

CJD and “Mad Cow Disease”: Risk for Blood Recipients?*

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Introduction

The human forms of transmissible spongiform encephalopathies (TSE), especially Creutzfeldt-Jakob disease (CJD) and a new variant CJD (nvCJD) thought to be the human form of bovine spongiform encephalitis (BSE), form a category of agents^[1-4] which affect both animals and humans. These diseases are rare forms of rapidly progressive neurodegenerative disorders that are distinguished by long incubation periods and neuronal loss within the central nervous system (CNS), are associated with characteristic spongiform changes and a failure to induce an inflammatory response, and end in death.

Extensive publicity on BSE and its possible relationship to nvCJD in the United Kingdom, and concerns that TSE's might be transmittable in blood or blood products have raised many questions about the transmissibility of these agents to man. For the hematologists and transfusion service personnel, these questions have focused on transmission by blood and blood products. However, it is not possible to adequately evaluate the risk of transmission by blood without discussion of some of the biological features of these unusual agents.

Biologic Determinants of Transmission Efficiency

The biologic features of the agent and the host, e.g., the route of infection, the titer of the infectious agent, the infective characteristics of the agents, and the susceptibility of the host, are important determinants in risk of transmission. In nature, the normal route of infection for the transmissible forms of TSE may be oral ingestion of infected tissue. For example, the cannibalistic ingestion of brains of dead relatives by the Fore tribe in New Guinea transmitted Kuru; among sheep, ingestion of infected afterbirth may transmit scrapie^[5-7]. BSE was transmitted to herds of cattle through ingestion of feed which had been supplemented with rendered infected sheep and cattle tissue; subsequently, nvCJD was most likely transmitted to humans through their eating products from BSE-infected cattle^[8-13]. The infective agent of the TSE diseases appears to be associated with (or to be) the prion protein which is found in many tissues, including CNS, corneal, pericardial, muscle, and placental tissue. It is found in highest concentration in the CNS. Other routes of infection appear to affect incubation

To give us some indication of this influence that the route of exposure has on incubation times and to examine the efficiency of other routes of infection, we must examine

iatrogenic transmissions to humans or studies in animals. Other than Kuru and nvCJD, virtually all other known TSE transmissions in humans have been iatrogenic and very infrequent, totaling approximately 100 cases worldwide^[14]. (**Table 1**) In humans, direct inoculation into the brain has produced the shortest incubation periods, ranging from 15 to 20 months, whereas peripheral injections of tissue extract, with subsequent hematogenous entry into the brain, has produced incubation times ranging from 5 to 30 years. Some of these latter patients are still followed, and it is possible that longer incubation times may be observed in the future.

The relative infectivity of various tissues, i.e., the level of inoculum required of various tissues to produce disease, has been difficult to assess from human data. Currently, experimental data on both infected human and animal tissues provide some information. The methods available to measure transmissibility are very tedious and time intensive. Most study methods rely on direct inoculation of suspected tissue into the brains of animals and then observing for the development of disease. Stain differences and species barriers have also made reproducibility and interpretation among laboratories very difficult. Introduction of susceptible strains of test animals and transgenic test animals have aided this process in more recent studies. **Table 2** shows some of the studies with positive results; however, it must be noted that an equal number or more studies were unable to demonstrate these effects^[15-21].

Several areas of conjecture have arisen from these animal studies. The first relates to level of infectious titer of CJD in various tissues. CJD is readily transmitted from infected CNS tissue and is thought to be present in high titer in these tissues. Blood and blood components are thought

Table 1. Influence of Exposure Route on CJD Incubation Time

Mode of Transmission	# Cases	Incubation Period (Range)
<i>Intracerebral Inoculation</i>		
1. Contaminated surgical instruments	4	20 months (15-28)
2. Contaminated EEG probes	2	18 months (16-20)
<i>Tissues Transplanted Adjacent to Brain</i>		
1. Corneal transplants	2	17 months (16-18)
2. Dura mater transplants	25	5.5 years (1.5-12)
<i>Peripheral Inoculation</i>		
1. Human Growth Hormone	76	12 years (5-30)
2. Gonadotropin	4	13 years (12-16)

* Adapted from Evatt BL, Prions and hemophilia: Assessment of risk. *Haemophilia* 1998;4:628-633.

to contain the infectious agent in much lower titer than tissue, since CJD is much less frequently transmitted to experimental animals when blood or components are injected directly into the brain.

