrine functioning [11] and/or of cognitive behaviour therapy [12]. As is typical of the patients seen in this tertiary care centre, they were markedly unwell. Few were working, and 19% were members of patient support groups for CFS/ME [12–14]. The levels of fatigue in this sample were high (mean Chalder Fatigue Scale, 26.3±5.4) [15], as were levels of disability (mean Work and Social Adjustment Scale, total score 28.2±7.2) [16]. The mean GHQ-12 score [17] was 19.7±8.1. Patients had been unwell for a median of 4.0 y (range 1–28 y). Of note was that 45% said their illness definitely related to a viral illness and 45% said it might relate to a viral illness. Overall, we conclude that this sample is typical of CFS patients seen in specialist clinical services in the UK. We also know from collaborative studies that our patients resemble those seen in other specialist CFS services in the United States and Australia [18].

PCR detection of XMRV and MLV sequences. DNA was extracted from EDTA whole blood using a standard phenol-based organic deproteinisation procedure [19]. DNA concentrations were determined by absorbance at 260 nm (A_{260}). Each sample was amplified in three nested PCRs using primers targeted to an XMRV-specific sequence, to a sequence conserved amongst most MLV and, as a control for sample addition and PCR-inhibition, to a human beta-globin (hBG) sequence (Table 1). Each first-round reaction was performed in a 25 µl volume containing 0.5 units TaqGold (Applied BioSystems, Warrington, UK), 1 x TaqGold reaction buffer (Applied BioSystems), 1.5 mM Mg^{2+}, 200 mM each dNTP, 2.5 pmol each primer to which 5 µl DNA extract or control was added. Reaction conditions were one cycle of 94°C, 8 minutes, 35 cycles of 94°C 30 seconds, 55°C 30 seconds, 72°C 30 seconds and one cycle 0f 72°C, 7 minutes. Second round reaction mixes were identical to the first round and the sample was a 1 µl transfer from the first round reactions. Second round reaction conditions were as for the first round over 30 cycles. PCR amplicons were visualised on a 1% agarose gel stained with ethidium bromide. Each PCR run consisted of test samples, six negative (water) and two positive controls. The positive control was a dilution of a plasmid with a full-length XMRV (isolate VP62) insert, generously gifted by Dr R. Silverman. To validate the sensitivity of the PCR, an end-point dilution of the plasmid was performed. To determine specificity of the PCR, a sample of human DNA from the LNCaP prostate cancer cell line (American Type Culture Collection, code CRL-1740) was amplified with the XMRV and MLV primer sets. To ensure integrity of the DNA extracts, three randomly selected samples were titrated to end-point using the hBG PCR to determine if the PCR copy number equated with the A_{260}. To determine if the DNA extracts exhibited low level non-specific inhibition of PCR, 10 samples were subjected to 30 cycles of the first round hBG PCR (reaction mix and conditions as above) followed by 40 cycles of a nested real-time SYBR-green PCR using the SYBR-green Fast PCR kit (Roche, Lewes UK) according to the manufacturer's instructions.

Table 1. Oligonucleotide Primers.

Target	Sequence		Location
XMRV	Forward outer	5'CATTCTGTATCAGTTAACCTAC 3'	411–432[1]
	Reverse outer	5' ATGATCTCGAGAACACTTAAAG 3'	606–588[1]
	Forward inner	5' GACTTTTTGGAGTGGCTTTGT 3'	441–461[1]
	Reverse inner	5' ACAGAAGAACAACAAAACAAATC 3'	566–544[1]
MLV	Forward outer	5' GGATCAAGCCCCACATACAG 3'	2796–2847[1]
	Reverse outer	5' CATCAAACAGGGTGGGACTG 3'	3179–3160[1]
	Forward inner	5' AGAAGTCAACAAGCGGGTGG 3'	2926–2945[1]
	Reverse inner	5' GGTGGAGTCTCAGGCAGAAA 3'	3062–3043[1]
hBG	Forward outer	5' TGGTGGTCTACCCTTGGACC 3'	148–162[2]
	Reverse outer	5' GAGGTTGTCCAGGTGAGCCA 3'	296–277[2]
	Forward inner	5' GAGGTTCTTTGAGTCCTTTGG 3'	170–190[2]
	Reverse inner	5' CATCACTAAAGGCACCGAGCA 3'	273–253[2]

Locations in GenBank accessions [1]EF185282, [2]NM000518.4.
doi:10.1371/journal.pone.0008519.t001

Results

Nested PCR Validation

Based on A_{260} of the purified plasmid, both primer sets (XMRV, MLV) were able to amplify a single target copy added to the reaction. Amplification of 600 ng of LNCaP cellular DNA added to XMRV and MLV PCRs yielded no non-specific bands when viewed on an ethidium bromide-stained agarose gel. Quantification of DNA samples from three randomly selected test samples by end-point dilution PCR with the hBG primer set showed concurrence of the PCR-determined copy number with A_{260}, thus indicating integrity of the DNA preparations. Nested real-time amplification of 10 samples showed no evidence of non-specific inhibition as determined by the slope of the amplification curves and the height of the signal plateau.

