医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2004 年 2 月 12 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①②③④人血清アルブミン ⑤乾燥濃縮人血液凝固第Ⅷ因子 ⑥乾燥濃縮人血液凝固第Ⅸ因子	研究報告の公表状況	Afssaps/communique de press/ 040210	公表国 フランス		
販売名 （企業名）	①献血アルブミン-Wf（ベネシス） ②献血アルブミン(5%)-Wf（ベネシス） ③アルブミン-Wf（ベネシス） ④アルブミン-ヨシトミ(20%)（ベネシス） ⑤コンコエイトーHT（ベネシス） ⑥クリスマシンーM（ベネシス）					

152 研究報告の概要

2004 年 2 月 7 日付号の Lancet 誌に掲載された、供血の数年後に vCJD で死亡した 15 人の患者の追跡調査から判明した、輸血の 6 年半後に vCJD を発症した事例の報告、及び霊長モデルにおいて静脈投与が経口投与と同じ体内分布を示し、且つ早く大量に沈着することを示した報告を受け、フランス保健製品衛生安全庁（Afssaps）から出された暫定的声明である。

血液の感染能：齧歯目モデル（ラット、ハムスター、マウス）と羊モデルに関するデータが確認されており、低い感染能（1ml あたりの感染ユニット 10 から 20 程度）を認めることができる。この一方で、調査対象モデルや検出方法にかかわらず、霊長類及びヒトの血液からは感染因子は依然として一切検出できなかった。これらのデータは、ヒトの血液に感染能があることは証明されていないが、その可能性は排除できず、また感染能がある場合、その程度は低い、むしろ極めて低いものと考えられるとした 2000 年 12 月の Afssaps 報告の結論を裏付けるものである。このように血液の感染能が低い、あるいは極めて低いと考えられることは、（輸血での注入量を考慮すると）人体における輸血による感染の可能性と相容れないものではなく、さらに、注入量が限られる動物モデルでは検出が難しいことの説明となるかもしれない。

疫学：現在報告されている vCJD の症例（英国での届出件数 147 件、フランス 6 件、イタリア 1 件）は新たな患者の出現が次第に少なくなっていることを示しているように見える。しかしながら専門家は、近年中に英国に第二のピークが訪れる可能性を排除できないとしている（より長い潜伏期間を経た、食品起因 BSE リスクにさらされた患者、外科処置後あるいは輸血後の、ヒトからヒトへの感染による二次感染に由来するケース）。また食品起因 BSE リスク（期間や程度は現時点では不明）にさらされた様々な国（南米大陸を含む）で vCJD 患者が発生することも考えられる。

血液及び二次製品に関するリスク分析：2003 年 12 月に輸血によって感染したと思われるケースが報告されたことにより、血液を介したヒトからヒトへの感染があり得るという仮説が強化された。

フランスはこの仮説を念頭に置き、これを根拠として以下のような輸血用製剤及び血漿分画による血液由来製剤（BDP）のリスク低減策を段階的に実施している。①CJD リスク因子を持った供血者の排除。②1980 年から 1996 年の期間、英国に 1 年以上滞在した供血者の排除。③輸血用細胞製剤からの白血球除去（血球濃厚液と血小板につき 1998 年から実施）。④輸血用血漿及び分画用血漿からの白血球除去（実験段階として 2000 年から実施、2003 年から公式に実施）⑤それが必要不可欠であり、かつ有効と認められている適応に属するケースに限って注入処置を行うための、不安定血液製剤の使用に関する勧告（2002 年と 2003 年に勧告が出された）。⑥輸血を受けたことのある供血者の排除（1996 年から実施）。⑦BDP の分画に関して、慢性的に使用される製品（凝固因子、免疫グロブリン）につきナノフィルトレーション補足工程を実施。

Afssaps の専門家グループによる最近の研究成果から、Lancet 誌に掲載された最新情報は、2000 年 12 月に提示したリスク分析及び結論に影響を及ぼすものではないと考えられる。数年前から段階的に実施している対策は、血液及びその二次製品を介した TSE 因子の感染リスクに対して依然最適のものであり、現時点で修正の必要はない。2 月 3 日の会議の報告書は現在まとめを行っており、専門家の承認を得た後に、2002 年及び 2003 年の更新と同様に公表する予定である。

使用上の注意記載状況・その他参考事項等

代表として献血アルブミン-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。

医薬品
医薬部外品　　研究報告　調査報告書
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報告企業の意見	今後の対応	
Lancet 誌に掲載された 2 つの論文（研究報告 No.1 および No.2）の発表を受け、フランス保健製品衛生安全庁（Afssaps）の専門家グループが、フランスが現在実施している血液安全対策について再評価した結果のプレス報告。当該論文は 2000 年 12 月に提示したリスク分析及び結論に影響を及ぼすものではないと考えられ、フランスが血液およびその二次製品を介した TSE 因子の感染リスクに対して数年前から段階的に実施している対策は依然、適切なものであり、現時点で修正の必要がないと評価している。 現時点で vCJD が報告されているのは英国と、英国滞在歴のない vCJD 患者についてはフランス、イタリアのみである。現在までに血漿分画製剤によって vCJD が伝播したとの報告はない。しかしながら、万一 vCJD 感染者の血液が本剤の原料に混入した場合には、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、vCJD の疫学情報については、今後とも注視することとする。	本剤の原料血漿の供給元は日本および米国であり、これらの国においてはこれまで vCJD 症例の報告はされていない。従って、英国における輸血による vCJD 伝播可能性例について検証した本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。	

afssaps
Agence française de sécurité sanitaire des produits de santé

Communiqué de presse
10 février 2004

Risque de transmission de la maladie de Creutzfeldt-Jakob par la transfusion sanguine

Dans le dernier numéro du Lancet publié le 7 février 2004, deux articles rapportent des éléments nouveaux sur les risques de transmission de la maladie de Creutzfeldt-Jakob (MCJ) et plus spécifiquement une forme variante de cette maladie (vMCJ) induite par la consommation de produits bovins contaminés par l'agent de l'encéphalopathie spongiforme bovine (ESB).

