

Variant Creutzfeldt–Jakob disease: risk of transmission by blood transfusion and blood therapies

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Summary. In the last decade, a new variant of the human prion disease Creutzfeldt–Jakob disease (now known as variant CJD or vCJD) was identified and causally linked to dietary exposure to bovine spongiform encephalopathy (BSE) during the 1980s and early 1990s. Preliminary studies in animal models suggest that prions can be transmitted by blood. Based on two recent reports of iatrogenic vCJD transmission by blood transfusion in humans, a Department of Health-sponsored risk assessment warned that recipients of plasma therapies are now at risk of contracting vCJD from potentially infected donors. It is believed that all the population may be susceptible to vCJD infection, although clinical cases have so far occurred only in methionine homozygotes at codon 129 in the human prion protein gene. A non-invasive blood-based diagnostic assay is urgently needed. Because the incubation period may be upwards of 40 years and there is no

reliable screening test, it is currently unknown how many people may be in an asymptomatic phase of vCJD infection in the UK. However, there remains a distinct possibility that some infected patients may never develop clinical symptoms but will remain asymptomatic carriers who can potentially transmit the disease to other individuals. Therefore, screening of infectious individuals will be a critical component for individuals who rely on blood transfusions and/or blood therapies. In the absence of screening tests or effective therapies to treat this disease, a formidable worldwide public health challenge lies ahead to prevent new infections, accurately assess infection rates and treat infected patients.

Keywords: blood transfusion, factor replacement, haemophilia, prion, transmission, variant Creutzfeldt–Jakob disease

Introduction

Variant Creutzfeldt–Jakob disease (vCJD) is a recently identified member of the transmissible spongiform encephalopathies (TSE) or prion diseases [1,2]. These disorders are fatal neurodegenerative conditions occurring in humans and other mammals, the best known examples in non-human species being bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep and chronic wasting disease in deer and elk [3]. Prion diseases are transmissible under both experimental and natural conditions. For many years, the nature of the transmissible agent was the subject of intense debate, and in 1982 the prion hypothesis was

proposed by Prusiner [4]. This postulated that the transmissible agent was composed entirely of a modified host protein (prion protein) that was partially resistant to proteolytic degradation, without a nucleic acid component.

The normal form of the prion protein (PrP^C) is expressed in many cells and tissues in the body, but is present at highest levels in neurones within the central nervous system [3]. The precise function of PrP^C is uncertain, but it has a short half life and is readily degraded by proteolytic enzymes [5]. An abnormal isoform of PrP (PrP^{Sc}) accumulates in the central nervous system in prion diseases. PrP^{Sc} has an identical amino acid sequence to PrP^C, but a different conformation, with an increased beta-sheet content that is associated with infectivity and neurotoxicity [3]. This abnormal conformation also confers a relative resistance to degradation by proteolytic enzymes. The precise cellular mechanisms that result in this conformational change, and their locations, have not yet been fully determined.

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The BSE epidemic in the UK

In 1987, a novel progressive neurological condition in cattle was reported in the UK [6]. The new disease was named bovine spongiform encephalopathy (BSE, or 'mad cow' disease) because of its similarity to other prion diseases by pathology and immunohistochemistry. By the early 1990s thousands of cattle were diagnosed with BSE and millions were incinerated to prevent the disease from spreading [7,8]. However, BSE has still not been fully eradicated in the UK. The BSE epidemic in the UK has been attributed to TSE-infected feeds made of meat and bone meal prepared from rendered sheep offal [9]. With the prohibition of specific feeding practices and specified offals, however, the number of reported cases declined to fewer than 500 by 2003 in UK (Fig. 1) [7,8].

Since the UK continued to export cattle offals after 1986, the BSE agent spread to over 20 European countries, as well as to Japan, Russia, Canada, Israel and the USA. Thus, the exportation of contaminated animal feed from the UK to many other countries across the world resulted not only in the spread of BSE but potentially widespread human exposure to BSE-positive animals through the consumption of BSE-contaminated meat products [10]. Public health concerns about the safety of meat products around the world since the BSE epidemic two decades ago have not diminished. On 24, June 2005, the US Department of Agriculture confirmed BSE in a cow that had conflicting screening test results the previous year. Fortunately, no part of the animal had entered the human or animal food supply; however, this case heightened the awareness of the need for better testing in this country and ongoing surveillance [8,11].

Table 1. Classification of human prion diseases [12].

