Review article

Folia Neuropathologica

Risks of transmission of variant Creutzfeldt-Jakob disease by blood transfusion

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Folia Neuropathol 2005; 43 (4): 271-278

Abstract

Variant Creutzfeldt-Jakob disease (vCJD) was first identified in 1996 in the UK, and results from human exposure to the bovine spongiform encephalopathy (BSE) agent. vCJD has subsequently been identified in 10 additional countries, and numbers continue to increase in the UK. Unlike other human prion diseases, infectivity and the disease-associated form of the prion protein are readily detected in lymphoid tissues in vCJD. In experimental BSE infection in a sheep model, infectivity has been transmitted by blood transfusion from asymptomatic infected animals to normal recipient animals, indicating that infectivity is present in blood during the incubation period. Recently, two cases of apparent iatrogenic vCJD infection by blood transfusion from asymptomatic donors who subsequently died from vCJD have been reported from the UK. The first case resulted in clinical illness identical to other cases of vCJD, while the second case was an asymptomatic infection detected at autopsy. Sensitive means of detection of disease-associated prion protein in the blood are required in order to be employed for screening purposes, both individually at the time of blood donation, and to help ascertain future numbers of vCJD cases in the UK and beyond.

Key words: *variant Creutzfeldt-Jakob disease, transmission, blood transfusion, infectivity.*

Introduction

Surveillance of human prion diseases was reinstituted in the UK in 1990 as a consequence of the epizootic of bovine spongiform encephalopathy (BSE) in UK cattle and its possible implications for human health. In 1996, variant Creutzfeldt-Jakob disease (vCJD) was reported in a series of 10 patients from the UK as a novel form of human prion disease with a unique clinical and neuropathological phenotype [41]. All affected individuals belonged to the same genetic subgroup as defined by the naturally occurring polymorphism at codon 129 in the prion protein gene (*PRNP*) on chromosome 20, since all were methionine homozygotes. In contrast, *PRNP* codon 129 genotype frequencies in the normal population and in sporadic Creutzfeldt-Jakob disease (sCJD) show marked differences in the distribution of *PRNP* codon 129 genotype frequencies [2] (Table I). Subsequent biochemical investigations found that all cases of vCJD contained a single isoform of the disease-associated prion protein (PrP^{Sc}) in the brain (type 2B), which was different from the 2 main isoforms occurring in sCJD (type 1 and type 2A) [17].

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Transmission studies to inbred and bovine transgenic mice have shown that the transmissible agent in vCJD has identical biological properties to the BSE agent, confirming that vCJD represents the consequence of BSE infection in humans [8,36]. vCJD is unique, since it represents the only example of a human prion disease acquired from another species (Table II).

Table I. Prion protein gene polymorphisms in the normal population and in prion diseases

	PRNP codon 129 polymorphisms (%)		
	MM	MV	VV
normal population	39	50	11
sCJD	71	13	16
vCJD	100	–	–

(M= methionine, V=valine)

Table II. Classification of human prion diseases

Idiopathic:		sporadic Creutzfeldt-Jakob disease sporadic fatal insomnia
Familial:		familial Creutzfeldt-Jakob disease Gerstmann-Straussler-Scheinker syndrome and variants fatal familial insomnia
Acquired:	Human:	Kuru iatrogenic Creutzfeldt-Jakob disease
	Bovine:	variant Creutzfeldt-Jakob disease

Table III. Numbers of vCJD cases worldwide (October 2005)

Country	Number of vCJD cases
UK	159
France	15
Ireland	3
Canada	1
Italy	1
Japan	1
Netherlands	1
Portugal	1
Saudi Arabia	1
Spain	1