Le premier article, de l'équipe du Pr. R. Will, rapporte le suivi de 48 patients ayant reçu une transfusion sanguine à partir de dons de sang issus de donneurs (15 donneurs identifiés jusqu'à présent) qui sont décédés quelques années plus tard de vMCJ. L'article rapporte essentiellement le cas d'un des 48 receveurs qui a développé une vMCJ, six ans et demi après la transfusion (ce cas avait été annoncé publiquement dès le 17 décembre par le ministre de la santé britannique). Les auteurs concluent que ce cas suggère que l'agent responsable de la vMCJ pourrait être transmis par transfusion, mais qu'il ne peut pas être exclu que la maladie développée par ce patient soit liée à une transmission par voie alimentaire, comme pour les 146 autres cas de patients qui ont été atteints de vMCJ en Angleterre depuis 1996.

Le second article, de l'équipe de C. Lasmézas (CEA, laboratoire de neurovirologie), étudie sur un modèle primate non-humain (macaque infecté par l'agent de l'ESB) la transmissibilité de l'agent infectieux (présent dans un homogénat de cerveau d'animal infecté) par différentes voies d'administration et compare la distribution de l'agent infectieux chez les animaux ainsi contaminés. Les résultats publiés montrent d'une part que la voie intraveineuse est une voie de transmission efficace et d'autre part que l'agent responsable de la vMCJ se distribue de la même façon dans les organes étudiés (cerveau, tissu lymphoïde, intestin et nerfs périphériques) quelle que soit la voie d'inoculation de l'infection. Il faut toutefois noter que cette étude n'a pas étudié l'infectiosité dans le sang des animaux infectés. Cette étude montre essentiellement l'efficacité de la voie intraveineuse dans la transmission de l'infectiosité, et rend ainsi plus probable l'hypothèse selon laquelle le patient vMCJ britannique ait contracté la maladie par transfusion. De plus, compte tenu de la répartition de l'infectiosité retrouvée dans les tissus, quelle que soit la voie d'inoculation (intraveineuse ou orale), les mesures de précaution à respecter dans les actes médicaux et chirurgicaux (et notamment endoscopiques) doivent être identiques pour réduire le risque de transmission secondaire quelle que soit la voie de contamination des sujets traités.

A la suite de l'annonce le 17 décembre 2003 d'un premier cas "probable" de transmission inter-humaine par transfusion, l'Agence française de sécurité sanitaire des produits de santé (Afssaps) a réuni une nouvelle fois le 3 février dernier son groupe d'experts tandis qu'une réunion de l'Agence européenne du médicament (EMEA) avait été organisée les 27 et 28 janvier dernier, pour confronter les données les plus récentes sur le risque de transmission de l'agent des encéphalopathies spongiformes transmissibles (ESST). De ces deux réunions, il peut être retenu essentiellement les éléments suivants.

Infectiosité dans le sang : les données acquises sur les modèles rongeurs (rat, hamster, souris) et sur le modèle du mouton sont confirmées ; une infectiosité peut être retrouvée, à un titre faible (environ 10 à 20 unités infectieuses par ml). En revanche, aucune infectiosité n'a encore pu être détectée, quel que soit le modèle étudié et la méthode de détection, dans le sang de primates ou des sujets humains. Ces données confirment les conclusions fournies dans le rapport Afssaps de décembre 2000 que la présence d'infectiosité dans le sang humain n'a pas été démontrée, mais qu'elle ne peut être exclue, et que si elle était présente elle le serait à un titre faible, voire très faible. Ce titre faible ou très faible n'est pas incompatible avec la possible transmission par transfusion sanguine chez l'homme (compte tenu des volumes injectés en transfusion) et expliquerait aussi la difficulté de détection dans modèles animaux qui sont limités par les volumes injectés.

Epidémiologie : le nombre de cas de vMCJ rapportés à ce jour (147 cas déclarés en Angleterre, 6 en France et 1 en Italie) semble montrer une décroissance dans la vitesse d'apparition des nouveaux cas. Cependant les experts ne peuvent pas exclure qu'un second pic puisse intervenir dans les années prochaines en Angleterre (sujets exposés au risque ESB alimentaire ayant développé une période d'incubation plus longue, cas issus du second passage par contamination interhumaine post chirurgie ou post transfusion). Il ne peut pas non plus être exclu que des cas de vMCJ puissent se déclarer dans différents pays (y compris le continent Nord-américain) ayant été exposés au risque ESB alimentaire (pour des périodes et à des niveaux inconnus actuellement).

Analyse de risque pour le sang et les produits dérivés : le cas possible rapporté en décembre 2003 de transmission par transfusion renforce la plausibilité d'une transmission inter-humaine, par voie sanguine. Cette hypothèse a toujours été prise en compte en France et a justifié la mise en place progressive de mesures de réduction du risque des produits transfusionnels et des médicaments dérivés du sang (MDS) issus du fractionnement du plasma, rappelées ci-après :

- Exclusion des donneurs de sang ayant un facteur de risque au regard de la MCJ.
- Exclusion des donneurs de sang ayant séjourné plus de un an en Grande-Bretagne dans la période 1980-1996.
- Déleucocytation des produits transfusionnels cellulaires (mise en place en 1998 pour les concentrés globulaires et les plaquettes).
- Déleucocytation du plasma pour transfusion et du plasma pour fractionnement (mise en place en 2000 dans le cadre d'une phase expérimentale et officialisation de la mesure en 2003.
- Rappels des recommandations d'utilisation des produits sanguins labiles, pour que l'acte transfusionnel soit réservé aux seuls cas indispensables et relevant d'indications validées (Recommandations publiées en 2002 et 2003).
- Exclusion de donneurs précédemment transfusés (mise en place en 1996).
- Pour le fractionnement des MDS, mise en place d'étapes supplémentaires de nanofiltration pour les produits utilisés de façon chronique (facteurs de la coagulation, Immunoglobulines).