At a recent TSE Advisory Committee meeting at the US Food and Drug Administration, Paul Brown, Robert Rohwer, and colleagues reported preliminary findings from animal experiments which examined the infectivity of various blood components (red cells, buffy coat, plasma) and Cohn plasma fractions (cryoprecipitate, fractions I+II+III, IV, and V)^[22]. These experiments examined both high-input infectivity in normal human blood which had been "spiked" with the scrapie agent and low-input infectivity in the blood of mice which had been inoculated with a mouse-adapted strain of human CJD, using, respectively, hamsters and mice as assay animals. These data suggested that CJD is present in low titer in the blood of infected animals and partitions into the various components, cryoprecipitate (starting material for FVIII concentrates) and Cohn fractions I, II (the starting fractions for immune globulins), and III, but has essentially no infectivity of fractions IV and V (the starting material for factor IX complex and albumin). Cryoprecipitate contained the highest titer of CJD of the plasma fractions. Red cells and white cells were reported to have 10 to 100 times higher infectivity titer than plasma or its fractions. While these data await further confirmation, they suggest that the starting material for the manufacture of factor VIII concentrates could have been contaminated with very low titer of the CJD agent. Each lot of concentrate contains material from approximately 20,000 to 60,000 donors. Although the disease is quite rare among the donor population, estimates have been made that the agent could be as frequent as 1 in 60,000 donors due to the long, asymptomatic incubation period during which an infected person could donate. These estimates, if accurate, would indicate that most individuals receiving long-term treatment with factor VIII concentrates have likely been exposed to the agent^[23] if further fractionation of cryoprecipitate does not eliminate the agent.

The second area of conjecture relates to the relative efficiency of the route of exposure. Animal experiments have indicated that direct brain inoculation is the most effective route, whereas inoculation into the blood stream is at least 10 to 100 times less efficient than brain inoculation^[22] in transmitting infection. Thus, whereas a high titer tissue ingested orally or introduced directly into the brain causes disease a substantial percentage of the time, low titer material, such as blood, introduced by a low efficiency route of transmission (e.g., intravenously) yields few or no infections in experimental animals. There is also probably a titer below which no infections occur. It is possible that further processing of cryoprecipitate into FVIII concentrate could result in further losses of any infectious titer, perhaps reducing the opportunity for transmission.

The third area of conjecture involves the nature of the infectious agent, its infectivity, and the species specificity as it relates to our ability to predict its effect in humans from its behavior in animals. Because the species barrier plays a significant role in assays, it is difficult to evaluate how animal experiments specifically apply to humans. In addition, agents such as BSE which readily cross species lines (sheep to cattle to humans) may represent a more aggressive agent. If true, this agent might be more infectious than what is thought about traditional CJD. Public health officials, in the United Kingdom are presently very concerned about the rise in the new epidemic of nvCJD and its influence on the blood supply^[24].

Not all humans are equally susceptible to the prion diseases because of genetic differences. For example, genetic studies on the phenotypes for codon 129 of the normal prion protein found in all brains show four primary phenotypes (**Table 3**)^[25-27]. Persons who are homozygous at this locus have a much higher incidence of sporadic CJD and iatrogenic CJD. Moreover, the recent outbreaks of BSE in the UK and France occurred at higher incidences among such persons. These genetic differences may ultimately be shown to be related to both susceptibility and/or length of incubation period.

Table 2. Infectivity of Human and Animal Blood Components.

Material	Source of Inoculum	Assay Animal	Inoculation	# Positive/ # Donors
Human Material				
Sporadic CJD	Buffy coat	Guinea pig/Hamster	ic	2/2
Sporadic CJD	Whole Blood	Mouse	ic	1/3
Sporadic CJD	Plasma (3x conc)	Mouse	ic	1/1
Animal Material				
Scrapie	Sheep serum	Rat	ic	1/1
Scrapie	Mouse	Rat serum	ic	1/1 (pool)
Scrapie	Mouse	Mouse serum	ic	1/1 (pool)
Scrapie	Mouse whole blood	Mouse	ic	3/13
CJD	Guinea pig buffy coat	Guinea pig	ic,sc, im, ip	10/28 (pairs)
CJD	Mouse buffy coat	Mouse	ip	4/7(pools)

sc=subcutaneous; im=intramuscular; ic= intracerebral; ip=intraperitoneal

Studies on Human Transmissions

The categories of exposure to CJD listed in Table I are presently accepted as probably routes of transmission for CJD. Still controversial is the risk of transmission by blood or blood products. The outcome of the debate about this issues is important for hematologists who use blood and blood products as primary therapeutic agents. Shortages of immunoglobulin and clotting factors have already resulted because of recalls of lots which contained plasma collected from donors at risk for CJD. Regulatory agencies are struggling to develop policies which will reduce the likelihood of product shortages and physicians are struggling with therapeutic decisions. Unfortunately existing data on transmission of CJD by blood or blood products is insufficient to allow definitive answers. Four types of data are available: data from case reports, from surveillance systems, from case control studies, and from cohort studies. To date, none have found transmission of CJD blood or blood products, but it is important to understand the strengths and weaknesses of each type of these data.