Class	Diseases
Idiopathic	Sporadic Creutzfeldt–Jakob disease Sporadic fatal insomnia
Familial	Familial Creutzfeldt–Jakob disease Gerstmann–Sträussler–Scheinker syndrome Fatal familial insomnia
Acquired	
Human origin	Kuru, iatrogenic Creutzfeldt–Jakob disease
Bovine origin	Variant Creutzfeldt–Jakob disease

Classification of human prion diseases

Human prion diseases are categorized into three distinct groups that reflect their different origin and range: idiopathic, inherited and acquired [2] (Table 1). The commonest of the idiopathic disorders is sporadic CJD (sCJD). Sporadic CJD is distributed worldwide and is the most common of all human prion diseases, accounting for around 85% of all cases [13]. It is associated with a highly aggressive clinical course with a mean duration of illness of approximately 4.5 months. Sporadic CJD occurs most frequently in middle-aged or elderly individuals and appears to be triggered by a somatic mutation of the prion gene, or by a spontaneous conformational change of the host prion protein from its normal cellular form (PrP^C) to its abnormal and pathogenic form (PrP^{Sc}) [3,14].

Inherited (familial) forms of prion diseases comprise up to 15% of all cases and are strongly linked to a series of pathogenic mutations and insertions in the prion protein gene [15,16]. The clinical course of these TSEs is characterized by a slow degeneration of the central nervous system, resulting in dementia, ataxia, motor difficulties and death. The inherited human prion diseases comprise three main groups of

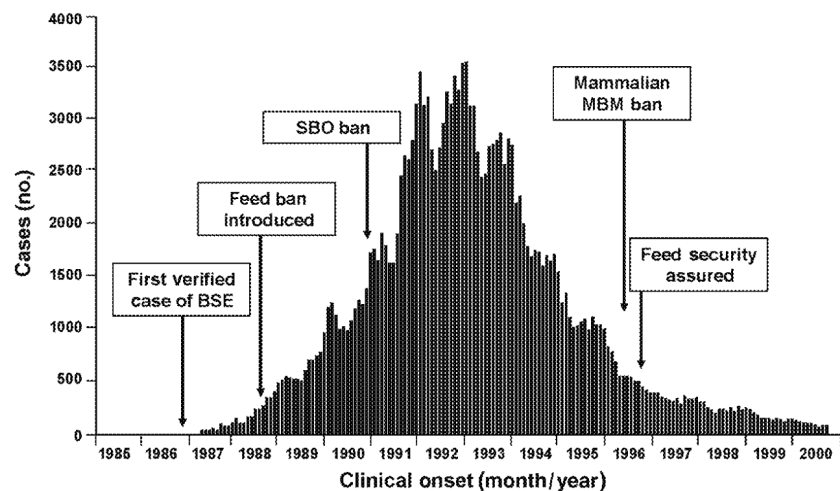


Fig. 1. Bovine spongiform encephalopathy epidemic in the UK [7].

disorders, each with a characteristic clinical and pathological phenotype: familial CJD, the Gerstmann-Sträussler-Scheinker syndrome and fatal familial insomnia [16]. All occur as autosomal dominant disorders [15].

The third group of human prion diseases, the acquired disorders, comprise <1% of all cases and are characterized by exposure to infectivity in brain or nervous system tissue either through human-to-human contact via contaminated neurosurgical instruments, tissue grafts or extracts (iatrogenic CJD) [17], or via the consumption of contaminated bovine meat products (vCJD). Experimental transmission studies have shown that the transmissible agent in vCJD has identical properties to the BSE agent, confirming the link between these 2 disorders [18,19].

Variant CJD was first described in the UK in 1996, but has now been identified in 10 other countries. Variant CJD tends to affect young adults, with a mean age of approximately 29 years (age range 12–74 years at disease onset) [1]. Interestingly, this corresponds with the general age group at which people become blood donors. The duration of the clinical illness is longer (mean duration of 13 months) than that of sCJD, and is characterized by psychiatric features and sensory symptoms at onset, followed by ataxia, myoclonus and other movement disorders; rapidly progressive dementia is very uncommon in this disease [1]. Thus, sCJD and vCJD are distinct disorders that are characterized by different geographical distributions, durations of illness, ages of onset and clinical course, and, most importantly, the causal association of vCJD with BSE.

