**医薬品**
**医薬部外品　研究報告 調査報告書**
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| 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 | 機構処理欄 |
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| | | | | 2004.6.3 | 該当なし | |

| 一般的名称 | 乾燥濃縮人血液凝固第Ⅷ因子 | 研究報告の公表状況 | First International Conference of the European Network of Excellence Neuroprion (Paris May 24-28, 2004) Dia-17 | 公表国 スイス |
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| 販売名(企業名) | クロスエイトM250　(日本赤十字社)<br>クロスエイトM500　(日本赤十字社)<br>クロスエイトM1000　(日本赤十字社) | | | |

**研究報告の概要**

vCJD では、PrP(Sc)が神経以外の部位、主にリンパ組織で検出されることから、他のヒトプリオン病とは異なると考えられている。しかし最近の報告から、スイスの孤発性クロイツフェルトヤコブ病（sCJD）患者の3分の1において、骨格筋、脾臓中に非常に低レベルのPrP(Sc)の蓄積が認められた。これはPrP(Sc)が中枢神経系から拡散する可能性や、これら特定のスイス人症例にみられる特殊な状況を示唆している可能性がある。この問題に取り組むために、燐タングステン酸Naによる高感度なウェスタンブロット法を用いて、英国vCJDサーベイランスユニットにおいて英国人のsCJD、vCJD筋肉検体をスクリーニングした。この結果、sCJD患者1例の少なくとも筋肉検体1件でPrP(Sc)の存在が確認された。PETブロット解析により、この結果は確定し、筋線維におけるPrP(Sc)の不均質分布を示した。これらの研究結果から、異なる組織におけるPrP(Sc)の有無を確認するためには、複数検体の採取と、高感度な手法を用いることの重要性が改めて確認された。今回の筋肉検体はサブタイプMV1型sCJD患者1例のもので、著しく長い罹患期間（4年間）など、複数の点が非定型であった。今回の結果から、筋肉中のPrP(Sc)の存在は、これらのスイス人症例のみに限定されるものではないこと、またsCJD患者の神経外組織におけるPrP(Sc)の蓄積は罹病期間と相関する可能性があることが示唆された。

| 報告企業の意見 | 今後の対応 |
|---|---|
| sCJDの患者の骨格筋、脾臓において低レベルのPrP(Sc)が検出されたことから、PrP(Sc)が中枢神経系から拡散する可能性があることとその蓄積は罹病期間と相関する可能性を示唆する報告である。 | これまでの疫学研究等では、ヒトにおいて、血漿分画製剤を介してvCJDが伝播するという証拠はない。また異常プリオンがアルブミン製剤の製造工程で効果的に除去されるとの報告もあるが、理論的リスクを完全には排除できないため、今後も情報の収集に努める。 |

**使用上の注意記載状況・その他参考事項等**

クロスエイトM250
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血液を原料とすることに由来する感染症伝播等
理論的なvCJD等の伝播のリスク

# Poster Session 2

## DIA-17 AN ANALYSIS OF MUSCLE SAMPLES FROM CASES OF SPORADIC AND VARIANT CREUTZFELDT-JAKOB DISEASE FOR THE PRESENCE OF PRP(SC)

AH PEDEN, M GLATZEL, DL RITCHIE, MW HEAD, A AGUZZI, JW IRONSIDE.
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Variant Creutzfeldt-Jakob disease (vCJD) is thought to differ from other human prion diseases in that PrP(Sc) can be detected at extraneural sites throughout the body, principally in the lymphoid tissues. However, a recent report shows PrP(Sc) at very low levels in skeletal muscle and/or spleen from a third of Swiss patients with sporadic Creutzfeldt-Jakob disease (sCJD). This may represent centrifugal spread from the CNS or may indicate something particular about these specific Swiss cases. In order to address this issue we have used the same high-sensitivity Western blot technique, involving precipitation with sodium phosphotungstic acid, to screen sCJD and vCJD muscle samples of UK origin at the National CJD Surveillance Unit. These studies have led to the identification of at least one positive muscle sample from a case of sCJD. PET blot analysis confirmed this result and showed a heterogeneous distribution of PrP(Sc) in muscle fibres. These findings highlight the importance of taking multiple samples and employing high sensitivity techniques to determine whether PrP(Sc) is present in different tissues. The positive muscle sample came from a case of sCJD with MV1 subtype and was atypical in a number of respects including an unusually long clinical phase (4 years). These results suggest that the presence of PrP(Sc) in muscle is not an exclusive characteristic of current Swiss cases and that accumulation of PrP(Sc) in extraneural tissues in sCJD may be a function of disease duration.