Both case reports and case series, have been useful in demonstrating the association of human growth hormone to CJD and of dura mater transplants to CJD (Table 1). The large number of cases and the uniqueness of the treatment contributed to these associations. Transfusion, on the other hand, is not a unique medical therapeutic procedure, and while CJD is very rare, the association between the two is much more difficult to establish. Only three reports of possible transmissions have appeared in the literature: one person developed CJD after a liver transplant; four persons in Australia developed CJD following transfusions; and one person from Canada who developed CJD 8 months (1/3 the incubation time of any known iatrogenic case) after receiving albumin from a plasma pool to which a CJD-infected donor had contributed^[28-31]. In none of these instances was there sufficient information to make a case association with the blood or blood product. Statistically, it is expected that a certain number of persons who receive transfusions during their lifetime will develop CJD. Without other collaborating evidence, however, it is impossible to establish a connection.

Surveillance reports have provided limited information concerning the probability of transmission of CJD by blood or blood products. During the past 40 years, the use of blood

and blood products has substantially increased. If blood transmission were a major cause of CJD, we would expect to see a rise in the number of CJD cases in countries with CJD surveillance. The incidence of CJD, however, has remained constant throughout the world. The surveillance systems, however, have had several weaknesses which affect interpretation of the data. Most countries with such surveillance instituted their systems only within the past 10 to 20 years and, thus, do not have the requisite background data for the past 40 years. In addition, surveillance systems that are designed to detect rare diseases have special requirements and are very expensive and labor intensive. The typical surveillance system uses death certificate data to find cases. However, such data is only about 80% to 85% effective in identifying CJD patients^[32]. Also, unless the patients have unusual clinical characteristics, e.g., onset at a young age or unusual neurological presentation, it may be very difficult to distinguish an association from background cases. Such unusual clinical features were very instrumental in drawing attention to the human growth hormone cases and the recent BSE cases in the United Kingdom. In the United States, the National Center for Health Statistics tracks mortality data from death certificates in the United States and has data, including demographic characteristics, on CJD and other diseases which might be associated with the use of blood and blood products such as hemophilia, sickle cell anemia, and thalassemia. From 1979 through 1994, there has been no increase in total CJD cases or cases occurring at younger ages nor has there been any cases reported in persons with hemophilia, sickle cell anemia, or thalassemia^[33].

Another category of surveillance is examination for signs of CJD of the autopsy tissues from persons who have used blood products over a number of years. Hemophilia patients who have had multiple exposures to whole blood, plasma, and cryoprecipitate and have received large quantities of factor concentrate would be expected to be a high risk population. Beginning in the mid-1980s, this population has had a excess number of deaths with CNS symptoms, thought to be secondary to HIV. If physicians were to confuse CJD illness for AIDS-related CNS problems, cases of CJD might be missed. The Centers for Disease Control has examined the CNS tissues from 30 patients who died with CNS symptoms since 1983; to date no cases of CJD have been found. Nearly all of these patients had severe hemophilia and had received factor concentrates for 15 to 23 years and other blood products for a much longer period^[34]. In addition, post mortem examination of the brains of 33 hemophilia patients in the United Kingdom who received products manufactured from UK-derived plasma between 1962 and 1995 were also negative for CJD^[35]. As yet, the numbers are too low to draw any conclusions; however, these data suggest that if CJD is transmitted through blood products, it is uncommon and/or has a extremely long incubation time.

Case control studies on CJD and blood transfusion provide very limited information, and only a few such studies

Table 3. Frequency of Phenotypes for Codon 129 in Patients with Iatrogenic CJD

Tested Phenotype	Met/Met(%)	Met/Val (%)	Val/Val(%)	(N)
All Iatrogenic	60	11	29	3
CNS route of inoculation	80	10	10	20
Peripheral route of inoculation	51	12	37	43
Healthy controls	48	42	10	1397

have been conducted: one in the United States, four in the United Kingdom, and one in Japan^[36-41]. In general, these studies examine CJD patients for a history of blood exposure as compared to matched controls who do not have CJD. None of these studies reported an association between receiving blood and CJD. Wientjens et al. used the pooled data from three of the studies (Japan, United States, and United Kingdom) consisting of 178 cases of CJD and 333 controls and did not find any association with blood transfusion^[42]. The weakness of this category of study design for CJD is due to the fact that CJD is a relatively rare occurrence in the donor population^[23]. Since only 0.0016% of the units transfused would carry a possible risk, more than 99.99% of transfused units have no risk. If only "history of transfusion" is used to represent exposure, the probability is quite high that actual exposure to the agent would be quite rare. A negative finding may simply represent a lack of exposure to the disease by transfusion. In spite of this major weakness, however, these studies do suggest that transmission by transfusion is not a common mode of CJD transmission.