A l'issue des récents travaux conduits par le groupe d'experts de l'Afssaps, il est considéré que les dernières informations publiées dans le Lancet ne remettent pas en cause l'analyse de risque et les conclusions proposées en décembre 2000. Les mesures mises en place progressivement depuis plusieurs années demeurent aujourd'hui les plus appropriées face au risque de transmission des agents ESST par le sang et ses dérivés, et n'ont pas à être modifiées actuellement.

Le rapport de la réunion du 3 février est en cours de finalisation et, après approbation par les experts sera rendu public comme les actualisations de 2002 et 2003.

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accueil nouveautés plan du site

医薬品
医薬部外品　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2004.7.1	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	ABC Newsletter, 2004.6.25	公表国 米国
販売名（企業名）	クロスエイトM250　（日本赤十字社） クロスエイトM500　（日本赤十字社） クロスエイトM1000　（日本赤十字社）			

研究報告の概要

米国赤十字社（米赤）は、変異型クロイツフェルトヤコブ病（vCJD）の輸血感染予防策の一環として講じている、英国滞在（旅行）歴を有する供血者の供血延期措置を緩和する方針を発表した。1980年1月から現在までに、合計3ヶ月以上の英国滞在（旅行）歴を有する供血者を供血延期としているが、この滞在期間を1980年1月から1996年12月31日までに短縮する予定である。この措置は、米国食品医薬品局（FDA）の勧告に則ったものである。しかし米赤は、欧州滞在（旅行）歴が累計5年以上の供血者の供血を延期するというFDAの勧告には従わず、1980年以降に、欧州（英国を含む）、オマーン、トルコに合計6ヶ月以上の滞在歴を有する供血者の供血延期措置は継続する予定である。2001年、米赤はFDAの勧告よりもはるかに厳格なvCJD対策方針を打ち出した。米赤の当初の試算では、154,000名（4%）の供血者が供血不可となっていたが、措置が緩和されることにより、多くの供血者が再び供血可能となる見込みである。供血延期措置の改訂は、FDAの承認を必要とするためまだ実施されていないが、2005年中に承認される予定である。

報告企業の意見	今後の対応
米国赤十字社が、変異型クロイツフェルトヤコブ病（vCJD）の輸血感染予防策の一環として講じている、英国滞在歴あるいは旅行歴を有する供血者からの供血制限を緩和する方針についての報告である。	日本赤十字社は問診時にvCJD対策のため過去の海外渡航歴（旅行及び居住）を質問して場所、期間、時期を確認し、英国を含む欧州36ヶ国に一定期間滞在歴を有するドナーを献血延期としている。また異常プリオンがアルブミン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。

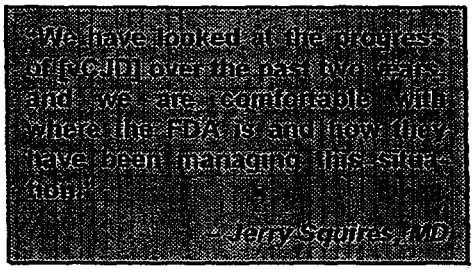
使用上の注意記載状況・その他参考事項等

クロスエイトM250
クロスエイトM500
クロスエイトM1000
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

23

American Red Cross to Modify some vCJD Donor Deferral Criteria

The American Red Cross will loosen its restrictions on blood donors who have lived or traveled in the United Kingdom because of concerns about variant Creutzfeldt-Jakob disease (vCJD), the organization announced this week. Instead of deferring donors who spent a total of three months or more from January 1, 1980 through the present, Red Cross now will end the residency period on December, 31, 1996.



The new policy will bring its deferral policy into line with recommendations by the Food and Drug Administration, Red Cross said in a statement (6/22/04). However, Red Cross will continue to defer donors who have spent a total of six months in Europe (including the UK), Oman, or Turkey since 1980, instead of following FDA's recommendation that a cumulative residence and/or travel of five years in Europe trigger the deferral.

"We have looked at the progress of the disease over the past two years, and we are comfortable with where the FDA is and how they have been managing this situation," said Jerry Squires, MD, American Red Cross vice president of Biomedical Services and chief scientific officer. "We are comfortable following their guidance in the matter." (The Red Cross did not respond to inquiries asking why it had adopted only one part of the FDA's geographical deferral recommendations.)

In 2001, the Red Cross instituted a vCJD policy that was much stricter than that recommended by FDA. Red Cross' initial estimates found that up to 4 percent, or 154,000 eligible donors, would no longer be able to donate under the tightened restrictions. "With this change, many of those donors will be able to donate blood again to the Red Cross," the organization said this week.

> **"The FDA continues to carefully investigate the situation, so the best approach we can take now is to closely follow their guidelines," Dr. Squires said.**

The revised deferral policy has not yet taken effect, Red Cross said. FDA approval is required, which is expected within the next year, Red Cross said. "Once the policy is enacted, tens of thousands more donors will be able to give blood," the organization noted. "Donors who were deferred should look for more information on these changes later this year." ♦

Sacramento Medical Foundation Closes Center for Blood Research; Transfers Projects to BloodSource

The Sacramento Medical Foundation (SMF) announced this week that it has closed operations of its Center for Blood Research (CBR) and transferred research responsibilities to BloodSource, its not-for-profit organization that provides blood services, transfusion medicine support, and research.