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| 識別番号・報告回数 | | | 報告日 | 第一報入手日<br>2004年7月23日 | 新医薬品等の区分<br>該当なし | 厚生労働省処理欄 |
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| 一般的名称 | ①②③④人血清アルブミン<br>⑤乾燥濃縮人血液凝固第Ⅷ因子<br>⑥乾燥濃縮人血液凝固第Ⅸ因子 | 研究報告の公表状況 | Department of Health / Publications and statistics/ Press releases / 2004/0270 | 公表国<br>イギリス |
| 販売名<br>（企業名） | ①献血アルブミン-Wf（ベネシス）<br>②献血アルブミン(5%)-Wf（ベネシス）<br>③アルブミン-Wf（ベネシス）<br>④アルブミン-ヨシトミ(20%)（ベネシス）<br>⑤コンコエイトーHT（ベネシス）<br>⑥クリスマシンーM（ベネシス） | | | |

**研究報告の概要**

英国保健省は2004年7月22日、輸血を介しての2例目のvCJD伝播が、National CJD Surveillance Unitによって確認されたことを公表した。この2例目の患者は1999年に、後にvCJDを発症したドナーからの輸血を受け、vCJDとは関係のない原因で亡くなったが、検死によって患者の脾臓からvCJD病原体の存在が明らかになったというものである。この2例目が特に科学的に興味を引くのは、この患者がこれまでvCJDを発症した患者から見つかったのとは異なるタイプの遺伝型を有していたことである。この症例の詳細は、Lancet誌に近々公表が予定されている。
輸血を介してのvCJD伝播を防ぐための新たな血液ドナー排除基準公表（※）についても記載されている。
※本年4月5日から開始された1980年1月以降に輸血を受けた血液ドナーを排除するとする基準に加えて、同じく1980年1月以降において、以前に輸血を受けたか否かが明確でない血液ドナーを排除するとするものと、以前に輸血を受けたことのあるアフェレーシスドナーを排除するとするもので、本年8月2日から実施される。

**使用上の注意記載状況・その他参考事項等**

本報告は献血ヴェノグロブリンーIH ヨシトミを代表製剤として、外国措置報告している(識別番号G-040000130、報告日 2004年7月30日)

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

| 報告企業の意見 | 今後の対応 |
|---|---|
| 本情報は、英国保健省によって公表された、英国における輸血を介したvCJD伝播の2例目の可能性例が確認されたとする報告である。<br>2003年12月に英国保健省によって公表された第1例目の可能性例に加え、本事例（2例目）によって、輸血を介したヒトからヒトへのvCJD伝播の可能性が更に高まった。これまで血漿分画製剤からのvCJD伝播の報告はされていない。しかしながら、万一vCJD感染者の血液が原料に混入した場合には、血漿分画製剤の製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ないため、弊社においても血漿分画製剤の製造工程におけるTSEの感染性の低減に関する検証実験を行っている。なお、弊社血漿分画製剤の原料供給元である日本及び米国においては現在のところ、vCJDの発症例はない。 | 今後、本情報に関連する各国の規制当局や専門家による分析・評価等の情報の収集に努め、監視を続ける。 |

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番号12 参考資料

Department of Health



# Update on precautions to protect blood supply

**Published:** Thursday 22 July 2004
**Reference number:** 2004/0270

Following advice from the Committee on the Microbiological Safety of Blood and Tissue (MSBT) further measures to reduce the risk of transmission of variant Creutzfeldt Jakob Disease (vCJD) via blood transfusion were announced today.

