

**New-Variant Creutzfeldt-Jakob Disease: The Risk of Transmission by  
Blood Transfusion.**

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**SUMMARY.**

New-variant Creutzfeldt-Jakob disease (nvCJD) was first described in the UK in 1996 and is thought to be related to the consumption of cattle suffering from bovine spongiform encephalopathy. Although only 25 cases have been confirmed to date, it is too early to predict the number of people who may currently be incubating the disease. Past experience suggests that sporadic CJD is rarely, if ever, spread by blood transfusion. However, it is unclear whether nvCJD may be transmissible by this route and if so, how easily. Assessing the potential risk of transmission of nvCJD by blood transfusion and evaluating the likely efficacy of proposed strategies to reduce this risk is therefore very difficult. This article summarises the spectrum of transmissible spongiform encephalopathies in animals and Man, the molecular and cellular biology of the prion protein and the continuing debate as to the nature of the infectious agent. The distribution of normal prion protein expression, the results of experimental transmission studies and the case reports and clinical studies on CJD transmission are reviewed. Finally, the extent of current knowledge and the potential utility of proposed strategies to reduce the risk of nvCJD transmission by blood transfusion are discussed.

## **INTRODUCTION.**

### **Transmissible spongiform encephalopathies in animals.**

Transmissible spongiform encephalopathies (TSE) are a spectrum of diseases which affect both humans and animals (Table I). The best characterised disease is scrapie which has been recognised for over two centuries in many countries and affects both sheep and goats. Icelandic farmers deduced that scrapie was infectious and this was demonstrated in 1936 when the disease was transmitted to uninfected sheep by intracerebral injection of infected sheep brain (1). Despite widespread and prolonged cohabitation with, and consumption of, scrapie-infected domestic animals, there is no evidence to indicate that transmission to man occurs (2). Chronic wasting disease of mule deer and Rocky Mountain elk occurs only in Wisconsin and Colorado. These are the only TSEs in which direct intra-species spread appears to occur. Transmissible mink encephalopathy was described in Wisconsin in 1947 and is thought to have resulted from ingestion of contaminated foodstuff, the source of which is uncertain.

Bovine spongiform encephalopathy (BSE) was identified as a novel TSE in British cattle in 1985-86 (3,4) and spread rapidly in the UK cattle population leading to over 175,000 recorded cases by February 1998. The cows become apprehensive, hyperaesthetic and uncoordinated and develop a deteriorating mental condition which makes them hard to handle. Neuropathological abnormalities are consistent with the symptoms of TSE and are reproducible in characteristics and distribution both in cattle and on passage in mice, suggesting a single strain of agent which differs from those of scrapie (*vide infra*). The BSE epidemic is thought to be related to the practice of feeding cattle on ruminant meat and bone meal, compounded by deregulation of the UK meat rendering process in 1981-82 which allowed reduction in the use of

organic solvents and high temperatures which previously may have reduced the titre of infectious agent. Ingestion of as little as 1 g of BSE-infected brain material has been shown to transmit disease to other cattle (5). Exotic ungulate encephalopathy (e.g. antelope, nyala, greater kudu, gemsbok) (6) was identified in British zoos in the mid-1980s and feline spongiform encephalopathy in domestic (7) and exotic cats in the early 1990s (8) and are thought to be due to transmission of BSE via foodstuff (9). BSE can be transmitted experimentally to other species including primates, rodents, sheep, goat and pigs (10-12). Some authorities believe it is possible that BSE has spread to commercial sheep by the consumption of ruminant meat and bone meal, but may be misdiagnosed due to confusion with scrapie. Transmission to pigs and chickens which have been fed in a similar way has not been reported, but may not be apparent because of the short life of commercial animals.

Concern over BSE led to restrictions in the feeding of ruminant-derived protein to other ruminants in July 1988 and the epidemic is now regressing although cases are still projected to be seen for at least another 4-5 years (13). Cases of BSE in other countries are thought to be due to imported UK cattle or meat and bone meal. Bovine offal was banned for human consumption in November 1989 and beef on the bone in December 1997. In total, over 700,000 infected cattle are thought to have entered the human food chain.

#### Transmissible spongiform encephalopathies in man.

Several forms of TSE are described in man and these are summarised in Table I. Although these can be classified as being of sporadic, infectious or familial / genetic aetiology, there is considerable overlap.

Creutzfeldt-Jakob Disease (CJD) was first described in the early 1920s (14,15) and manifests with a range of clinical neurological manifestations including rapidly progressive presenile dementia, myoclonus and progressive motor dysfunction and a characteristic electroencephalogram (EEG) which, together, usually allow a confident clinical diagnosis (16). No disease-specific immunological response has been described and there are no consistent haematological, biochemical or systemic pathological changes. Definitive diagnosis is reliant on brain biopsy or post-mortem examination. The average age at presentation is 65 years with an average clinical course of 2-6 months between presentation and death (17). Sporadic CJD is not associated with mutations in the *PRNP* gene, but a naturally occurring polymorphism at codon 129 influences disease susceptibility (*vide infra*). There is no evidence that CJD is contagious.

Kuru was described in the Fore tribe of the Papua New Guinea highlands in 1957 (18) and was transmitted by the practice of ritualistic cannibalism of the brains of dead relatives. Patients presented with cerebellar ataxia and progressive neurological incapacity leading to death within 12 months. The incubation period was thought to have been between 5 and 30 years. At the peak of the epidemic the incidence was about 1% of the population though this disease has now almost entirely disappeared.