The decision was reached following internal audits and a Food and Drug Administration (FDA) inspection, SMF said yesterday in a press release (6/24/04). A BloodSource quality assurance team is examining CBR records and research projects, the Foundation said. "Projects that were under way at CBR have been closed or moved to BloodSource to assure that procedures and records are in compliance with all regulations and quality plans."

(continued on page 3)

別紙様式第2-1
番号9

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2004年5月21日	新医薬品等の区分 該当なし	厚生労働省処理欄

一般的名称	①②③④人血清アルブミン ⑤乾燥濃縮人血液凝固第Ⅷ因子 ⑥乾燥濃縮人血液凝固第Ⅸ因子	研究報告の公表状況	Jounal of Pathology, 203(3), 733-739,2004	公表国 イギリス
販売名 （企業名）	①献血アルブミン-Wf（ベネシス） ②献血アルブミン(5%)-Wf（ベネシス） ③アルブミン-Wf（ベネシス） ④アルブミン-ヨシトミ(20%)（ベネシス） ⑤コンコエイトーHT（ベネシス） ⑥クリスマシンーM（ベネシス）			

研究報告の概要

本研究は、英国における変異型クロイツフェルト・ヤコブ（vCJD）病に潜伏感染し、医原性伝播をさせる可能性のある人の数を見積もることを目的としている。プリオンタンパク質のリンパ網内系への蓄積は、剖検における vCJD の一致した特徴であり、症状が出る前においても現れる。リンパ網内系へのプリオンタンパク質の免疫組織化学的蓄積は、動物のプリオン疾患の神経学的疾病を確実に予測することのできる唯一の技術である。本研究において、モノクローナル抗体 KG9 及び 3F4 を用いて外科的に切除された扁桃摘出検体及び虫垂摘出検体におけるプリオンタンパク質の存在を確認するために、免疫組織化学が用いられた。検体は患者 16,703 人から得られ（虫垂摘出検体 14,964、扁桃摘出検体 1,739）、患者の約 60%は手術時 20～29 才であった。検体の 25％は、不適当な量のリンパ組織を含んでいたので、最終的な解析から除外された。虫垂摘出の 3 検体は、プリオンタンパク質のリンパ網内系への蓄積を示し、3/12,674 もしくは 237/100 万（95％CI 49～692/100 万）が罹患していると見積もられた。これらのうち 2 検体のリンパ網内系蓄積パターンは、vCJD の既知症例でみられるそれと異なっていた。リンパ網内系でのプリオンタンパク質の免疫組織化学的蓄積が vCJD に特異的であるかどうか不確定であるけれども、それは他の形態のヒトプリオン病もしくは広範な炎症性及び感染性状態を含む他のどの疾病においても報告されたことはない。これらの発見によって、血液製剤及び外科用器具を介した vCJD の伝播リスクを減らすために英国保健省がとる措置の重要性及びプリオンタンパク質の検出のために新しい扁桃検体の大規模なスクリーニングを緊急に進める重要性が強固なものとなった。

報告企業の意見	今後の対応
英国の 12,674 の虫垂及び扁桃摘出検体のうち、虫垂摘出の 3 検体からプリオンタンパク質が検出されたとする報告である。 現時点で vCJD が報告されているのは英国と、英国滞在歴のない vCJD 患者についてはフランス、イタリアのみである。現在までに血漿分画製剤によって vCJD が伝播したとの報告はない。しかしながら、万一 vCJD 感染者の血液が本剤の原料に混入した場合には、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、vCJD の疫学情報については今後とも注視することとする。	本剤の原料血漿の供給元は日本および米国であり、これらの国においてはこれまで vCJD 症例の報告はされていない。従って、本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

代表として献血アルブミン-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。

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Rapid Communication

Prevalence of lymphoreticular prion protein accumulation in UK tissue samples

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Abstract

This study aims to provide an estimate of the number of individuals in the UK who may be incubating variant Creutzfeldt-Jakob disease and at risk of causing iatrogenic spread of the disease. Lymphoreticular accumulation of prion protein is a consistent feature of variant Creutzfeldt-Jakob at autopsy and has also been demonstrated in the pre-clinical phase. Immunohistochemical accumulation of prion protein in the lymphoreticular system remains the only technique that has been shown to predict neurological disease reliably in animal prion disorders. In this study, immunohistochemistry was used to demonstrate the presence of prion protein, with monoclonal antibodies KG9 and 3F4, in surgically removed tonsillectomy and appendicectomy specimens. The samples were collected from histopathology departments across the UK and anonymised prior to testing. Samples were tested from 16703 patients (14964 appendectomies, 1739 tonsillectomies), approximately 60% of whom were from the age group 20–29 years at operation. Twenty-five per cent of the samples were excluded from the final analyses because they contained inadequate amounts of lymphoid tissue. Three appendicectomy samples showed lymphoreticular accumulation of prion protein, giving an estimated prevalence of 3/12674 or 237 per million (95% CI 49–692 per million). The pattern of lymphoreticular accumulation in two of these samples was dissimilar from that seen in known cases of variant Creutzfeldt-Jakob disease. Although it is uncertain whether immunohistochemical accumulation of prion protein in the lymphoreticular system is specific for variant Creutzfeldt-Jakob disease, it has not been described in any other disease, including other forms of human prion disease or a range of inflammatory and infective conditions. These findings reinforce the importance of measures taken by the UK Department of Health to reduce the risk of spread of variant Creutzfeldt-Jakob via blood products and surgical instruments, and of the urgency to proceed with large-scale screening of fresh tonsil specimens for the presence of prion protein.