Following the first resport of a possible transmission of vCJD from person to person via blood in December 2003 it was recommended that recipients of blood transfusions since January 1980 be excluded from donating blood in the future. This precautionary measure was implemented from April 5th this year.

Today two further groups who have received transfusions since January 1980 will be added to those cluded from giving blood in the future. They are:

donors who are unsure if they have previously had a blood transfusion; and

apheresis donors who have previously had a blood transfusion. Apheresis donors are a small pool of committed donors who make frequent attendances to centres to donate blood, where machine processing removes only certain blood components, and the rest is returned to the donor.

When actions were taken in April 2004 neither of these groups were excluded until any potential impact on the blood supply became clearer. However, it has become apparent that the impact on blood supplies is small and MSBT has therefore recommended that these additional groups can be excluded. These new exclusions will take effect from 2nd August 2004.

In a separate development, a second case of possible transmission of vCJD from person to person via blood tranfusion has now been confirmed by the National CJD Surveillance Unit. A patient in the UK received a blood transfusion in 1999 from a donor who later went on to develop vCJD. The patient died of causes unrelated to vCJD but a post mortem revealed the presence of the vCJD agent in the patient's spleen.

After the first person to person transmission of vCJD was indentified it was expected that further cases may follow. This second case is of particular scientific interest as the patient had a different genetic type to that far found in patients who have developed vCJD. A detailed account of the case will be appearing in The Lancet medical journal shortly.

Precautions already in place to protect the blood supply include:

Since 1997 all cases of vCJD that are reported to the National CJD Surveillance Unit and diagnosed as having 'probable' vCJD, result in a search of the National Blood Service blood donor records. If the patient has given blood, subsequently any stocks of that blood are immediately destroyed.

Since 1998, plasma derivatives, such as clotting factors, have been prepared from plasma imported from the USA.

Since October 1999, white blood cells (which may carry the greatest risk of transmitting vCJD) have been removed from all blood used for transfusion.

In August 2002 we announced that fresh frozen plasma for treating babies and young children born after 1st January 1996 would be obtained from the USA.

In December 2002, the Department of Health completed its purchase of the largest remaining independent US plasma collector, Life Resources Incorporated. This secures long-term supplies of non-UK blood plasma for the benefit of NHS patients.

The Secretary of State for Health John Reid said:

"We are continuing to follow a highly precautionary approach. Although people may have concerns about the implications of this announcement, I would emphasise again that the exclusion crieteria are being tightened because of a small but unquantifiable risk. People should continue to have a blood transfusion when it is really necessary. Any slight risk associated with receiving blood must be balanced against the significant risk of not receiving that blood when it is most needed.

"People who can, should continue to give blood. Blood donation is a safe procedure and people should continue to donate blood regularly. We place great value on those who already donate and would welcome new donors."

For media enquiries ONLY please contact the Department of Health press office on the numbers provided:

**Contact** Press Office

**Phone** **Press Officers**
020 7210 5649/5656/5282.

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|---|---|---|---|---|---|---|
| | | | | 2004年8月10日 | 該当なし | |

| 一般的名称 | ①乾燥抗HBs人免疫グロブリン<br>②ポリエチレングリコール処理抗HBs人免疫グロブリン | 研究報告の公表状況 | The Lancet, 364,527-529, 2004 | 公表国<br>イギリス |
|---|---|---|---|---|
| 販売名<br>（企業名） | ①ヘブスブリン（ベネシス）<br>②静注用ヘブスブリンーIH（ベネシス） | | | |