Epidemiological studies have indicated that the most likely source of human exposure to BSE is the consumption of contaminated meat products, although other possibilities (such as occupation exposure in abattoir workers and butchers) cannot yet be excluded. By October 2005 there have been over 150 cases of vCJD in the UK, with additional cases in 10 other countries (Table III). The cases outside the UK represent a combination of individuals who had previously visited or resided in the UK (and therefore were presumably infected in the UK), and others who had never visited the UK and thus apparently represent endogenous infections with BSE. Although the incidence of vCJD has declined in the UK since 1999-2000, there have been more new cases identified in 2004 than in 2003, making it difficult to predict the likely number of future cases. Furthermore, a retrospective prevalence study of vCJD infection using immunohistochemistry to detect disease-associated prion protein accumulation in surgically resected appendix and tonsil tissues found 3 positive cases out of 12,674 samples, far higher than current clinical cases of vCJD would suggest [23]. This finding suggests that the current patients with vCJD may represent only those with the shortest incubation periods; alternatively, not all BSE infections may result in clinical disease, but instead may produce an asymptomatic carrier state.

Peripheral tissue involvement in vCJD

Another major difference between vCJD and other human prion diseases is the consistent presence of PrP^{Sc} within tissues outside the central nervous system, particularly lymphoid tissues and the peripheral nervous system [26]. PrP^{Sc} was first identified in the tonsil [21], and was demonstrated subsequently in the spleen, lymph nodes, thymus and gut-associated lymphoid tissues e.g. in the appendix and rectum [19,22,26,27,39]. In lymphoid tissues, PrP^{Sc} accumulates in follicular dendritic cells and macrophages within germinal centres (Figure 1), which may act as a site for the replication of the infectious agent prior to invasion of the central nervous system [20]. PrP^{Sc} has been identified by the western blot analysis and immunohistochemistry in the tonsils of patients within the clinical phase of vCJD, allowing tonsil biopsy to be used as a diagnostic tool in some cases, since PrP^{Sc} does not accumulate in the tonsil in sCJD [20]. PrP^{Sc} accumulation has also been identified by immunohistochemistry in germinal centres in the surgically resected appendix specimens from patients who subsequently developed vCJD up to two years prior to the onset of their illness [23]. PrP^{Sc} has been identified in the peripheral nervous system in vCJD, particularly in the autonomic and sensory ganglia by both immunohistochemistry and western blot techniques [16,19].

Infectivity has been demonstrated in the spleen and tonsil from variant CJD on intracerebral inoculation into inbred mice, at levels around 2 logs lower than in the brain [7]. Studies in experimental models of prion diseases indicate that involvement of the lymphoid tissues can occur early after exposure to the transmissible agent, and may persist throughout the incubation period [40]. The spread of infection between lymphoid tissues may involve circulating lymphocytes, although their precise role is uncertain; the role of lymphocytes in the peripheral pathogenesis prion diseases has been extensively researched and reviewed by Aguzzi et al [1]. These findings have lead to the suggestion that circulating lymphocytes may carry infectivity in blood [38,42]. Despite this suggestion, intracerebral inoculation of buffy coat from vCJD cases into susceptible mice failed to demonstrate infectivity [7], and PrP^{Sc} could not be detected by the Western blot examination of buffy coat in vCJD [39]. However, the sensitivity of the techniques used in these studies may be insufficient to detect low levels of PrP^{Sc} and infectivity, and only small numbers of vCJD blood samples have been available for study.

Blood infectivity in experimental prion diseases

Infectivity in blood or in its components during both the incubation period and the clinical phase of the experimental prion diseases has been demonstrated in a range of rodent models [5]. One major problem in assessing the potential for vCJD infectivity by blood transfusion in these studies is that the prion strain used in many of these experiments was not derived from either BSE or vCJD, although recent studies of a mouse-adapted vCJD model have demonstrated endogenous infectivity in blood [11]. Furthermore, only relatively small volumes of blood are available for transfusion in rodent models, which makes it difficult to identify the distribution of infectivity in blood components.