Keywords: Creutzfeldt-Jakob disease (CJD); prion; screening; immunohistochemistry

Introduction

Variant Creutzfeldt-Jakob disease (vCJD) was first recognized as a new and distinctive disease in the UK in 1996 [1]. Subsequent transmission studies and strain typing have shown that the transmissible agent causing vCJD exhibits identical characteristics to the bovine spongiform encephalopathy (BSE) agent [2–4] and there is no evidence that vCJD occurred prior to 1995 [5,6]. These data indicate that vCJD is a new disease, almost certainly caused by exposure to the BSE agent. This conclusion has led to concern about a possible human epidemic of vCJD, particularly as it is likely that over 400000 infected cattle entered the human food chain in the UK prior to the introduction of the specified bovine offal ban in November 1989 and as the ban was not fully effective for several years, a large number of infected cattle also entered the food chain in the early 1990s [7]. There have been a number of attempts to predict future numbers of vCJD cases using mathematical models and extrapolating from vCJD cases seen to date [8–13]. Recent estimates based on the pattern of clinical cases suggest that the epidemic of vCJD will be relatively small, with an upper 95% confidence interval of 540 future cases [13]. However, remaining uncertainties, including the possibility that other genetic loci affect susceptibility [14], make the distribution and timing of any human epidemic unclear. Furthermore, such models are unable to estimate the prevalence of asymptomatic infection and hence provide any estimate of the potential number of future infections and cases that could arise from secondary (human-to-human) transmission of vCJD. In addition, questions have been raised as to the

safety of some food products not covered by the specified bovine offal ban [15,16] and it is not known if BSE has entered the British sheep flock, factors which could alter predicted numbers of vCJD cases [12]. These uncertainties make decisions about health care planning problematic, particularly measures to reduce the risk of iatrogenic spread of vCJD. In order to reduce these uncertainties, some form of population screening is required. However, the lack of a conventional immune response and the failure to date to demonstrate abnormal prion protein (PrP) in blood in vCJD [17] have made the development of a diagnostic blood test difficult. If a blood test becomes available for symptomatic vCJD, it may be several years before it is known whether pre-clinical disease could be reliably detected.

It has been known for some time that lymphoreticular accumulation of PrP occurs early in murine models of scrapie [18], even when incubation periods are long [19]. This lymphoreticular involvement has been successfully used in the development of a tonsillar biopsy as a pre-clinical test for scrapie in sheep [20]. Although widespread lymphoreticular involvement is not a feature of BSE in cattle [21], extensive lymphoreticular PrP deposition has been found in all cases of symptomatic vCJD examined to date [22,23] and in two cases in appendicectomy specimens removed prior to the onset of symptoms [24,25]. On the basis of these data, we have screened large numbers of appendicectomy and tonsillectomy specimens for the presence of abnormal lymphoreticular PrP deposition. Although the antibodies used in this study cannot distinguish PrP^{c} from PrP^{Sc}, immunohistochemical accumulation of PrP within lymphoid tissue correlates with the detection of protease-resistant PrP by western blot analyses in human tissues [22] and immunohistochemistry remains the only technique that has been shown to predict disease in animals reliably [26,27]. This study was primarily designed to look for evidence of a large epidemic, but also to provide information about how many individuals are at high risk of developing vCJD and causing iatrogenic spread. Interim results from this study have been published previously [25,28]. However, the study has now been completed following the examination of additional cases.

Materials and methods

Tissue samples

Appendicectomy and tonsillectomy samples were identified by Systematized Nomenclature of Medicine (SNOMED) searching of the computerized databases of 63 histopathology departments across the UK. Initially, samples from the age range 10–50 years were included. However, following negative findings in the first 3000 cases [28], it was decided only to examine appendix samples from individuals aged 20–29 years, as this represents the highest risk age group for vCJD. Tonsil samples included all ages, as fewer samples were available for examination (most tonsillectomy samples are discarded rather than sent to histopathology departments for diagnosis and archiving). A maximum of two tissue blocks was examined for each case. Only samples removed from 1995 onwards were included, as these represent a longer time from possible BSE exposure than earlier samples and therefore a greater likelihood of PrP being detectable. Tissue samples were collected into batches of at least 1000 cases and given a randomly obtained study number prior to testing, in order to protect the anonymity of positive individuals. Batches of samples from England were tested at Plymouth and, from Scotland, at Edinburgh.

The study received approval from the South and West Multi-centre Research Ethics Committee (MREC reference 99/6/32) and for each of the centres included, appropriate local research ethics committee approval. The ethical approach has been discussed previously [29] and in view of the lack of direct patient consent and uncertainty of the significance of a positive result, the study design was anonymous.

Immunohistochemistry

Four-micrometre sections were cut from tissue blocks at two levels 100 μm apart. Sections were pretreated by autoclaving at 121 °C for 10 min, followed by immersion in 96% formic acid for 5 min and digestion with proteinase K (10 μg/ml) for 5 min at room temperature, in order to enhance PrP^{Sc} detection and reduce PrP^{c} detection. PrP was detected using the well-characterized and widely used monoclonal antibodies 3F4 (Dako, UK) and KG9 (IAH, TSE Resource Centre, UK) [22,24] and visualized using the CSA kit (Dako, UK), which gives superior results in terms of sensitivity to most other immunohistochemical detection systems [30]. A section from each case was stained with haematoxylin and eosin for morphological assessment. Autopsy tonsil tissues from confirmed cases of vCJD were used as a positive control for each group of slides stained by immunohistochemistry for PrP; negative controls were performed by omitting the primary antiserum. Thirty cases from each batch of 1000 were exchanged between the study centres and tested 'blinded' to the findings of the other centre, for quality control and validation of results. In order to minimize the possibility of human error, the samples were tested and analysed with each of the antibodies on separate dates.