## 研究報告の概要

1999年に、1例の高齢患者が1ユニットの白血球除去していない赤血球の輸注を受け、その赤血球はドネーションの18ヶ月後にvCJDの症状を発症したドナーからのものであった。そのドナーは2001年に死亡し、vCJDであったことは解剖後が確認された。レシピエントはこの輸注の5年後に死亡したが、その際神経疾患を疑わせる証拠は認められなかった。直接の死因は腹部動脈瘤破裂であった。
この患者のプリオンタンパク質遺伝子(PRNP)のコドン129はヘテロ接合(メチオニン/バリン)であることが判明した。ウエスタンブロット分析では、脾臓中に$PrP^{res}$が存在することが示された。脾臓のシグナルの移動度と糖型比は、慢性vCJD患者から得た脾臓およびCJDでない脾臓中にvCJDの脳を希釈したものと同様であり、それらのシグナルは孤発性CJD症例のサブセットで報告されているものとは全く別なものであった。脳(1,337g)に見られた変化は加齢に伴うものであり、病理学的にはvCJDの特徴は示していなかった。$PrP^{res}$は脳および脊髄中に検出されなかった。扁桃、もう一方の頚部リンパ節、脊髄後根神経節、および筋肉のサンプルについてはウエスタンブロットでは$PrP^{res}$は認められず、扁桃、虫垂、および大腸内のリンパ濾胞でも免疫組織化学的方法で$PrP^{res}$は検出されなかった。
この報告は英国で臨床症状出現前にvCJD感染が解剖で検出された最初の症例記録である。我々はこれまでに、vCJD発症の8ヶ月前および2年前に虫垂摘出術を受けた2例の患者から得た虫垂組織の胚中心内に症状発現前であってもPrP免疫反応性が見られたことを示した。今回の症例の脾臓および頚部リンパ節の胚中心内へのPrP蓄積のパターンは、大規模な検体の由来を匿名化した遡及的研究で調べた3検体の外科的に切除した虫垂中に認められたものと類似しており、このことはこれらの所見が症状出現前にvCJD感染を示す可能性を示唆している。我々の所見はPRNPのコドン129がヘテロ接合である人に$PrP^{res}$のウエスタンブロット分析を行うことによってvCJD感染を確認しうることを示している。
この患者のPRNPのコドン129がヘテロ接合であることはvCJD感染がPRNPのメチオニンホモ接合の遺伝子型のヒトに限定されているわけではないことを示している。これらの知見は英国におけるvCJDの今後の発症予測とサーベイランスに大きな意味がある。

## 使用上の注意記載状況・その他参考事項等

本報告は献血ヴェノグロブリンーIH ヨシトミを代表製剤として、外国措置報告している(識別番号 G-040000130、報告日 2004年7月30日)。
代表として静注用ヘブスブリンーIHの記載を示す。

代表として静注用ヘブスブリンーIHの記載を示す。
2. 重要な基本的注意
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## 報告企業の意見

英国における輸血を介した2例目のvCJD伝播可能性例（研究報告No.9）の患者のPRNPコドン129がヘテロ結合であり、vCJD感染がPRNPのメチオニンホモ接合の遺伝子型のヒトに限定されているわけではないことを示唆する報告である。
これまで血漿分画製剤からのvCJD伝播の報告はされていない。しかしながら、万一vCJD感染者の血液が原料に混入した場合には、血漿分画製剤の製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ないため、弊社においても血漿分画製剤の製造工程におけるTSEの感染性の低減に関する検証実験を行っている。なお、本剤の原料供給元である米国においては現在のところ、vCJDの発症例はない。

## 今後の対応

今後、本情報に関連する各国の規制当局や専門家による分析・評価等の情報の収集に努め、監視を続ける。

# Preclinical vCJD after blood transfusion in a *PRNP* codon 129 heterozygous patient

*Alexander H Peden, Mark W Head, Diane L Ritchie, Jeanne E Bell, James W Ironside*