At least 150 cases of iatrogenic transmission of CJD have been described from inadequately sterilised neurosurgical instruments and stereotactic EEG

electrodes, dura mater grafts, corneal grafts and cadaveric pituitary derived gonadotrophins and growth hormones (19-21). Unfortunately, the denominator for these figures does not appear to be readily available; in the United Kingdom 26 cases of iatrogenic CJD have occurred amongst 1,908 who had received cadaveric pituitary hormones (1.4%) (22). In France, 54 probable cases of CJD have been recorded amongst 968 recipients of cadaveric growth hormone (5.6%) (23). Iatrogenic disease is particularly informative in that time and route of infection can be defined. Incubation periods and distribution of disease have been found to differ depending on the route of infection. Central inoculation (neurosurgical instrumentation, corneal grafts) leads to a short incubation period (median 18 months, range 1-2 years) with predominant demential clinical pattern. Parenteral inoculation by pituitary-derived hormones, on the other hand, tends to present with a longer incubation period (median 12 years, range 5-30 years) and a predominant cerebellar clinical pattern. These clinical differences are reflected in the distribution of neuropathological lesions (21,24).

The prevalence of human TSE is around  $0.7 / 10^6$  in most countries. Of these around 87% represent sporadic CJD, 8% genetic and 5% iatrogenic infection (25). The overall incidence in the UK has increased slightly over the 1990s, mainly in those over 75 years of age which is thought most likely to reflect improved case ascertainment (26,27).

#### New variant Creutzfeldt-Jakob disease.

Routine epidemiological surveillance was re-instituted in the UK in May 1990 to identify possible changes in the occurrence of CJD following the BSE

epidemic. Similar surveillance was established in France, Germany, Italy and the Netherlands between 1993 and 1995. In 1996 Will and colleagues (28) reported a cluster of CJD in the UK with atypical clinical and neuropathological features (Figure 1). The patients were 10 young adults (median age 29 years, range 19-41), with a longer duration of clinical disease than classical CJD (7.5 to 22.5 months), presenting with behavioural change / depression, dysaesthesia and ataxia, with progressive dementia, myoclonus and choreoathetosis as late features. None had the EEG changes associated with classical CJD. They termed this condition new variant CJD (nvCJD) and proposed on epidemiological and neuropathological grounds that it was linked to dietary exposure to BSE. Since then 14 further individuals in the UK (27,29-31) and one in France (32,33) have been diagnosed with the same condition. It has been suggested that the French case may be related to use of bovine growth hormone (34). No specific risk factors have been identified apart from dietary consumption of UK beef products. Four cases of CJD have been reported in dairy farmers with BSE in their herds; these exhibited the features of sporadic CJD, which is known to be more common than average in this occupational group, even in countries with no BSE (28).

The proposed aetiological relationship between BSE and nvCJD has been supported by several lines of evidence. Experimental transmission of BSE to rhesus macaques reveals neuropathological similarities to nvCJD (10). Collinge *et al* have demonstrated that nvCJD is associated with a specific pattern of protease-resistant prion protein fragment size on Western Blot (glycosylation dependent) which distinguishes it from other forms of CJD and resembles that found in BSE and in BSE transmitted to other animals (35).

The same group recently extended these observations with similar observations on transmission of nvCJD to wild-type and PrP-transgenic mice (36). Bruce *et al* demonstrated that the strain characteristics (incubation period and pathological phenotype) were similar in 3 cases of nvCJD, distinct from that of classical CJD and indistinguishable from that of BSE (12). Taken together, these studies provide very solid support for the proposition that BSE has spread to man as nvCJD.

Several authors have attempted to project the number of individuals who may develop nvCJD. Exposure of the UK general population to BSE is likely to have commenced in 1982, have been greatest in the years leading up to the ban on bovine offal for human consumption in November 1989 and should decline to negligible levels over the next 4-5 years (13). The overall rate of new cases of nvCJD does not appear to be increasing at present but it will be several years before one can be confident that there will not be an epidemic (27,37). Prediction of the possible size of any future epidemic from the number of cases of BSE which have entered the food chain is virtually impossible because the dose of BSE required to infect man when administered orally is unknown. Dealler uses an estimated range of between  $10^3$  and  $10^9$  iu as the oral dose of BSE infective for humans to project between 36,000 and 34 million possible cases of nvCJD in the UK (38,39). Mathematical prediction from current incidence gives rise to smaller figures but a similarly wide range, because the estimates are highly dependent on the average incubation time of nvCJD and this is currently unknown. Calculations based on a range of mean incubation periods (10 to 25 years) and a 90-100% effective ban on bovine offal re-entering the food chain points



to an epidemic of between 75 cases and 80,000 cases (40). This projection should be treated extremely cautiously at this stage because, as the authors themselves say, it is based on very little data and a number of unverifiable assumptions. Even if no further people are being infected at present we could still see new cases of nvCJD presenting up into the 2020s. Of course, it is possible that people will continue to be infected and present beyond this, either because they continue to eat bovine offal, or because the strategy to eliminate BSE from cattle may not be fully effective, or because BSE may have spread to cattle or other commercial animals in other countries, or because secondary human to human transmission may occur via blood transfusion or organ transplantation.