All sections were examined by an experienced neuropathologist (DAH at Plymouth and JWI at Edinburgh). Cases with fewer than five secondary lymphoid follicles were excluded from the final analyses because in the original reported case [24] and those examined at autopsy (personal observation JWI), PrP could be demonstrated in only approximately 20% of follicles. Sections were recorded as positive if PrP staining was detected in follicular dendritic cells or tingible body macrophages in lymphoid follicles.

Statistical methods

Simple summary statistics were calculated in Microsoft Excel. Exact binomial confidence intervals were calculated for the prevalence estimates. The expected number of individuals incubating vCJD was calculated using estimates of the UK population size stratified by age (http://www.statistics.gov.uk/downloads/theme_population/PT114.pdf).

Results

Tissue samples

The numbers of cases examined and the age distribution are summarized in Table 1 and Figure 1. The age distribution of our sample is heavily weighted towards the high-risk age group (based on cases of vCJD to date, see Figure 2). The majority of the specimens examined were appendicectomies, reflecting the availability of samples within histopathology departments (most tonsillectomy specimens are discarded after surgery in the UK).

The number of secondary lymphoid follicles varied considerably between appendicectomy cases, but in about 25%, fewer than five were present on the first level and these were therefore excluded from the figures for analyses. Most of these excluded cases were severely inflamed, although some showed fibrous obliteration, and none was considered positive. The median number of secondary lymphoid follicles in the remaining appendicectomy cases, which were included in the study, was 22 at the first level and most had several additional follicles examined at the second level. Most tonsil specimens included over 100 secondary lymphoid follicles, although in about 10% of samples, fewer than five were present.

Table 1. Summary of the samples used in the study

Specimens	Number
Appendicectomy specimens tested	14964
Tonsillectomy specimens tested	1739
Excluded from analysis*	4029
Total included in analysis	12674†

* Due to inadequate amounts of lymphoid tissue.
† 10260 from England and 2414 from Scotland.

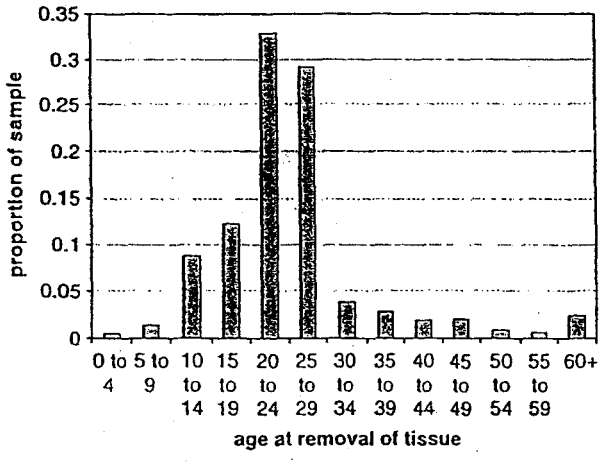


Figure 1. Age distribution of the samples included in the study

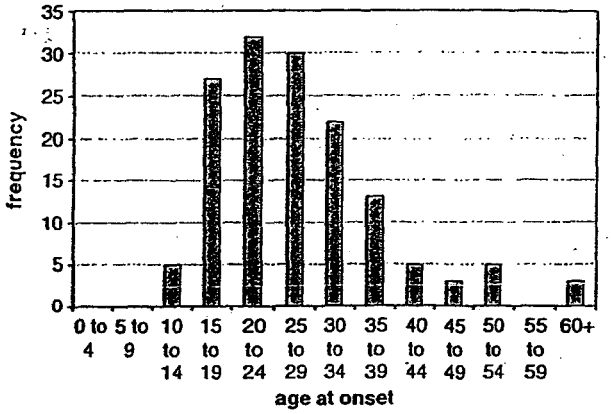


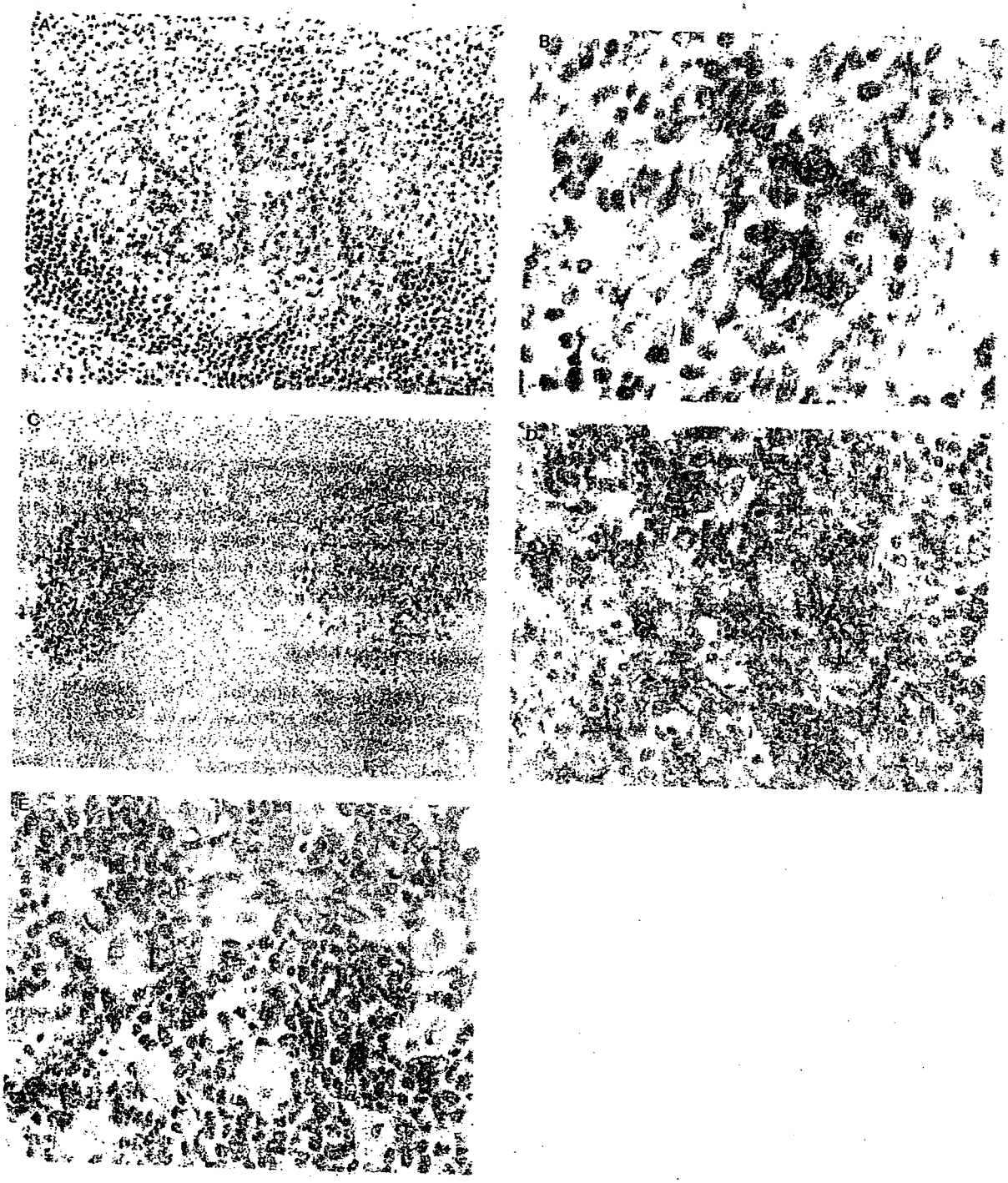
Figure 2. Age distribution at onset of vCJD cases to end of 2003