*Lancet* 2004; 264: 527–29

See Comment page 477

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**We report a case of preclinical variant Creutzfeldt-Jakob disease (vCJD) in a patient who died from a non-neurological disorder 5 years after receiving a blood transfusion from a donor who subsequently developed vCJD. Protease-resistant prion protein ($PrP^{res}$) was detected by western blot, paraffin-embedded tissue blot, and immunohistochemistry in the spleen, but not in the brain. Immunohistochemistry for prion protein was also positive in a cervical lymph node. The patient was a heterozygote at codon 129 of *PRNP*, suggesting that susceptibility to vCJD infection is not confined to the methionine homozygous *PRNP* genotype. These findings have major implications for future estimates and surveillance of vCJD in the UK.**

In 2003, an elderly patient in the UK was diagnosed with variant Creutzfeldt-Jakob disease (vCJD) that seemed to have been transmitted by a transfusion of non-leucodepleted red cells from a patient who developed vCJD after the donation.[1] The same investigation also reported 17 individuals alive in December, 2003, who had received labile blood components from donors who subsequently developed vCJD.[1] We report an autopsy detection of a preclinical case of vCJD infection, which appears to have been transmitted by blood transfusion in one of this cohort.

In 1999, an elderly patient received a unit of non-leucodepleted red blood cells from a donor who developed symptoms of vCJD 18 months after donation. The donor died in 2001 and vCJD was confirmed after autopsy. The recipient died 5 years after receiving the transfusion, with no evidence of a neurological disorder. Medicolegal instruction for autopsy was issued. The immediate cause of death was a ruptured abdominal aortic aneurysm. We are bound by a medicolegal restriction regarding disclosure of the patient's age, sex, and geographical location.

We assessed samples of frozen brain, spinal cord, dorsal root ganglion, lymphoid tissues, and muscle for the presence of protease-resistant prion protein ($PrP^{res}$) by western blot with phosphotungstic acid precipitation and the monoclonal antibody 3F4.[2] Immunohistochemistry and paraffin-embedded tissue blotting was done on protease-treated tissue sections from a wide range of tissues, with a panel of four antibodies raised against different epitopes of prion protein (PrP).[3] Restriction fragment length polymorphism analysis of DNA extracted from frozen brain material identified the patient as being heterozygous (methionine/valine) at codon 129 of the prion protein gene (*PRNP*). Consent for full sequence analysis of *PRNP* had not been obtained. Western blot analysis showed the presence of $PrP^{res}$ in spleen (figure 1). The mobility and glycoform ratio of the signals in spleen were similar to those seen in spleen from patients with clinical vCJD and in vCJD brain diluted in non-CJD spleen (figure 1), and were distinct from those described in a subset of sporadic CJD cases, usually with a relatively lengthy clinical illness.[2] We found that $PrP^{res}$ positivity by this method was a consistent feature of four autopsy specimens of spleen from patients who had vCJD, but was absent from a series of nine spleens from controls without CJD (data not shown).

The brain (1337 g) showed only age-related changes, with no pathological features of vCJD. $PrP^{res}$ was undetectable in the brain and spinal cord by western blotting, paraffin embedded tissue blotting, and immunohistochemistry. Immunoreactivity for PrP was found in a few germinal centres in the spleen, in a pattern consistent with staining of follicular dendritic cells (figure 2, A). The number of positive follicles was far lower than in clinical cases of vCJD, with a less aggregated accumulation of immunoreactivity.[3] Immunoreactivity for PrP was also found in a germinal centre within a cervical lymph node, with similar pattern of positivity to that noted in the spleen (figure 2, B). $PrP^{res}$ was not detectable by western