## **MOLECULAR AND CELLULAR BIOLOGY OF PRION PROTEIN.**

### Normal prion protein.

TSEs are associated with a conformational change in the secondary structure of a normal cell surface sialoglycoprotein termed prion protein (PrP). By convention the normal form of the protein is termed PrP<sup>C</sup> and the abnormal form PrP<sup>Sc</sup>. In the "sporadic" and "infectious" forms of TSE abnormalities in the genetic structure and primary amino acid sequence do not occur. PrP is encoded by a gene on chromosome 20 termed *PRNP*. *PRNP* has a single uninterrupted open reading frame with two additional exons (41,42). The gene shows extensive homology across vertebrate species. It encodes for a 30-35kDa, 250 amino acid glycoprotein with two N-linked glycosylation sites. The carboxy-terminal half of the molecule consists of three  $\alpha$  chains and a single  $\beta$  pleated sheet which form a stable globular structure, whilst the amino-

terminal half is a random coil (43). PrP<sup>C</sup> is synthesised through the endoplasmic reticulum and Golgi in the normal fashion and is expressed on the surface membrane. It is internalised via clathrin-coated pits with a 1/2 life of 3-6 hours and cycles through the endosomal compartment from where 95% is returned to the surface with a transit time of 60 minutes (44). Sulphated glycosaminoglycans have been reported to increase the rate of turnover (45). The predominant form of the protein is glycosyl-phosphatidylinositol (GPI) anchored to the cell membrane and when cells are stained for PrP<sup>C</sup> without prior fixing, capping can be seen. However, transmembrane anchorage of the protein has also been described (46). Small amounts of the protein are shed from the surface of the cell: soluble PrP<sup>C</sup> has been identified in cell culture supernatants and human CSF (47,48). The function of PrP remains unknown although different groups have identified binding to a 37kDa laminin receptor precursor protein (49) and to a 66kDa membrane protein (50) which may be a homodimer of the former (51). PrP can also act as a copper metalloprotein (52).

#### The abnormal prion conformer.

The infectious agent in TSE is either identical to or closely associated with PrP<sup>Sc</sup> and the abnormal protein is regarded as a sensitive and specific marker in both animals and man. PrP<sup>C</sup> is completely digested by Proteinase K whereas the 33-35kDa PrP<sup>Sc</sup> is shortened to 27-30kDa, probably as a result of degradation of the amino-terminal segment. There is a characteristic conformational change in the secondary structure of PrP<sup>Sc</sup> with an increase in the proportion of  $\beta$ -pleated sheets from 3% to 43% of the molecule and a

slight decrease in the proportion of  $\alpha$ -helices (43% to 34%). The increased  $\beta$  structure arises mainly, therefore, at the expense of the unstructured amino-terminal region (53). The mechanism by which this conformational change occurs is unclear but the most popular theory suggests that this is propagated by homodimerisation between PrP<sup>Sc</sup> and PrP<sup>C</sup> (*vide infra*). The conformational alteration is not thought to occur in the synthetic pathway but at the surface membrane or in the endocytic pathway (54). Unlike the normal protein, PrP<sup>Sc</sup> is not cleaved from the plasma membrane by proteases or phosphatidylinositol-specific phospholipase C (55-57), has a much longer 1/2 life and is predominantly internalised in calveolar zones where it forms amyloid plaques in association with sulphated glycosaminoglycans. In neurons, more surface membrane and extracellular matrix deposition appears to occur - so it is possible that the structure or cellular handling of PrP<sup>Sc</sup> differs, depending on the cell type. Highly sulphated glycosaminoglycans such as Congo red and pentosan sulphate have been shown to inhibit the conversion of PrP<sup>C</sup> to PrP<sup>Sc</sup> in *in vitro* cell culture systems (54,58) and to prolong incubation periods and increase infective dose required in rodents following inoculation of infected material (59). The mechanism of action is unclear but may reflect competitive inhibition of binding to endogenous glycoaminoglycans required for the conformational change.

The genetic structure of *PRNP* can contribute to PrP<sup>Sc</sup> formation. To date some 19 different point mutations or insertions in the open reading frame have been associated with familial TSE. For example mutations at codons 102, 117, 198 and 217 are associated with GSS, whilst those at 178, 200 and 210 are associated with familial CJD and 178 is also associated with FFI.

These are autosomal dominant conditions with almost complete penetrance, albeit that the incubation periods may be prolonged and the clinical phenotype highly variable (60). Mutational experiments in Syrian hamster cells have demonstrated that aberrant glycosylation of PrP leads to intracellular accumulation (61). It appears that both primary amino acid sequence and glycosylation may contribute to formation of abnormal secondary structure in genetically determined disease. In comparison sporadic and iatrogenic CJD are not associated with allelic point mutations. Both genetic and epigenetic TSEs can, however, be modified by a common polymorphism at codon 129 in *PRNP*. 37% of the general population are homozygous for the allele encoding methionine at this site, 11% are homozygous for the allele encoding valine and 52% are heterozygous. Patients with a codon 178 point mutation present either as FFI or as familial CJD depending on their codon 129 genotype (met/met or met/val phenotype respectively). Patients with sporadic and iatrogenic CJD are more frequently homozygous at this locus (over 90% in most studies) (22,23, 62-64). Deslys *et al* calculated the number of cases in heterozygotes to be 7.5 times lower than that in homozygotes, with a 5 year delay in clinical presentation of the first cases (23). All cases of nvCJD have so far occurred in met/met homozygotes (65), if cases do occur in heterozygotes we should probably not expect them to present before the year 2000. Why the codon 129 polymorphism should have such a marked effect on disease phenotype is unclear. In acquired disease, conformational similarity between incoming PrP<sup>Sc</sup> and endogenous PrP<sup>C</sup> may influence the rate of propagation of the abnormal isoform and thereby the overall propensity to become infected, the

ease of lymphoreticular - neural transmission, the incubation period or the neurological distribution of lesions. Bovine PrP is methionine homozygous at codon 129, as are the prion proteins of most animal species.

#### The nature of the infectious agent.

Two hypotheses have been proposed to explain the hybrid genetic / infectious features of this agent.