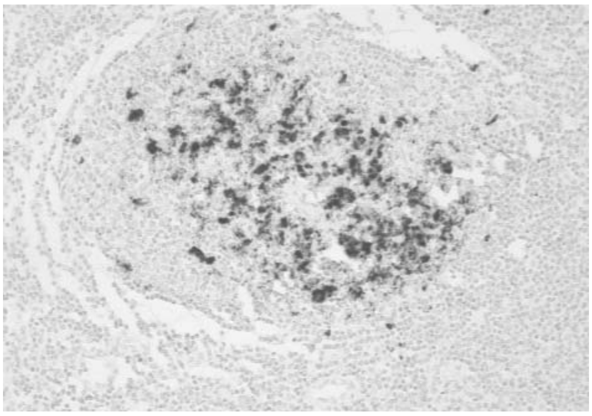

Fig. 1. Immunohistochemistry for PrP gives a positive reaction in follicular dendritic cells and macrophages within a germinal centre in the tonsil from a patient with vCJD (KG9 antibody with haematoxylin counterstain, original magnification x 200)

For these reasons, a study of infectivity transmitted by blood transfusion in an experimental BSE model in sheep has attracted attention, particularly since the tissue distribution of PrP^{Sc} and infectivity outside the central nervous system in this model is very similar to vCJD in humans. The preliminary results indicated transmission of BSE by whole blood transfusion from one infected sheep in the pre-clinical stage of the disease [24]. Subsequent data has indicated that additional BSE transmissions by transfusion of whole blood from infected donor animals during both the pre-clinical and clinical stages of infection have occurred, and the experiment is still incomplete [25]. These recent results give a minimum rate of BSE infection by blood transfusion of at least 17%; the same study also investigated the possibility of scrapie transmission by blood transfusion, which was achieved at a rate of around 19% at the time of publication [25]. One positive scrapie transmission also occurred in a sheep transfused with a relatively small volume of a buffy coat preparation made from blood taken at the clinical end point of disease from the donor. The lengthy timescale of these experiments means that it will be some considerable time before all the experimental data is complete. This model has

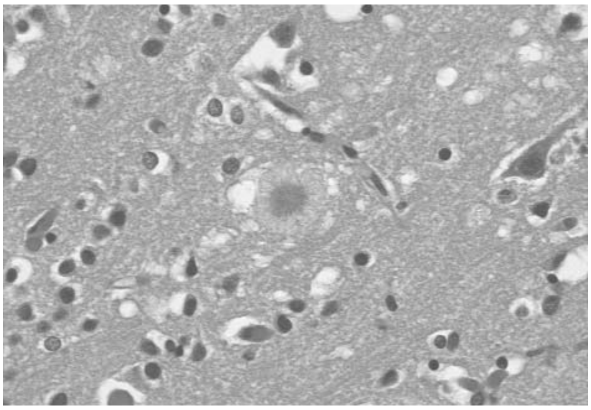

Fig. 2. The cerebral cortex in the patient who developed vCJD after receiving a red blood cell transfusion shows a characteristic florid plaque (haematoxylin and eosin, original magnification x 250)

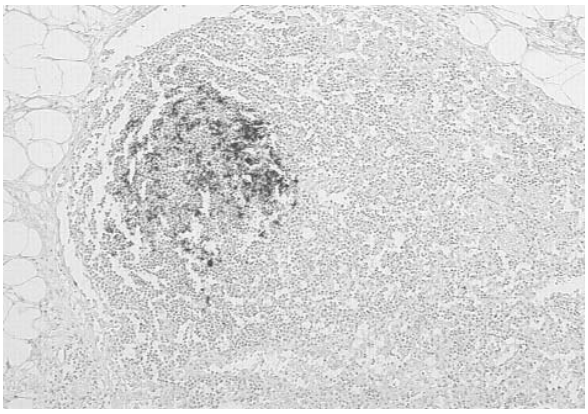

Fig. 3. A cervical lymph node from the elderly patient who developed asymptomatic vCJD infection following a red blood cell transfusion shows positive staining on immunohistochemistry for PrP in a germinal centre (KG9 antibody with haematoxylin counterstain, original magnification x 200)

the considerable advantage of having the potential to determine the distribution of infectivity in the BSE and scrapie-infected sheep blood components. The transmission of scrapie by buffy coat indicates that the white-cell fraction of blood is infectious, but further data is required on infectivity in other cellular components of the blood, plasma and plasma fractions in this experimental model. This model could also be used to study the effect of various interventions to reduce or abolish infectivity in blood, blood components and blood products, particularly those such as leucodepletion which can be, or are currently employed as risk-reduction measures to prevent the spread of vCJD in humans.