PCR Analysis of Test Samples

Input DNA ranged from 10 to 600 ng (1.6×10^3 to 1.1×10^5 cell equivalents) as determined by A_{260} of which 149 samples had an input of >100 ng and 106 samples >200 ng. None of the 186 test samples analysed yielded a specific PCR product with either the XMRV or MLV primer sets and no non-specific PCR products were observed. A specific hBG product was amplified from all 186 test samples. The positive control was amplified in each run by the

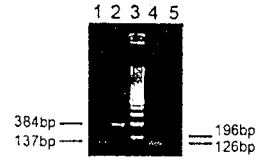

Figure 1. PCR products of the XMRV VP62 clone. Primers are generic to MLV (lanes 1 and 2) or specific to XMRV (lanes 4 and 5). The sizes of the respective fragments are shown. Lane 3–200 bp molecular size ladder.
doi:10.1371/journal.pone.0008519.g001

XMRV and MLV primer sets. A stained gel of the XMRV and MLV PCR products is shown in figure 1 and a representative sample of our results with CFS DNA and MLV primers is shown in figure 2.

Discussion

Unlike the study of Lombardi *et al.*, we have failed to detect XMRV or closely related MRV proviral DNA sequences in any sample from CFS cases. There have been numerous claims for an infective aetiology to CFS over the years, not least because, as in this sample, many patients report that their symptoms were triggered by an infective episode. Prospective epidemiological studies have confirmed that certain infective agents, for example Epstein Barr virus, are unequivocally associated with subsequent CFS [20], even if the mechanisms are unclear and almost certainly multi factorial. Nearly two decades ago, sequences from another retrovirus, the human T-lymphotropic virus type II, were amplified from the PBMCs of 10/12 (83%) adult and 13/18 paediatric CFS patients, but not from healthy control subjects [21]. However, subsequent studies carried out on small numbers (20–30) of CFS patients, failed to confirm evidence for HTLV (type I or II) [22–25] or other retroviruses, including the closely-related simian T lymphotropic virus type I, the prototype foamy virus, simian retrovirus, bovine and feline leukaemia viruses [26] and HIV-1 [23].

The Lombardi paper is the first to study a significantly larger number of people than that in any previous study and to detect a virus only recently discovered. Our study resembles that of Lombardi *et al.* in certain respects. Both studies use the widely accepted 1994 clinical case definition of CFS[10]. Lombardi *et al.* reported that their cases "presented with severe disability" and we provide quantifiable evidence confirming high levels of disability in our subjects. Our subjects were also typical of those seen in secondary and tertiary care in other centres.

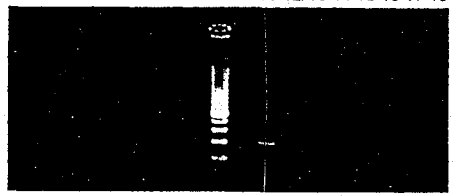

Figure 2. Nested PCR from the DNA of 8 CFS patients. Products of generic MLV primers (including XMRV) are shown. Lanes 1–8, CFS patient DNA (2nd round); lanes 9 and 10, XMRV 2nd round and 1st round positive controls; lanes 11 and 12, DNA of uninfected cell line LNCaP; lanes 13–18, water controls.
doi:10.1371/journal.pone.0008519.g002

Our own study also differs from that of Lombardi in other respects. Firstly, the PCR operator was blinded to the provenance of the DNA samples. In fact, with the exception of the PCR controls, all 186 DNA test samples originated from CFS patients. Care was taken to grow the XMRV plasmid in a laboratory in which no MLV had been cultured and no MLV vectors used and the PCR was carried out in a CPA-accredited Molecular Diagnostics Unit which processes only human tissue. Multiple (six) water (negative) controls were included in every run to detect low level contamination and a PCR to amplify a sequence that is conserved in most murine leukaemia viruses was included in order to expose any circulating MLV contamination and to detect any variant of XMRV that might be circulating in the UK CFS population.

Based on our molecular data, we do not share the conviction that XMRV may be a contributory factor in the pathogenesis of CFS, at least in the U.K.

Acknowledgments

The assistance of Sarah Bull in data collection and processing is gratefully acknowledged.

Author Contributions

Conceived and designed the experiments: SK MM. Performed the experiments: OWE SK. Analyzed the data: SK MM. Contributed reagents/materials/analysis tools: SK GW DC SW AC. Wrote the paper: SK MM. Facilitated the study by setting up the collaboration: JW. Responsible for providing samples and associated data from a well characterised and valuable cohort of subjects: SW.

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別紙様式第２－１

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医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日 2010年1月25日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	別紙のとおり		研究報告の公表状況	2009-2010 Influenza Season Week 1 ending January 9, 2010 : 3	公表国 米国	
販売名（企業名）	別紙のとおり					

研究報告の概要	問題点：the Iowa Department of Public Health から、ヒトにおける初のブタインフルエンザA(H3N2)感染事例が報告された。 ヒトにおける新規インフルエンザAウイルスの感染事例1例が the Iowa Department of Public Health から報告された。患者は2009年9月に発症したが、入院の必要は無く、回復した。同ウイルスはブタインフルエンザA（H3N2）と同定され、2009年11月に精査された。ブタからの暴露は不明である一方、同ウイルスのヒトーヒト感染の証拠は認められていない。新規インフルエンザA感染事例の速やかな同定及び精査は流行の拡大規模及びヒトーヒト感染の可能性の評価に重要である。新規インフルエンザAウイルスのヒト感染における調査は通年で実施されている。

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

使用上の注意記載状況・その他参考事項等
記載なし。

MedDRA/J ver.12.0