Transmission of prion diseases by blood

While the transmission of prion infectivity through blood in rodent models of scrapie is well established, recent reports have also found evidence of infectivity in the blood of a rodent model of vCJD and in sheep experimentally infected with BSE [20,21]. These findings have raised questions over the potential transmission of vCJD by blood or blood components. Therefore, concern over safeguarding the blood supply has been gradually mounting given the potentially large number of asymptomatic carriers of vCJD who may unknowingly donate blood. This threat to the blood supply poses a unique challenge to public health officials and raises concerns for patients – especially individuals with haemophilia and other bleeding disorders – who routinely rely on the blood supply and blood therapies. Retrospective studies of haemophilia patients who died from other diseases, including

HIV, have not identified any cases of sCJD that were missed or misdiagnosed, either in the UK or in the USA [22,23]. However, although epidemiological studies of sCJD have found no convincing evidence of its transmission by blood [24], the different pathogenesis of vCJD does not allow reassurance to be taken from these studies focusing on sCJD.

Genetic susceptibility to vCJD

Progress in the understanding of human prion diseases was accelerated following the identification of the PrP gene on the short arm of chromosome 20. The identification of pathogenic mutations and insertions in the PrP gene provided evidence to support the prion hypothesis, as familial prion disorders are both genetic and transmissible. Furthermore, it is now recognized that a polymorphism at codon 129 in the human PrP gene may influence susceptibility to prion disease.

Three genetic subgroups have been identified at codon 129 of the PrP gene: methionine homozygous (M/M), valine homozygous (V/V) and heterozygous (M/V). All clinical cases of vCJD have so far occurred in individuals with the methionine homozygous genotype [25,26]. This finding is important because only around 40% of the total human population are methionine homozygotes; approximately 10% are valine homozygotes and 50% are heterozygotes [27,28,29] (Table 2). However, among sCJD cases, only 65% are methionine homozygotes. Thus the methionine homozygous genotype is more susceptible to developing both sporadic and vCJD.

Diagnostic assays for vCJD

One of the largest issues that confront clinicians trying to manage this disease is the absence of a diagnostic screening test for vCJD. Confirmation of a clinical diagnosis of vCJD requires neuropathological examination of the brain following autopsy, with demonstration of the characteristic type 2B isoform of PrP^{Sc} in the brain and lymphoid tissues [25].

Table 2. PRNP codon 129 genotype frequencies [29].

	Genotype		
	M/M	M/V	V/V
Normal population	37%	51%	12%
Sporadic CJD	65%	17%	18%
Variant CJD	100%	–	–

CJD, Creutzfeldt-Jakob disease; M/M, methionine homozygous; M/V, valine heterozygous; V/V, valine homozygous.

Therefore, diagnostic assays are urgently needed for vCJD that are blood based and do not require an invasive brain or tonsil biopsy [30].

A major challenge to the development of such a test is that prions are devoid of nucleic acid, unlike bacteria or viruses, making rapid polymerase chain reaction-based diagnostics non-viable. In addition, as prions are modified cellular proteins and not foreign, there is an absence of a measurable host immune response; hence, an enzyme-linked immunoadsorbent assay (ELISA) diagnostic test is not feasible. The best diagnostic marker for prion diseases is the presence of the disease-associated isoform of the prion protein, PrP^{Sc} [30]. This is generally detected by western blot assay in the brain and in lymphoid tissues in vCJD [31], but attempts to detect PrP^{Sc} in blood from patients with vCJD have so far been unsuccessful, probably because of limitations in the sensitivity of this assay [32]. However, a conformation-dependent immunoassay was recently described that measures both the protease-resistant and protease-sensitive forms of PrP^{Sc} [33] and appears to be far more sensitive than western blot assays. Whether this method will be applicable to blood samples remains to be seen. Another technique that has recently been developed for enhanced detection of PrP^{Sc} is the cyclical amplification method [34]. This relies on a repeated series of incubation with normal PrP and subsequent cycles of sonication, and has recently detected PrP^{Sc} in blood from a rodent model of TSE [35].

Probable pattern of tissue infectivity in vCJD

In the UK, it is presumed that most of the adult population was exposed to the BSE agent through the ingestion of contaminated meat products in the late 1980s and early 1990s. However, because the incubation period of BSE in humans is unknown (incubation periods of 40 years or longer have been documented for other human TSE) [17], and because of the lack of a reliable screening test, it is currently unknown how many people may be in an asymptomatic phase of vCJD infection in UK.