Is infectivity present in blood in vCJD?

Epidemiological studies have indicated that blood transfusion is not a risk factor for sCJD, despite early reports of the detection of infectivity in blood in sporadic CJD using rodent bioassay models [30]. However, this finding has not been replicated in other models, even on intravenous infusion of a whole unit of blood from a sCJD patient into a chimpanzee [6], which is probably the experimental model that most closely replicates human susceptibility to prion diseases. The more widespread tissue distribution of infectivity in vCJD in comparison to sCJD [7,19], and the experimental transmission of infectivity by blood transfusion in BSE-infected sheep [24] have suggested that blood transfusion is more likely to potentially transmit infectivity in vCJD than in sCJD or in other human prion diseases.

In the UK, a collaborative study known as the Transfusion Medicine Epidemiology Review between the various blood authorities and the National CJD Surveillance Unit reviews the transfusion histories of patients who have given blood or received blood and subsequently develop variant CJD. In 2003, this study identified a case of vCJD that occurred in a patient who had received one unit of non-leucodepleted red blood cells from a donor who, although asymptomatic at the time of donation, developed vCJD and died 3 years later [28]. The recipient developed symptoms of vCJD 6.5 years after the transfusion and died one year later. The clinical and neuropathological features of the illness in the recipient were closely similar to those of other cases of vCJD (Figure 2), and biochemical studies found the type 2B isoform of PrP^{Sc} in the brain. Genetic analysis of the *PRNP* showed that the recipient was a methionine homozygote at codon 129, as have been all patients with vCJD in whom a similar analysis has been performed.

In 2004, the UK National CJD Surveillance Unit reported the pathological findings following autopsy in an elderly patient who had undergone transfusion of one unit of non-leucodepleted red blood cells from another donor who was asymptomatic at the time of donation, but who subsequently died from vCJD [32]. The recipient died 5 years later of an unrelated illness, with no symptoms of vCJD during life. Neuropathological examination of the central nervous system found no pathological changes of vCJD and immunohistochemistry for PrP^{Sc} was negative. The Western blot analysis for PrP^{Sc} was also negative in the brain. Immunohistochemistry for PrP^{Sc} found positivity in germinal centres within the spleen and a cervical lymph node (Figure 3), but not in the tonsil or the appendix. A high sensitivity western blot analysis confirmed the presence of PrP^{Sc} in the spleen (Figure 4) with variable levels in several samples, indicating a heterogeneous distribution [32]. The PrP^{Sc} isoform in the spleen closely resembled that found in the spleen in clinical cases of vCJD. Of particular interest, a genetic analysis of the *PRNP* found that this patient was a codon 129 heterozygote (methionine/valine), unlike all clinical cases of vCJD.

This unique case raises a number of important questions, in particular the influence of the *PRNP* codon 129 genotype on the incubation period of the disease, and the distribution of PrP^{Sc} in lymphoid tissues. In kuru, another acquired human prion disease that was likely to have been transmitted by the oral route, the *PRNP* codon 129 polymorphism has been found to exert a major influence on the disease incubation period, with heterozygotes having the longest incubation period [15]. In this case of preclinical vCJD infection, it is also possible that the restricted distribution of PrP^{Sc} within lymphoid tissues might reflect the route of transmission of infectivity. In primary cases of vCJD, infection is likely to be acquired by the oral route, with PrP^{Sc} and infectivity present in tonsil and gut-associated lymphoid tissue possibly before the spleen and lymph nodes. Since the vCJD infection in the preclinical case is likely to have been acquired by the intravenous route, the lack of PrP^{Sc} in the tonsil and appendix may reflect the absence of exposure to oral infectivity.