Immunohistochemistry

In the majority of samples, fine granular PrP immunoreactivity was noted in nerve fibres and the myenteric ganglia with both antibodies, and in a few cases, PrP immunoreactivity was also noted in epithelial cells immediately adjacent to acute inflammation. In three appendicectomy cases, we identified PrP immunoreactivity in lymphoid follicles, which was seen in sections tested at both centres. None of the tonsillectomy samples was positive.

In the first positive case (previously published [25]), immunoreactivity was seen in the sections stained using KG9 and was limited to one of the six secondary lymphoid follicles present, with a distribution suggesting that it was within follicular dendritic cells (Figure 3A). The pattern of staining, in particular the coarse granularity (Figure 3B), was very similar to that seen in the two other cases who subsequently developed vCJD [24,25]. However, staining was less evident in sections immunostained with the 3F4 antibody. The reason for this discrepancy is not entirely clear, although we feel that the most likely explanation is sampling error due to the focal nature of the PrP deposition. This positive case also showed evidence of acute appendicitis in adjacent tissue, but there was no morphological evidence of any other disease process in an adjacent haematoxylin and eosin-stained section.

The second positive sample showed extensive staining in 31 of 68 secondary lymphoid follicles (Figure 3C); this was seen with both antibodies, although it was less intense with 3F4. The staining had a finer granular pattern and appeared confined to follicular dendritic cells (Figure 3D). The appendix did not show any acute inflammation. A very occasional



Color Figure - Print and Online

Figure 3. Immunoreactivity with monoclonal anti-PrP antibody KG9 in the three study cases. The first positive case shows granular staining of follicular dendritic cells in one follicle (A), including numerous coarse granular aggregates (B). The second positive case shows intense PrP immunoreactivity in two follicles (C), with a predominantly finely granular pattern in follicular dendritic cells (D). The third case shows a mixture of granular follicular dendritic cell staining and accumulation within the cytoplasm of macrophages (E)

multinucleate cell was noted in the submucosa of this case, but not within germinal centres.

The remaining positive case showed staining in three of 46 secondary lymphoid follicles, which was similar with both antibodies. Fine granular immunoreactivity was present in cells with the morphology of follicular dendritic cells, and within the cytoplasm of cells with abundant eosinophilic cytoplasm, presumably macrophages (Figure 3E). Acute inflammation was not present.

If lymphoreticular immunoreactivity for PrP is a reliable marker of pre-clinical infection, the estimated prevalence of vCJD based on these three positive samples in 12674 tested will be 237 infections per

million population (exact 95% CI 49–692 per million). If we assume that this estimate relates to those aged 10–30 years (83% of the sample), then this translates to a best estimate of 3808 individuals (95% CI 785–11 128) aged 10–30 years incubating vCJD. If only the one case with a similar pattern to that seen in previous cases of vCJD is considered, then the estimates will be correspondingly lower (prevalence of 79 infections per million population, 95% CI 2–440). In contrast to these high estimates, clinical case numbers remain at a much lower level and have been declining since 2000, with only 18 deaths in 2003.

Discussion

This study provides an estimate of the prevalence in the UK population of abnormal lymphoreticular accumulation of PrP. However, our findings need to be interpreted cautiously, in terms of the clinical significance of both negative and positive results.