In contrast to sCJD, vCJD infectivity is more widely distributed outside the CNS, and can readily be found in the peripheral nervous system and lymphoid tissues (tonsil, spleen, lymph node and gut) [31]. The levels of infectivity in these tissues are lower than in the CNS, but they still represent possible sources of person-to-person spread of infectivity (Fig. 2) [36]. As the asymptomatic phase of infection in vCJD may last for at least several years, infected individuals may represent a potential source

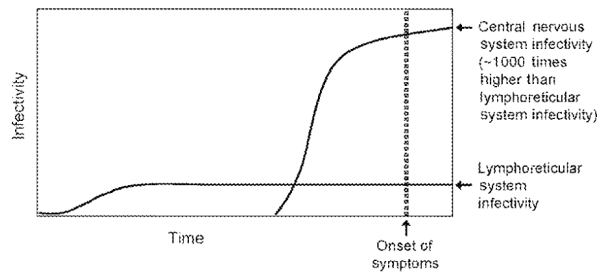


Fig. 2. Probable pattern of tissue infectivity in variant Creutzfeldt-Jakob disease [36].

of secondary spread of vCJD to others via contaminated surgical instruments (such as tonsillectomy instruments) or by blood transfusion.

Variant CJD prevalence study in UK

To estimate the number of individuals in the UK who are asymptomatic for vCJD and who could potentially contribute to the iatrogenic spread of the disease, a retrospective study of lymphoid tissues was recently performed using immunohistochemistry for prion protein in surgically removed tonsillectomy and appendectomy specimens. Researchers reported three positive samples out of 12 674 tested, or an estimated prevalence of 237 vCJD cases per million in the UK (CI 95%) [37,38].

These findings indicate a far higher prevalence than clinical cases would predict, suggesting that additional cases of vCJD are likely to emerge in the UK. Furthermore, they emphasize the importance of preventive measures already instituted by the UK Department of Health to reduce the potential spread of vCJD through blood therapies. These findings also point to the urgent need for large-scale screening of lymphoreticular tissue samples to determine with greater precision the incidence of vCJD infection in the asymptomatic UK population [38].

However, there remains a distinct possibility that some infected patients may never develop clinical symptoms but will remain asymptomatic carriers who can potentially transmit the disease to other individuals. Therefore, screening of infectious individuals will be a critical component for individuals who rely on blood transfusions and/or blood therapies.

Transmission of vCJD infectivity via blood transfusion in humans

Two cases of probable iatrogenic vCJD transmission through blood transfusion have been reported. The first case was a 69-year-old male who presented with

clinical symptoms typical of vCJD in 2002, 6.5 years after receiving one unit of non-leucodepleted packed red blood cells [39]. This patient died 1 year later. Sequencing of the prion protein gene revealed that he was methionine homozygous at codon 129 of the prion protein gene. The asymptomatic donor developed symptoms 3.5 years after donation and subsequently died.

The second case was an elderly female patient who was a known recipient of a blood transfusion from an asymptomatic donor who later developed vCJD [40]. The female patient died of an unrelated illness and without any vCJD clinical symptoms. Because of her known exposure, a medicolegal autopsy was performed. Abnormal prion protein was detected in the spleen and lymph nodes; however, PrP^{Sc} was not detected in the CNS and there were no other significant abnormalities in the CNS. Interestingly, this patient was heterozygous (M/V) at codon 129 in the prion protein gene.

Because that was the first identified case of vCJD infection occurring in the heterozygous subgroup [40], this case raises many important issues regarding the disease, including whether this genotype may have influenced either its incubation period or distribution of infectivity in this patient. These findings underscore the importance of developing effective screening tools and techniques to identify blood donors who may be asymptomatic. In addition, they highlight the need to ascertain whether all vCJD/BSE infections result in clinical disease or whether a subclinical carrier state may occur.

Epidemiological considerations

In the absence of a transfusion-transmitted infection, one statistical analysis has estimated that the probability of acquiring vCJD is approximately 1 in 15,000 to 1 in 30,000 [39]. Therefore, while dietary exposure can never entirely be ruled out, in the aforementioned cases, the infections were far more likely associated with vCJD-contaminated blood transfusions.

To examine a probable link between transfusion and vCJD infection, a review of blood transfusion policies in the UK and a risk assessment on the implications for plasma therapy recipients was commissioned by the Department of Health [41]. The commissioned research concluded that the infectivity concentrations in blood were likely to be highest in the buffy coat fraction, followed by those in plasma and whole blood (Table 3). Moreover, the report stated that levels of the infectious agent present in a full unit of blood would probably be sufficient to

Table 3. Selected infectivity of blood components [41].