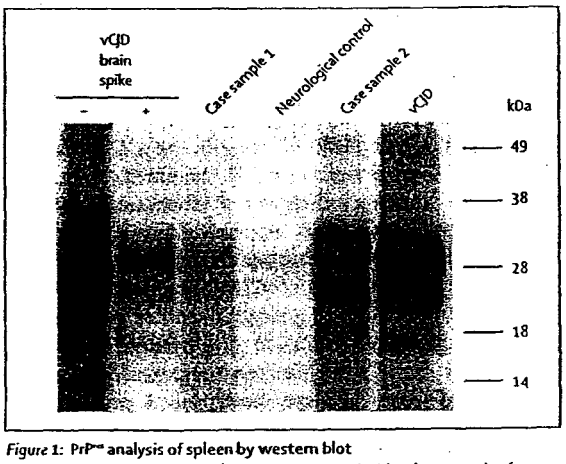


*Figure 1:* $PrP^{res}$ analysis of spleen by western blot
Two samples of the patient's spleen were compared with spleen samples from a control with non-CJD neurological disease and from a patient with vCJD, and with vCJD brain homogenate (10μg) diluted in non-CJD spleen, with (+) or without (–) proteinase K digestion. Every lane represents the phosphotungstate precipitate from 50 mg wet weight of spleen. Horizontal lines indicate positions of molecular weight markers. Amounts of $PrP^{res}$ in eight samples of spleen from the patient were undetectable in two samples (not shown), intermediate in five (sample 1), and similar to those found in the spleen of a patient with vCJD at autopsy in one (sample 2).

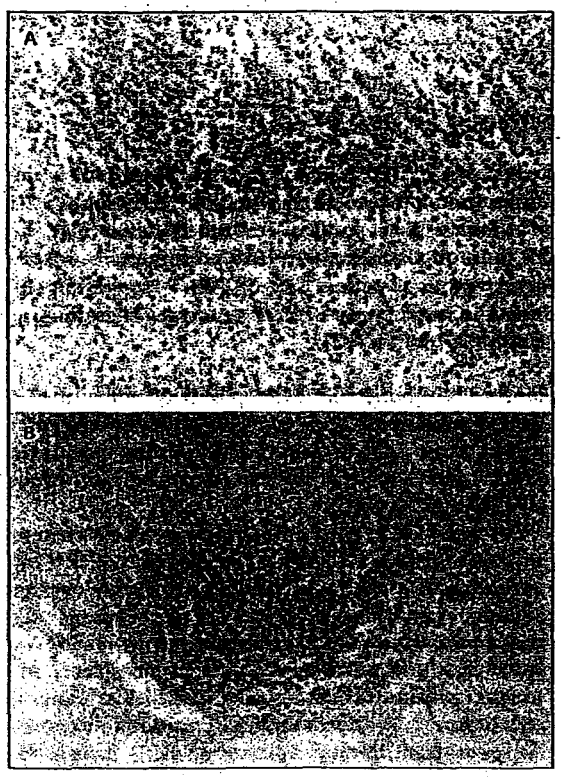

Figure 2: PrP in germinal centres within the spleen and cervical lymph node
Germinal centres are labelled (brown) with the anti-PrP antibodies 3F4 in spleen (A) and 12F10 in a cervical lymph node (B) in a pattern similar to that noted in the follicular dendritic cell network, with less aggregated positivity than in cases of clinical vCJD. Original magnifications (A) × 20 and (B) × 10.

blotting in samples of tonsil, another cervical lymph node, dorsal root ganglion, and muscle; neither was it detected in the lymphoid follicles within the tonsil, appendix, and large intestine by immunohistochemistry.

This is the first recorded case in the UK of autopsy detection of preclinical vCJD infection. We have previously shown preclinical PrP immunoreactivity in germinal centres within appendix tissue from two patients who underwent appendectomy 8 months and 2 years before the onset of vCJD.[4] The patterns of PrP accumulation within the germinal centres in the spleen and cervical lymph node in the present case were similar to those seen in three surgically removed appendices from a large anonymised retrospective study, suggesting that these findings might also represent preclinical vCJD infection.[4]

Our findings also show that vCJD infection can be confirmed by western blot analysis of $PrP^{res}$ in an individual who is a heterozygote at codon 129 of *PRNP*.[1,3] This finding has major implications for future estimations of numbers of vCJD cases in the UK, since individuals with this genotype constitute the largest genetic subgroup in the population.[4] This subgroup might have a different incubation period after exposure to either primary infection by the bovine spongiform encephalopathy (BSE) agent or secondary infection by blood transfusion. A very lengthy incubation period might explain why no clinical cases of vCJD have yet been observed in this subgroup. Such preclinical cases might also represent a source of iatrogenic infection themselves, either by blood donation or by contamination of surgical instruments coming into contact with lymphoid tissues, even in the absence of infectivity in the brain.