One major limitation of this study in estimating the prevalence of asymptomatic infection and predicting future numbers of vCJD cases is that it is not known at what stage during the incubation period PrP can be detected in lymphoid tissue. In murine models of scrapie, infectivity can be demonstrated in Peyer's patches as early as 1 week after oral inoculation [19] and immunohistochemistry can detect PrP in Peyer's patches 1 month after intraperitoneal inoculation [31]. In the tonsils of scrapie-infected sheep, immunohistochemical detection of PrP occurs from 4 months of age in those homozygous for a susceptibility PrP gene polymorphism, and by 15 months in heterozygotes, reliably predicting future neurological disease [26]. A further study examining tissue from the third eye of sheep at risk of scrapie found that immunohistochemical detection of PrP in lymphoid follicles predicts neurological disease with an estimated 87% sensitivity and 94% specificity [27]. Data are only available in the pre-clinical phase from three cases of vCJD [25]; the two appendicectomy samples removed in the 1990s (up to 2 years before symptoms and 4 years before death) were positive and a third case, removed in 1987, 10 years before the onset of symptoms, was negative. This retrospective study has only examined samples taken from 1995 to 1999, several years after the peak human exposure to BSE, which is likely to have occurred between 1988 and 1992, in order to maximize the chances of identifying positive individuals. Furthermore, we have used a highly sensitive immunohistochemical technique [30] and because of the focal nature of PrP deposition, extensive sampling of appendix tissue, with a minimum of five (and an average of more than 20) secondary lymphoid follicles assessed in each case. Using this approach, we have found that 95% of autopsy appendicectomy samples from cases of vCJD, with adequate amounts of lymphoid tissue, test positive [25]. The finding of fine granular PrP in the myenteric plexus of most samples (and some epithelial cells adjacent to inflammation in a few samples) suggests that the proteolytic digestion used during immunocytochemistry does not completely remove PrP^c and also reflects the high levels of PrP^c in autonomic nerves [32].

Although immunohistochemical accumulation of PrP in lymphoreticular tissues has not been demonstrated in any disease other than vCJD [22,33,34], the significance of the positive samples in this study is not certain. In one case, the immunohistochemical pattern of immunoreactivity resembled that seen in appendix tissue from pre-clinical [24,25] and autopsied cases of vCJD, but in the other two cases, a more finely granular pattern of staining was present in relation to follicular dendritic cells, raising the possibility that these may be false positives. However, we have been unable to demonstrate PrP immunoreactivity in a range of other disorders including other human prion diseases, neoplastic disease, or a range of inflammatory conditions [33]. Other explanations for our finding of cases with an unusual pattern of lymphoreticular PrP immunoreactivity include involvement of other genotypes (genotype is known to affect the morphological patterns of PrP deposition in the brain [35]) or differing strains of BSE [36]. The anonymous study design prevents detailed investigation of the positive cases. However, spare paraffin wax sections were available from the second and third positive cases and have been used for transmission studies, but these may be inconclusive if negative, because of the small amount of tissue available and the difficulty in transmitting from fixed tissue [37]. Commercially available anti-PrP antibodies for immunohistochemistry detect both PrP^c and PrP^{Sc}, and although two groups have developed PrP^{Sc}-specific antibodies [38,39], they do not appear to work for immunohistochemistry (JWI, personal communication).

If our positive cases represent pre-clinical cases of vCJD, then this will be of some concern, as the prevalence is much higher than expected from the observed incidence of clinical cases, either indicating a future increase in numbers of vCJD cases or a significant number of individuals with a 'carrier state' [40]. In the latter context, it is of interest to note that inoculation of the BSE agent into transgenic mice which express only the human PrP gene with methionine homozygosity at codon 129 has revealed a high incidence of sub-clinical infection [41]. In vCJD, immunohistochemical accumulation of PrP correlates with the presence of protease-resistant PrP, as determined by western blot examination [22] and infectivity [42]. Individuals with sufficient PrP accumulation to be detected by immunohistochemistry may therefore pose a health risk to others by causing iatrogenic spread via surgical instruments, blood transfusion or organ donation. Infectivity is not fully inactivated by autoclaving [43] and CJD has been transmitted by re-use of surgical instruments [44], although this risk is likely to be small (http://www.doh.gov.uk/cjd/consultation).

However, there has been a recent increase in concern about surgical transmission of CJD, following the demonstration of low levels of PrP^{Sc} in the skeletal muscle and spleen of some patients with sporadic CJD [45,46] and epidemiological studies that have shown an increased incidence of sporadic CJD following surgical procedures [47,48]. Abnormal PrP has not yet been demonstrated in the blood of patients with vCJD [17], but the most sensitive test for infectivity remains intra-species inoculation and data from sheep infected with BSE indicate that blood-borne transmission is possible [49]. A recent case of vCJD occurring in an individual 6 years after receiving a blood transfusion from a patient who later developed vCJD suggests that human blood is also able to transmit the disease [50]. Our findings therefore reinforce the importance of recent steps taken by the Department of Health to reduce these potential risks, which include the leucodepletion of all UK-sourced blood and the introduction of more stringent decontamination procedures for surgical instruments.

The incubation period of vCJD is not known and although numbers of cases are currently in decline, the possibility of further rises cannot be excluded. The average incubation period of kuru and iatrogenic CJD following peripheral inoculation has been estimated to be about 12 years, with some cases of kuru occurring more than 40 years after the cessation of cannibalism [44,51], but these diseases did not have to cross a species barrier. Data from a geographically associated cluster suggested that they resulted from exposure to BSE prior to 1986 (http://www.leics-ha.org.uk/Publics/cjdrep.pdf), indicating an incubation period for these cases of 10–16 years.

Our study has demonstrated how a better understanding of the pathology of vCJD has allowed the investigation of an important epidemiological question about this disease using archival tissue collections. However, the techniques used in our study have been limited to immunohistochemistry, because of the use of formalin-fixed tissue sections, and by the study design, which prevents return to a positive tissue sample for further verification. These factors have limited the interpretation of our findings. However, we believe that they are of some concern and require urgent further investigation by prospective screening of tissue from tonsillectomies. By analysing fresh tissue, samples could be tested with a sensitive assay that allows for automation [17] and positive findings could be reliably confirmed by transmission studies. However, about half of tonsillectomies are performed on children under 10 years of age, so many individuals undergoing this procedure will soon have had little or no exposure to BSE and therefore the window of opportunity for such a study will diminish over time.

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