The protein-only hypothesis was advanced by Stanley Prusiner (66,67) who received a Nobel prize for his work on prion diseases in 1997. It maintains that prions are devoid of nucleic acid and that the essential pathogenic mechanism is alteration in the secondary conformational structure of PrP<sup>C</sup> by direct homodimerisation with abnormal PrP<sup>Sc</sup>. It is thought that molecular chaperones (protein X or heat shock proteins) (68,69) may contribute to the change of isoform. Some groups have managed to partially replicate this process in cell-free experimental systems (70). In support of this thesis, infectivity is closely associated with the PrP<sup>Sc</sup> glycoprotein, no nucleic acid has been demonstrated and infectivity survives most procedures known to inactivate nucleic acids but is reduced by processes which denature protein (71).

The virus / virino hypothesis maintains that the large number of strain phenotypes identified (particularly in scrapie) cannot be explained simply on the basis of protein conformation and holds that there must be a small amount of nucleic acid associated with PrP<sup>Sc</sup>, albeit that this may be very difficult to detect (72,73). In support of this proposition, solubilisation of PrP<sup>Sc</sup> is accompanied by denaturation of the protein and loss of infectivity. PrP<sup>Sc</sup>

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can be recovered on renaturation, but infectivity has not been regained. One series of experiments has demonstrated apparent transmission of disease in the absence of detectable PrP<sup>Sc</sup> (74).

This debate is effectively unresolved at present (75). Infectivity partitions with, and is inseparable from, PrP<sup>Sc</sup> and it seems appropriate to assume, for practical purposes, that cells or tissues expressing PrP may be involved in the pathogenesis or transmission of nvCJD.

## **DISTRIBUTION OF PrP EXPRESSION AND THE PATTERN OF DISEASE.**

### Neurological disease.

The major pathological manifestations of TSEs are restricted to the central nervous system and consist of neuronal cell death with vacuolation (spongiform change), reactive astrocytosis and deposition of amyloid plaques.

The pathophysiology has not been fully resolved. PrP<sup>C</sup> is expressed by neurons, astrocytes and microglia. A synthetic peptide corresponding to residues 106-126 of the human PrP is toxic to neurons and trophic to astrocytes *in vitro* (76). Transgenic PrP-null mice survive normally, though some develop subtle neurological abnormalities such as defective GABA<sub>A</sub> receptor-mediated fast inhibition and impaired long-term potentiation, altered circadian rhythms and loss of cerebellar Purkinje cells. They are resistant to infection with scrapie (77,78). Studies in PrP-deficient mice subject to grafting with PrP<sup>C</sup>-overexpressing neural tissue and secondarily infected with scrapie, have demonstrated that all PrP-positive neurons eventually die with a reactive

gliosis, whilst the surrounding PrP-negative neural tissue survives despite dissemination of PrP<sup>Sc</sup> and formation of amyloid plaques (79). This suggests that it is the intracellular accumulation of a PrP<sup>Sc</sup> which kills the cell rather than absence of PrP<sup>C</sup> or deposition of exogenous PrP<sup>Sc</sup>. Alterations in neuronal cell membrane function have been described and cells probably die through apoptosis. Microglial generation of free radicals may be essential to this process (80). No systemic or local immunological response to PrP<sup>Sc</sup> is demonstrable, though the lymphoid system may itself play an important role in the pathogenesis of the disease (*vide infra*).

#### Presence of PrP in extra-neural tissues.

Until recently, very little work has been done on PrP<sup>C</sup> expression outwith the CNS and in particular in the peripheral blood in man. One group have reported PrP<sup>C</sup> expression by normal human lymphocytes and lymphoid cell lines which was upregulated by cell activation, using flow cytometry and Northern blot (81). Similar observations have been made in mice (82). A second group have reported PrP expression by monocytes and fibroblasts using Western blot and immunocytochemistry (83). Recent studies of human bone marrow have demonstrated expression of PrP by CD34+ haemopoietic progenitors, which is maintained during lymphocytic and monocytic differentiation, but lost during granulocytic differentiation (84). Similarly, *in vitro* retinoic acid-induced differentiation of the PrP+ haemopoietic cell line HL-60 into granulocytic cells led to loss of PrP expression, whilst expression was maintained during phorbol ester-induced differentiation into monocytic cells (84). PrP fragment 106-126 has been shown to activate human leucocytes (76) and subtle abnormalities of T cell subsets have been

described in 3 patients with CJD (85). Finally, Pernini *et al* have demonstrated PrP<sup>C</sup> in supernatants following platelet activation and treatment with PI-PLC (86,87).

The involvement of the immune system in the pathogenesis of the disease is supported by studies on the infection of mice with scrapie or BSE.

Transmission studies in rodents show that following peripheral inoculation a phase of peripheral lymphoreticular replication occurs prior to neurological replication and that first-passage intracerebral inoculation is also frequently preceded by peripheral replication (88). PrP<sup>Sc</sup> replication persists in peripheral lymphocytes and dendritic cells throughout the course of the disease (89,90).

There are differences between TSEs in this regard, which may reflect strain-dependent characteristics. It has been known for some time that mice with severe combined immune deficiency (SCID), which lack B, T and possibly dendritic cell function, do not develop scrapie following peripheral inoculation (91) although they do so after central inoculation and that this situation is normalised if an allogeneic bone marrow transplant is carried out (92). The work of Aguzzi *et al* points to B lymphocytes being the critical lymphoid sub-population for onward transmission from the periphery to the nervous system (93-95). In sheep with scrapie, fairly widespread infectivity has been demonstrated in the central nervous system, placenta, spleen and other lymphoreticular tissues (96). In cattle with BSE, on the other hand, infectivity has been demonstrated in brain, spinal cord, cervical and thoracic dorsal root ganglia, distal ileum and bone marrow (97,98).