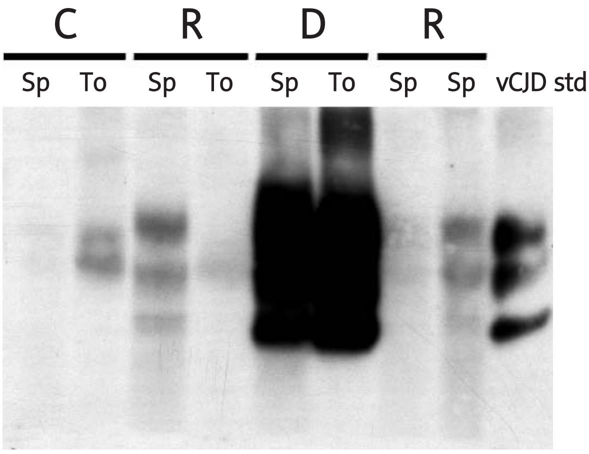

Fig. 4. Western blot analysis of spleen (Sp) and tonsil (To) samples from patients C, R and D. C, non-CJD neurological control patient; R, the patient who developed asymptomatic vCJD infection following a red blood cell transfusion; D, the relevant donor to the asymptomatic vCJD infection patient. Tissue samples (50mg) were homogenised, precipitated with phosphotungstate and proteinase K digested as described previously [11,27]. Samples were immunoblotted alongside standard vCJD brain homogenate (250 μg) using anti-PrP antibody 3F4 as the primary reagent

The identification of vCJD infection in two individuals who received blood transfusions from vCJD-infected donors is highly unlikely to have occurred by chance, and indicates that blood is infectious in the asymptomatic preclinical phase of vCJD. Since vCJD was first identified in 1996, a number of precautionary steps have been taken to reduce the likelihood of transmission of infectivity from blood and blood products in the UK (Table IV). None of these measures are likely to completely remove the risks of vCJD transmission, and it is possible that other precautionary steps will be introduced to further reduce any residual risk.

The Department of Health in the UK commissioned a risk assessment of the potential for transmission of vCJD by blood and blood products [13]. This concluded that in addition to the recipients of blood and certain blood components from donors infected with vCJD, recipients of UK plasma products in the 1980s and early 1990s might also be at increased risk of vCJD infection. Following a joint decision between the CJD Incidents Panel and clinicians responsible for the treatment of haemophilia and primary immunodeficiency, the recipients of certain plasma products (including most UK adult haemophiliacs) have been notified that they are at increased risk of vCJD, and their clinicians and general practitioners have been requested to undertake certain precautionary measures (Table V). Similar measures have also been undertaken for patients who were known to have received blood or certain blood components from donors who subsequently developed vCJD, and for donors of blood to patients who have subsequently developed vCJD. The problem of infectivity in vCJD blood donations is not confined to the UK, since some vCJD cases in France were also found to have donated blood prior to the onset of their illness.

Table IV. Measures taken in the UK to reduce the risk of vCJD transmission by blood and blood products

Year	Measure introduced
1997	withdrawal and recall of any blood components, plasma products or tissues obtained from any individual who develops vCJD
1998	importation of plasma from the USA for fractionation
1998-9	leucodepletion of all blood used for transfusion
2002	importation of fresh plasma from the USA for patients born on or after January 1st 1996
2004	blood donation is not accepted from people who have previously received a blood transfusion in the UK since 1980, or are unsure of this
2005	donors of blood to patients who have developed vCJD following transfusion have been advised that they are "at risk" of vCJD (see Table V)
Since 1997	promotion of more appropriate use of blood and alternatives in the National Health Service

Table V. Public Health precautions for patients "at risk" of vCJD in the UK

1. Patients should not donate blood, organs or tissues.
2. Patients should inform their clinicians if they need medical, surgical or dental treatment so that infection control measures can be taken.
3. The patient's "at risk" status should be recorded on their medical records.
4. Clinicians responsible for these patients should contact the patient's General Practitioner, who should:
   a Be aware that their patient is being informed of their "at risk" status,
   b. Record this status in the primary health record,
   c. Provide information on the patient's recent surgical history at other hospitals.