	Volume (mL unit ⁻¹)	Infectivity (ID ₅₀ /unit)	Infectivity concentration (ID ₅₀ /unit)
Whole blood	450	900	2.0
Plasma	225	480	2.1
Filtered plasma	225	480	2.1
Red cells	212	219	1.0
Buffy coat	14	201	14.9

cause infection in recipients [41]. The Department of Health's Health Protection Agency also evaluated the risk of different plasma products in an attempt to determine which were most likely to carry the greatest degree of vCJD infectivity. Recipients of factor VIII, factor IX and antithrombin were estimated to have the highest risks: administration of even a single one-vial dose of these products was determined to be sufficient to cause transmission of the disease [42]. Intravenous immunoglobulin (IVIG) and large doses of albumin were concluded to be of medium risk, and anti-D and IVIG were determined to be of low-risk of infectivity.

The risk of contracting vCJD from plasma therapies

As recipients of plasma therapies appear to possess the highest risk of contracting vCJD, it is theoretically possible that many patients with bleeding disorders in the UK have already been exposed to the agent responsible for vCJD. Patient groups and the UK Haemophilia Centre Doctors' Organisation believed that the Health Protection Agency's CJD Incidents Panel should recommend that all patients with bleeding disorders in the UK who were treated with UK-source pooled factor concentrates between 1980 and 2001 be considered at potential additional risk for public health purposes [42].

The risk of contracting vCJD has implications for the overall safety of the worldwide blood supply. To address this concern, various measures have been taken to protect the blood supply in the UK, including the sourcing of plasma from the United States (Table 4). Future efforts to minimize the risk of prion contamination of the blood supply might include improved filtration steps to more effectively remove this pathogen.

Variant CJD worldwide as of October 2005

As of October of 2005, 184 confirmed cases of vCJD have been reported worldwide. Individual countries include: UK (158), France (15), Ireland (3), Italy (1),

Table 4. Measures taken to reduce the risk of variant Creutzfeldt–Jakob disease (vCJD) transmission via blood and blood therapies in the UK.

Date	Measure
1997	Withdrawal and recall of any blood components, plasma therapies or tissues obtained from any individual who develops vCJD
1998	Importation of plasma from the USA for fractionation
1998–1999	Leucodepletion of all blood used for transfusion
2002	Importation of fresh plasma from the USA for patients born on or after 1, January 1996
2004	Blood donation is not accepted from people who have received a blood transfusion in the UK since 1980, or who are unsure of this
2005	Donors of blood to patients who have subsequently developed vCJD are advised that they may be at 'increased risk' of vCJD and should not continue to donate blood
Today	Promotion of appropriate use of blood and alternatives in NHS
The future?	Use of 'prion filters'?

USA (1), Canada (1), Saudi Arabia (1), Japan (1), the Netherlands (1), Spain (1) and Portugal (1). The individuals in the USA, Canada and Japan who contracted vCJD and one person in Ireland had all lived in the UK; therefore, these four cases are considered as UK infections.

Japan confirmed its first case of vCJD in 2005. This patient had briefly visited the UK in the late 1980s, fell ill in 2001 and died in 2004. While BSE has been identified in 15 Japanese cattle, officials contend that the patient most likely contracted the disease while in the UK [43]. Because the patient is believed to have visited the UK for less than a month, the Japanese government has changed its blood donation policy to ban donations from anyone who visited UK for a day or more between 1980 and 1996. Previously its policy had been to accept blood donors who had visited the UK for up to 1 month [44].

The fact that cases of vCJD have been reported in many different countries suggest that the disease has spread from the UK to other continents. Although the number of deaths per annum of vCJD in the UK has steadily declined from 28 in the year 2000 to only two by the middle of 2005, the onset of new cases has gradually risen to nine in 2004 from five in 2003 [45]. These data suggest that the disease may become endemic at a low level in the UK population.

Research priorities for vCJD

There are four immediate research priorities. First, to reduce the potential spread of vCJD, there is an urgent

need for development of a new screening assay that is applicable to blood and is both highly specific and sensitive. Second, enhanced epidemiological surveillance of potentially infected donors should be broadened to encompass all age groups in the UK. Third, improved methods of decontamination of surgical and laboratory instruments must be developed and implemented across the country to reduce further iatrogenic infections. Finally, progress in the treatment and prophylaxis of vCJD is desperately needed.

Conclusions

In the last decade, a variant of CJD has emerged in many countries that has been causally linked to dietary exposure to BSE during the 1980s and early 1990s. Preliminary studies in animal models suggest that prions, including the BSE agent, can be transmitted by blood. Based on two recent reports of iatrogenic vCJD transmission by blood transfusion in humans, a UK DOH-sponsored risk assessment warned that recipients of plasma therapies are now at risk of contracting vCJD from potentially infected donors. In the absence of screening tests or effective therapies to treat this disease, a formidable worldwide public health challenge lies ahead to prevent new infections, accurately assess infection rates and treat infected patients.

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