In man, PrP<sup>Sc</sup> has been demonstrated in human tonsillar tissue (probably follicular dendritic cells) in patients suffering from nvCJD, but not in those suffering from sporadic CJD (99,100) (Figure 2).

## **TSE TRANSMISSION STUDIES.**

### **Scrapie / BSE transmission to experimental animals.**

There is a complex 30 year literature on the transmission of animal TSEs (particularly scrapie) to rodents, mainly by intracerebral inoculation. The most germane points relate to the species barrier and strain characteristics.

Primary scrapie intracerebral inoculation leads to clinical disease in only a minority of recipients. With sequential intracerebral transmission the incidence of successful transmission increases to 100% and the incubation period falls, sometimes quite dramatically e.g. from >700 days to @120 days in some models. BSE leads to clinical disease in all animals on primary intracerebral transmission, but the same effect on incubation periods is seen. It might be conjectured that the species barrier reflects the extent of homology between incoming foreign PrP<sup>Sc</sup> and host PrP<sup>C</sup>, though how evolution of the disease may occur is not clear. In man, one might expect that secondary transmission of nvCJD by blood will prove more efficient than primary transmission from infected beef products - allowing for differences in infectious dose and route of transmission. Different inbred strains of mouse (with the same *Prnp* structure) differ in their susceptibility and incubation periods for TSEs, suggesting that other genetic factors modify the pathophysiology of the disease. In addition, multiple strains of TSEs have been identified which differ in their clinical phenotype in the same strain of mouse (101). In some

conditions - such as scrapie, up to 20 different strains have been identified, whereas in BSE only a single strain has been demonstrated. Again, the explanation is not clear, with some authorities seeing this as evidence for multiple conformational PrP isoforms and others as demonstrating the presence of nucleic acid.

CJD transmission to experimental animals.

CJD has been transmitted to a large number of experimental rodents by intracerebral inoculation of infected brain. Intracerebral inoculation of mice and hamsters with whole blood and buffy coat from CJD infected patients has been demonstrated, but infectivity appears to be lower and more patchy (102,103). The majority of studies have not demonstrated transmission by intra-cerebral inoculation of plasma. The recent study discussed by Paul Brown at the WHO meeting in March 1997 which has suggested the presence of infectivity in plasma is currently awaiting publication and therefore cannot be fairly evaluated at this stage (104). The species barrier clearly provides an important limiting factor to the sensitivity of these assays.

Of 440 experimental attempts to transmit human TSEs to primates by intracerebral inoculation reviewed by Brown (17), 300 transmitted infection with highest rates for iatrogenic CJD (100%), kuru (95%) and sporadic CJD (90%). Incubation periods, duration and character of clinical illness showed great variability even in animals receiving the same inoculum, but age at infection did not seem to be a determining factor.

No primate transmission studies have been published on nvCJD, though the disease is transmissible to both routine laboratory and transgenic mice (35,36).

It should be noted that all but one of the transmissions from animal blood and all transmissions from human blood have resulted from the highly efficient but artificial method of intracerebral inoculation. The only three attempts to transmit disease by intravenous infusion have failed.

The infectious dose of TSE is calculated to be  $1 \text{iu} / 10^6 \text{PrP}^{\text{Sc}}$  molecules. However, this relates to intracerebral inoculation of rodents with infected brain, so that both the species barrier and the abnormal route of inoculation need to be considered. How this infectious dose relates to the intravenous inoculation of peripheral blood components derived from human CJD or nvCJD is unclear.

#### Evidence for the transmission of CJD in man.

There is little evidence for transmission of sporadic CJD from blood or blood products in man.

Two epidemiological case control studies carried out in Japan (105) and the US (106) in the 1980s found that the percentage of patients with CJD who had received blood transfusions was no greater than in healthy controls.

A report from Australia describing 4 patients who received transfusions 5 years before the onset of peripheral iatrogenic-type CJD is regarded as anecdotal because there was no information about the existence of CJD patients amongst the blood donors or about the frequency of blood transfusion in a non-CJD control population (107).

An epidemiological surveillance study of CJD in the UK identified 21 patients who had received a blood transfusion and 29 who had donated blood out of a total of 202 cases. Frequency of blood donation or transfusion was not significantly different from that in age and sex matched controls and the clinical features in those with a history of blood transfusion were similar to those of sporadic CJD and dissimilar to those of peripheral iatrogenic CJD (108).

A lookback of a regular blood donor in Germany who developed CJD identified 55 recipients, 35 of which were traceable, 21 of whom had died from non-CJD illness up to 22 years after transfusion and 14 of whom were still alive with no evidence of neurological disease (109).

A retrospective study in the US following identification of a long-term plasma donor with CJD in 1994 showed no excess of neurological manifestations amongst HIV infected haemophiliacs compared to HIV infected homosexual men and blood transfusion recipients (110).

A single individual has been reported in France who died from CJD two years after undergoing a liver transplant. The clinical pattern was consistent with peripheral iatrogenic transmission. The liver donor had no history of neurological disease, but one albumin donor developed CJD 3 years later. The incubation period is extremely short and the authors concluded that a random association of two very rare conditions was the most likely possibility (111-113).

A second large epidemiological study in Europe from 1993-1995 also failed to demonstrate any difference in exposure to surgery or blood transfusion in 405 patients with CJD compared to case-controls (114).

A further case report of CJD in a donor and recipient similarly concluded that the association was circumstantial (115).

Finally, no excess of case of CJD has been identified amongst patients who have received multiple exposures to blood or blood components (116).