This patient was a UK resident and might therefore have had dietary exposure to the BSE agent. However, the chance of observing vCJD transmission in the absence of a transfusion infection in a second recipient of blood from a donor with vCJD must be far less likely than the 1 in 15 000 to 1 in 30 000 chance for the first reported case.[1] $PrP^{res}$ was not detected in the nine patients without CJD used as negative controls in this study, and in a previous study we and others did not detect PrP accumulation in lymphoid tissues in 56 cases of other forms of human prion disease and in 85 non-CJD cases.[5] The restriction of $PrP^{res}$ to the spleen and cervical lymph node (but not the tonsil or gut-associated lymphoid tissue) in this case is consistent with an intravenous rather than oral route of exposure. It is also possible that the *PRNP* codon 129 genotype might affect the distribution of PrPres in tissues.

This case highlights the need for continuing surveillance for CJD in the UK, and strongly reinforces the role of the autopsy in the investigation and diagnosis of both clinical and preclinical forms of human prion disease.

**Conflict of interest statement**
None declared.

**Contributors**
A H Peden did biochemical analysis and photography, and contributed to drafting of the manuscript. M W Head did biochemical analysis, and contributed to the drafting of the manuscript. D L Ritchie did histological analysis and photography, and contributed to the drafting of the manuscript. J E Bell did the autopsy, provided the autopsy data, and contributed to the drafting of the manuscript. J W Ironside did the histological analysis and coordinated the preparation and drafting of the manuscript.

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**References**

1 Llewelyn CA, Hewitt PE, Knight RS, et al. Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. *Lancet* 2004; 363: 417–21.

2 Glatzel M, Abela E, Maissen M, Aguzzi A. Extraneural pathologic prion protein in sporadic Creutzfeldt-Jakob disease. *N Engl J Med* 2003; 349: 1812–20.

3 Head MW, Ritchie D, Smith N, et al. Peripheral tissue involvement in sporadic, iatrogenic, and variant Creutzfeldt-Jakob disease: an immunohistochemical, quantitative, and biochemical study. *Am J Pathol* 2004; 164: 143–53.

4 Hilton DA, Ghani A, Conyers L, et al. Prevalence of lymphoreticular prion protein accumulation in UK tissue samples. *J Pathol* 2004; 203: 733–39.

5 Hilton DA, Sutak J, Smith MEF, et al. Specificity of lymphoreticular accumulation of prion protein for variant Creutzfeldt-Jakob disease. *J Clin Pathol* 2004; 57: 300–02.

# Effectiveness of leucoreduction for removal of infectivity of transmissible spongiform encephalopathies from blood

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**In 1999, the UK implemented universal leucoreduction as a precaution against transmission of variant Creutzfeldt-Jakob disease by transfusion of domestic blood or red blood cells. We aimed to assess how effectively leucoreduction reduced infectivity of transmissible spongiform encephalopathies (TSEs) in blood. 450 mL of whole blood collected and pooled from scrapie-infected hamsters was leucoreduced with a commercial filter. Blood cell concentrations were quantified, and infectivity titres measured. Blood cell recovery and white blood cell removal complied with American Association of Blood Banks standards. Leucofiltration removed 42% (SD 12) of the total TSE infectivity in endogenously infected blood. Leucoreduction is necessary for the removal of white-cell-associated TSE infectivity from blood; however, it is not, by itself, sufficient to remove all blood-borne TSE infectivity.**