Future prospects and needs

Many of the current concerns over the transmission of vCJD by blood and blood products could be alleviated if there was a sensitive and specific screening test available that could be used on blood donors [29]. At present no such test is available, although a number of groups are undertaking research in this field. Most of these investigations are based on the detection of PrP^{Sc} in blood, but given the considerable scientific and technical difficulties involved, it does not seem likely that such a screening technique will be available in the immediate future. The potential approaches to detect PrP^{Sc} in tissues and blood have varying degrees of sensitivity and specificity, many of which have been reviewed by MacGregor [29]. Data from experimental models in which infectivity is detectable in blood indicate that the levels of PrP^{Sc} that are likely to be present in blood will be very low, requiring extremely sensitive detection techniques. Specificity for detection of PrP^{Sc} is also essential, since PrP^{C} is present in blood and may be present at increased levels in patients with prion diseases [14]. The relative sensitivity of current assays for PrP^{Sc} is summarised in Table VI.

One additional technique that has recently been found capable of detecting low levels of PrP^{Sc} is the cyclical amplification technique [33]. This method utilises a combination of PrP^{Sc} amplification by incubation with excess normal PrP^{C} and sonication in repeated sequence to produce a highly sensitive detection system. This method has been used with success primarily in rodent models of prion disease, in which it has been claimed that infectivity can be amplified by this method, thereby supporting the prion hypothesis [9]. PrP^{Sc} has recently been detected in the blood of a scrapie-infected hamster model by this method, and although further work is required to confirm this finding in other models and in humans, this report demonstrates the potential for considerable short-term technical advances in this field [10].

Even before a technically validated, sensitive and specific screening test for vCJD becomes available, consideration should be given to the potential use of such a test [37]. Possible uses could include anonymised screening of large sections of populations

Table VI. Detection limits of the most sensitive PrP^{Sc} assays currently available. Detection limits for the assays are expressed as the maximum dilution of a 10% (w/v) homogenate of prion-diseased brain in which PrP^{Sc} is still detectable. (Updated from MacGregor [30])

Assay	Test sample	Published detection limit	Reference
Enfer®-ELISA	BSE brain homogenate	$10^{-1.5}$	31
Prionics®-Check western blot	BSE brain homogenate	$10^{-1.5}$	31
Bio-rad® ELISA	BSE brain homogenate	10^{-3}	12
NaPTA/Western blotting	vCJD brain homogenate	$10^{-2.3}$-$10^{-3.3}$	39
Prionics®-Check LIA	BSE brain homogenate	10^{-4}	4
CDI/DELFIA	vCJD brain homogenate	10^{-4}	3
CDI/DELFIA	BSE brain homogenate	10^{-5}	34
CDI/DELFIA (InPro®)	sCJD brain homogenate	10^{-5}-10^{-6}	35

in order to establish the prevalence of vCJD infection, and testing of individuals prior to blood donation and/or surgical operations. These potential uses will raise a number of major ethical considerations, given the current lack of any proven effective prophylaxis or treatment for prion diseases. The need for informed consent for such investigations (particularly in individual cases) is currently under debate in the UK, since the benefits for any one individual undergoing testing might be questionable. There are already a number of potential therapeutic compounds under investigation for prion diseases that, if proven to be effective, might eventually allow a reappraisal of the current situation [18]. However, it is unlikely that an effective prophylactic or therapeutic compound will become available in the near future, reinforcing the need for continued measures to reduce the risk of secondary transmission of vCJD by blood components and blood products. Continued surveillance for vCJD in the general population and in those identified as "at increased risk" of vCJD because of exposure to potential- infectivity via blood components and blood products will be required in order to assess the magnitude of these risks.

Acknowledgements

The National Creutzfeldt-Jakob Disease Surveillance Unit is supported by the Department of Health and the Scottish Executive. This work forms part of the EU NeuroPrion network of excellence (FOOD-CT-2004-506579) subproject PRIOGEN.

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