#### **EVALUATING THE RISK OF TRANSMISSION BY BLOOD TRANSFUSION.**

There are several key issues which need to be addressed in assessing the likely risk of nvCJD transmission by blood transfusion namely: the possible number of infected donors, the level of infectivity in peripheral blood during the preclinical phase of the disease, the efficiency of transmission by the intravenous route, the potential numbers, age and general health of infected recipients and the probable clinical manifestations of disease, particularly the effect of passage in man on efficiency of transmission and incubation periods.

##### Possible numbers of infected donors.

Four (15%) of the current 26 patients in the UK with nvCJD have donated blood, a figure which is similar to the proportion of the general population who have been blood donors at some stage in their lives. However, it may be more relevant to estimate the proportion of current UK blood donors who may be incubating the disease. If one takes the best estimate of the number of people in the country who may have been infected with nvCJD with its attendant caveats (40), and assume that 5% of these are current blood donors, then somewhere between 4 and 4,000 out of @ 4 million UK donors (*i.e.* between  $1/10^6$  and  $1/1,000$  donors) may be incubating the disease. Matching of the age groups of blood donors and patients with nvCJD gives rise to a higher worst-case figure of  $1/250$  donors (S Dealler, personal

communication). The best estimate for the number of donors incubating sporadic CJD is  $1/10^6$ .

#### Levels of infectivity in peripheral blood.

In principle, it is clear that nvCJD could be transmitted by blood components. PrP is expressed by human lymphocytes and monocytes (81-85) and by platelets (86,87). Lymphoreticular replication occurs at an early stage in the transmission of TSEs to experimental animals (88-90) and appears to be essential for peripheral transmission of disease (91-96). Scrapie-infected sheep, but not BSE-infected cattle, demonstrate infectivity in peripheral lymphoid tissues (97,98). CJD can be transmitted to experimental animals by intracerebral inoculation of whole blood and buffy coat from patients with clinical disease (17,102,103). Where infectivity is present in peripheral blood, it appears to be several orders of magnitude less than that of neural tissue and is probably species and strain-dependent.

#### The efficiency of the intravenous route of infection.

In general, peripheral routes appear to be less efficient at transmitting infection than central inoculation, resulting in fewer infected individuals, longer incubation periods and a different pattern of clinical disease (19-21). Disease can be transmitted by oral consumption of CJD-infected human brain (18), oral consumption of BSE-infected bovine brain (28-31) and parenteral administration of infected human cadaveric pituitary hormone preparations (19-21). Intravenous administration of infected neural tissue to experimental animals has, however, failed to transmit infection. It is clear that peripheral

transmission of CJD can occur when the infectivity of the source material is sufficient to overcome the relative inefficiency of the route of inoculation.

Potential for nvCJD transmission by blood transfusion.

No clinical cases of CJD have so far been definitively ascribed to transmission from blood components or products. The case reports (107, 111,112,115) are suggestive because of the cerebellar phenotype of the clinical disease, but there are important caveats (113). The epidemiological case control (105,106,108,114) and lookback (109,110,116) studies have proved negative, though sporadic CJD is a rare condition and occasional cases of transmission arising through blood transfusion may have been numerically insufficient to achieve statistical significance. One can, therefore, conclude that sporadic CJD is rarely, if ever, transmitted by blood transfusion (117). This may not necessarily hold true for nvCJD - the strain of agent is different and the prevalence in the donor population may be significantly higher. Furthermore, there has probably been insufficient time since the appearance of the disease for clinical presentation of cases of secondary transmission to occur. In conclusion, it remains unclear whether the level of infectivity in the peripheral blood of people in the preincubation phase of nvCJD is sufficient to permit transmission by intravenous infusion.

The potential number of infected recipients.

It is very difficult to project the number of patients at risk of becoming infected and of developing clinical disease. If one accepts the previous estimates of the number of potential infected donors and further assume that the mean number of blood donations / donor is in the order of 1.5 / annum with an

average of 2 recipients per donation, somewhere between 12 and 48,000 patients might be exposed to infected blood components per annum. However, not all of these are likely to become infected or develop clinical disease. Most UK adults have consumed infected beef products, but Cousens' estimates do not project the incidence of nvCJD to be much above 0.2% of the population (40). One might expect secondary transmission via blood transfusion to prove more efficient than primary transmission from infected beef. However, even with parenteral administration of infected cadaveric pituitary hormones only 1-5% of the exposed population developed iatrogenic CJD (22,23). Only 1% of the Fore people developed Kuru even though apparently most women and children participated in this ritual. All cases of nvCJD have thus far occurred in patients below the age of 55, though whether this represents differences in exposure to infected beef products or an inherent age-related difference in the vulnerability to develop disease is unclear. In addition, it should be recalled that the majority of blood recipients are over 60 years old and that 50% of blood components are administered to patients who do not survive more than 12 months. It is probable, therefore, that if nvCJD is transmissible by blood, only a proportion of the recipients will become infected and only a further proportion of these will survive sufficiently long to develop clinical disease. Younger recipients are likely to be at more risk than the elderly.

The population at risk from plasma products is potentially more worrisome because pooling of up to 20,000 donations occurs and large numbers of recipients are exposed to each batch. The impact of a single infected



donation is therefore potentially magnified. In addition, a greater proportion of recipients are young healthy individuals.

In summary, although the number of recipients potentially exposed to nvCJD-infected blood can be calculated in principle, it is virtually impossible to estimate with any degree of confidence how many of these would go on to develop clinical disease because of uncertainty over the level of infectivity in different blood components and products.