Transmissible spongiform encephalopathies (TSEs) are fatal CNS infections that can incubate asymptomatically for a decade or more in human beings before the appearance of clinical disease. People in the asymptomatic phase of variant Creutzfeldt-Jakob disease (vCJD) appear healthy and donate blood with the same frequency as any healthy person. Transmission of vCJD by transfusion was recently recognised in Great Britain.[1] To reduce the risk of transfusion transmission of such diseases in human beings, the UK implemented universal leucoreduction of donated blood in 1999. This measure was based on the expectation that infectivity would be associated with white blood cells.[2] However, findings in blood from infected mice and hamsters suggested otherwise; at least 40% of the infectivity was plasma-associated, suggesting that leucoreduction would not eliminate infectivity (Rohwer laboratory, unpublished).[3] Other investigations showed no loss of infectivity when small amounts of TSE-infected plasma were passed through scaled-down filters.[4] Similarly, no significant removal of abnormal prion protein was detected when units of human whole blood, spiked with a microsomal fraction from TSE-infected brain, were passed through leucoreduction filters from any of the four major suppliers.[5] Because of reservations about the relevance of these experiments, none of these findings aroused concern.

We investigated the effectiveness of leucoreduction in removal of TSE infectivity from a human-sized unit of pooled hamster blood. To ensure that the 150 hamsters needed for a 450 mL blood pool were at the same symptomatic stage of disease (wobbling gait and head bobbing) for each of two separate experiments, 400 weanling golden Syrian hamsters (Harlan, Madison, WI, USA) were inoculated intracranially with 50 µL of brain homogenate containing about 250 infectious $dose_{50}$ ($ID_{50}$) of hamster-adapted scrapie-strain 263K. A low dose of infectivity was used to preclude re-isolation of the inoculum in the blood. This animal protocol was approved by the University of Maryland Institutional Animal Care and Use Committee.

We obtained two pools of blood from the hamsters, one at 106 days and one at 111 days after inoculation. Under carbon dioxide anaesthesia, 3·5 mL of blood was drawn from the right ventricle into 0·5 mL of CP2D anticoagulant. Care was taken not to touch any other tissue. Only perfect bleeds containing 12·5% CP2D with no visible clots were pooled.

Two in-line leucofiltration systems from Pall Corporation (Port Washington, NY, USA) were evaluated. We selected the Leukotrap WB collection set for the infectivity study because filtration and component separation of hamster blood was fully compliant with American Association of Blood Banks (AABB)[6] specifications, and required only two titrations for interpretation. The Leukotrap RC-PL system

| | Volume (mL)* | White blood cells† | | Red blood cells, total (% of total) | Platelets, total (% of total) |
|---|---|---|---|---|---|
| | | Total (% of total) | $Log_{10}$ reduction | | |
| Whole blood | 448·5 | $2·1\times10^{9}$ (100%) | 0 | $3·7\times10^{12}$ (100%) | $1·4\times10^{11}$ (100%) |
| Leucoreduced blood | 424·2 | $3·0\times10^{6}$ (0·15%) | 2·9 | $3·6\times10^{11}$ (100%) | $1·5\times10^{11}$ (100%) |
| Plasma | 179 | $3·0\times10^{5}$ (0·02%) | 3·8 | 0 (0%) | $1·1\times10^{10}$ (8%) |
| Red blood cells + AS3 | 305·9 | $2·0\times10^{6}$ (0·15%) | 3 | $3·1\times10^{12}$ (86%) | $1\times10^{11}$ (71%) |

*Volume measurements were obtained by weight using experimentally determined densities of whole hamster blood, 1·04 g/mL. †Values are average of at least three separate microscopic determinations using a haemocytometer and by flow cytometric measurements with white cells stained with propidium iodide. AS3 is a preservative and stabiliser.

*Table 1:* Blood component cell numbers and volumes before and after leucoreduction