#### Clinical manifestations of disease.

If nvCJD is transmissible by blood transfusion we should expect patients to present with typical features of peripheral iatrogenic disease. The efficiency of transmission is likely to be higher, and the incubation period shorter, than that seen in primary oral transmission of BSE due to the passage effect. It may not be possible to determine definitively the source of infection in patients alleged to have contracted nvCJD by blood transfusion. Lookback studies may give an indicative answer, but false negatives may occur if the original donor has died or is untraceable and false positives if there is a high background prevalence of dietary-acquired nvCJD in the general population.

#### **STRATEGIES WHICH MAY REDUCE THE RISK OF TRANSMISSION BY BLOOD TRANSFUSION.**

Strategies which may reduce the risk of transmission of nvCJD include: development of donor selection or screening procedures, removal or inactivation of the infectious agent from blood, reduced clinical use of blood components and products with more widespread use of alternative

therapeutic measures and the possible future development of therapeutic agents which could prevent transmission.

#### Donor selection.

UK donor selection procedures currently screen out individuals who have a first degree relative with CJD or a history of corneal grafting, human pituitary-derived hormone exposure prior to 1986 or neurosurgical intervention prior to 1993. However, none of these steps is likely to exclude donors in the incubation phase of nvCJD. There is no evidence to support excluding those with occupational exposure to cattle.

Consideration has been given to excluding those donors who themselves have received blood components or products on the grounds that this is the only likely route for continued passage in man (*i.e.* third generation transmissions and greater) and continued strain evolution (organ and tissue transplant recipients are permanently deferred from blood donation). However, it is estimated that 8% of the donor population have themselves received blood components and 20-25% have received plasma products. Implementation of such an exclusion policy would therefore seriously undermine the UK blood supply.

#### Donor screening assays.

Since CJD does not elicit a systemic immunological response and no nucleic acid has so far been identified in association with PrP<sup>Sc</sup>, standard serological or molecular biological screening assays are not applicable. Surrogate markers of neurological disease such as S100 are unlikely to prove

sufficiently sensitive to act as screening assays for preclinical disease (118). Infectivity bioassays are too prolonged to be of practical value (17,101-104). Immunoblot detection of protease-resistant PrP<sup>Sc</sup> from peripheral blood cell lysates is a possible approach, but likely to be difficult to control and very labour intensive (35,36,83). Most monoclonal antibodies do not differentiate between the normal and abnormal conformations of PrP, but the 15B3 antibody recently described by Korth et al does appear to do so (119). An important reservation is that peripheral blood PrP<sup>Sc</sup> may be biochemically different (e.g. in terms of glycosylation or conformational structure) (84) or may remain bound to the cell membrane or internalised and may not be accessible to plasma based assays. In addition, there are substantial difficulties to be overcome in validating any novel screening assay - in particular establishing the significance of a positive result in terms of infectivity and development of clinical disease (120).

#### Leucocyte depletion of blood components.

The studies of PrP expression by peripheral blood leucocytes and those demonstrating CJD infectivity on intracerebral inoculation of whole blood and buffy coat from infected individuals support the contention that leucocytes represent the main source of infectivity in the peripheral blood of CJD-infected individuals.

Modern leucocyte depletion filters reduce the leucocyte concentration of cellular components by about 3-4 log ( $1 \times 10^9$  to  $<1 \times 10^6$ ) and in principle may reduce PrP load and / or infectivity by a similar amount. However, issues requiring urgent resolution include the nature of the leucocyte populations

passing through the filter (*i.e.* selective subsets) and the degree of cell fragmentation. Platelets also express large amounts of PrP<sup>C</sup> and could be a source of infectivity (86,87). PrP<sup>C</sup> is shed from the surface of cells in culture and *in vivo* (47,48), is secreted following platelet activation (86,87) and is present in plasma (unpublished observations) and could also potentially prove to be a source of infectivity (104). However, PrP<sup>Sc</sup> may behave differently to PrP<sup>C</sup> in that it is more tightly adherent to the cell membrane and in cell culture the main accumulations are intracellular. It is therefore feasible, but not certain at this stage that leucocyte depletion will reduce the infectivity of peripheral blood. The issue may take some time to resolve because of the difficulty in designing representative transmission studies and the duration of the read-outs.

A secondary argument in favour of leucocyte depletion is that non-leucocyte depleted red cell concentrates are known to induce immune activation (121). Given that lymphocyte activation has been shown to correlate with increased PrP<sup>C</sup> expression (81,82), it is possible that allogeneic leucocytes may increase the vulnerability of the recipient to transmission of PrP<sup>Sc</sup> or increase its rate of propagation (*i.e.* reduce the incubation period) independent of the infectious dose.

However, a potential deleterious effect of early leucodepletion on bacterial growth in blood components requires further investigation.

Universal leucocyte depletion is not currently standard practice in the UK (122), though several European countries including Austria, Norway, Spain, France, Ireland and Portugal have elected to implement this policy (123). In November 1997 the UK Spongiform Encephalopathy Advisory Committee

(SEAC) expressed the view that it was logical to seek to reduce the risk of transmission of nvCJD by minimising the number of leucocytes in blood components and recommended that consideration be given to introducing universal leucocyte depletion as a precautionary policy. In February 1998 the UK Department of Health commissioned an independent risk assessment and asked the Blood Transfusion Services to develop a plan to support the introduction of universal leucodepletion. The decision to proceed was announced in July 1998 though it is likely to take up to 12 months to implement and cost between £70-80 million per annum. There are likely to be collateral benefits which may offset some of the cost of leucodepletion, including reduction in the transmission of cell-associated viruses, in rates of alloimmunisation and in immunomodulatory effects.

#### Fractionated plasma products.

Fractionated plasma products are a particular concern because up to 20,000 donations are pooled per batch and because of the widespread use of human plasma products in healthy individuals both as prophylactic and therapeutic agents in their own right and as excipients in a variety of other medicinal products.

It would appear from animal experiments that plasma is unlikely to be a source of high levels of infectivity (103,104). In addition, evaluation of the physicochemical characteristics of PrP<sup>Sc</sup> suggests that most plasma products are likely to undergo very substantial reductions in infectivity compared to feedstock during the fractionation process due to partitioning (P Foster:

personal communication). It is unlikely that steps currently taken to inactivate viruses in plasma products will have an effect on prion infectivity. Irradiation has proved entirely ineffective, whilst the most active chemical agents are concentrated solutions of sodium hypochlorite or sodium hydroxide (71). Exposure to steam heat at 134°C for 1 hour is effective, but exposure to dry heat at temperatures up to 360°C for 1 hour may leave residual infectivity. Thus, measures likely to be effective in reducing nvCJD infectivity, are also likely to destroy the plasma product itself.

In 1997, the UK Haemophilia Directors recommended that recombinant Factor VIII was the therapeutic product of choice for all those with haemophilia A and, where it was not possible to provide this, priority should be given to children, previously untreated and HCV negative patients (124). In December 1997, in response to concern over the possibility of nvCJD transmission, they reinforced their recommendation that all those with haemophilia A should be offered recombinant Factor VIII and that recombinant Factor IX should be the treatment of choice for those with haemophilia B when licensed. They further suggested that, for those patients for whom recombinant products are not available, consideration should be given to using products derived from plasma from countries where cases of nvCJD or BSE have not been identified (125,126). In the same month the UK authorities recommended that in view of the uncertainty as to whether nvCJD could be transmitted by plasma products, such products should be recalled where a donor confirmed as suffering from nvCJD is found to have contributed to the pool. This has led to 2 subsequent product recalls. In

February 1998 the European Committee for Proprietary Medicinal Products (CPMP) recommended recall of plasma products where a donor subsequently diagnosed as a probable or confirmed case of nvCJD is found to have contributed to the pool. In view of the widespread use of human albumin as an excipient in medicinal products, they further advised discontinuation of the use of human albumin from countries where clusters of nvCJD are known to have occurred (127). In the same month the UK Committee for the Safety of Medicines (CSM) initiated a review of licensed plasma products and in May 1998 advised that these should not be sourced from UK plasma for the present time as a precautionary measure (128). The UK Blood Transfusion Services will import plasma from outside the UK until such time as a test is developed to screen for the possibility of infection, or it is proven that nvCJD cannot be transmitted by blood products or it can be proven that the manufacturing process destroys any infective agent. There are concerns that pressure to reduce the potential risk of exposure to nvCJD could result in shortages of plasma products or engender an increased risk of exposure to other infectious agents (129).

#### Effective use of blood components and products.

There is clear evidence of widespread variation in the clinical use of blood components and products (130). Rationalisation of clinical practice in terms of the establishment of evidence-based clinical guidelines, education and clinical audit, more widespread use of autologous transfusion and the use of alternative therapeutic agents, are likely to be the most practical and cost effective way of reducing the risk of exposure to nvCJD in the short term and

may reduce demand at a time when maintaining the blood supply is likely to become increasingly problematic.

**Prevention of transmission.**

It might prove possible to reduce infectivity by spiking blood components or pretreating patients with agents which may interfere with replication of PrP<sup>Sc</sup>. Possibilities include sulphated glycosaminoglycans (58,59), phenothiazines (131) and amphotericin (132). These possibilities require further investigation.

**CONCLUDING REMARKS.**

nvCJD presents the most serious of challenges to the UK Blood Transfusion Services. Although the clinical manifestations and neuropathology of the disease are well described, it remains uncertain whether peripheral blood from individuals in the preclinical phase of disease may transmit infection following intravenous administration and if so what, if anything, will be effective in reducing that infectivity. Much fundamental work is required to understand the physiology of PrP<sup>C</sup> in haemopoietic cells, the pathophysiology of lymphoreticular replication and to delineate the level and distribution of infectivity in blood. In practical terms, there is a pressing need to develop screening assays and to evaluate the potential impact of proposed prophylactic intervention strategies. It is important that in attempting to reduce the potential risk of transmission of nvCJD the overall risk to patients is not inadvertently increased through shortages of essential blood products or through an increased risk of transmission of other infections. Unfortunately,



many policy decisions will continue to have to be reached in the face of profound uncertainty.

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**Table I: Spectrum of Transmissible Spongiform Encephalopathies.**

<i>Disease</i>	<i>Species</i>	<i>Distribution</i>
Scrapie	Sheep, Goats	Widespread

Chronic Wasting Disease	Rocky Mountain Elk Mule Deer	Wisconsin and Colorado, USA.
Transmissible Mink Encephalopathy	Mink	Wisconsin, USA.
Bovine Spongiform Encephalopathy (BSE)	Cattle	UK, Europe.
Exotic Ungulate Encephalopathy	Antelope, Greater Kudu Nyala, Gamsbock	UK Zoos.
Feline Transmissible Encephalopathy	Domestic and exotic cats.	UK Domestic and Zoos
Creutzfeldt-Jakob Disease (CJD)	Man	Ubiquitous
Kuru	Man	New Guinea Highlands
Iatrogenic Creutzfeldt-Jakob Disease	Man	Europe, USA, Australia, Japan.
new-variant Creutzfeldt-Jakob Disease (nvCJD)	Man	UK, France.
familial Creutzfeldt-Jakob Disease	Man	Selected families
Gerstmann-Straussler-Scheinker Syndrome (GSS)	Man	Selected families
Fatal Familial Insomnia (FFI).	Man	Selected families
Atypical prion diseases	Man	Uncertain