Finally, cohort studies have been used to look for evidence that CJD develop in patients who have received blood from donors who subsequently develop CJD. Two studies are notable. One study from Germany examined 27 recipients of 35 units of blood donated over a period of 20 years. Eighteen recipients had died, and none of the recipients had exhibited neurologic symptoms. Only eight of the recipients had lived longer than 5 years^[43].

More recently, the Red Cross working in collaboration with CDC has conducted a look-back study which identified 178 recipients of blood collected from CJD donors. None of these recipients had developed CJD; 41 of the recipients lived more than 5 years and 9 as long as 13 to 24 years after the transfusion. These cohort studies support the theory that transmission of CJD by blood transfusion is uncommon; however, incubation periods longer than 30 years are still unobserved^[31,44].

Bovine Spongiform Encephalopathy and new-variant Creutzfeldt-Jakob Disease

In 1985, a number of dairy cows in the United Kingdom developed a fatal illness characterized by usual symptoms of abnormal and aggressive behavior and ataxia. Autopsies on these cows showed findings resembling scrapie in sheep, and the disease was named bovine spongiform encephalopathy^[8-9]. The epidemic that followed affected more than 170,000 cows in more than 34,000 herds (**Figure 1**). The epidemiologic evidence all pointed to a common source cause, most likely a food supplement made from meat and bone meal produced by commercial rendering plants. Dead cattle from the initial infections were used by the rendering plants in the food supplement and thus quickly spread the disease. The disease has been experimentally transmitted to a variety of different animals including nonhuman primates and laboratory rodents by various routes. The number of affected cattle began to decline after an imposed ban

on feeding food supplements derived from ruminants was imposed in 1988.

In the United Kingdom, a surveillance program for CJD was established in 1990, in part because of the concern of cross-species transmission of BSE. Because of this program, 38 cases of what is currently known as new-variant CJD (nvCJD) were detected between 1994 and the present. These patients are younger than those with classical CJD, develop early psychiatric and behavioral changes, and have persistent paresthesia and dysesthesia, followed by ataxia^[45]. All had eaten meat prior to 1991, and it was suggested that the disease was the result of cross-species transmission of BSE. Supporting this hypothesis were the observations that no cases had appeared before 1993; only one case had appeared outside the UK; the cases followed the BSE epidemic; the nvCJD prion resembles the BSE prion; and the pathologic patterns seen in nvCJD and mice infected with BSE are similar to each other but distinct from those of classical CJD^[10-13].

The suspected transmissions to humans raised the possibility that nvCJD may be transmitted more easily than classical CJD or may be in higher titer in tissues. This possibility has caused concern about the transmission of nvCJD by blood or blood products. Little data are available. To date a Transfusion Service Look back study has identified four nvCJD patients who were known blood donors in the United Kingdom. Of six known recipients of components from these donations, none have developed nvCJD. The United Kingdom, however, is following a precautionary principle and health authorities have implemented a ban on plasma from UK donors in fractionated products. Other countries are considering a precautionary ban on UK donors.

Discussion

Based on information accumulated to date, it is still difficult to judge absolutely the risk of CJD and blood transfusion. It is reasonable to conclude that CJD is produced by a

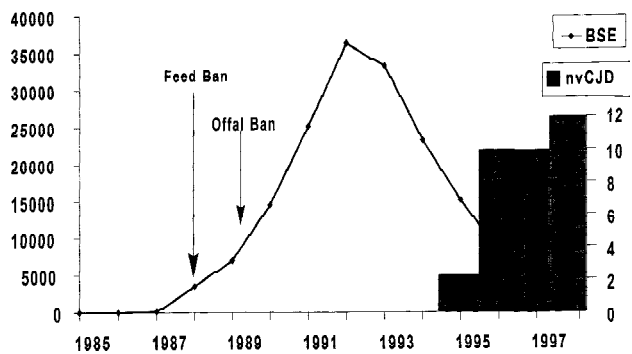


Figure 1. Cases of BSE (Linear Graph) and nvCJD (Bar Graph) in the United Kingdom.

The number of cases of BSE are shown on the left axis and the number of cases of nvCJD on the right axis. Dates represent years reported. (Adapted from Johnson RT, Gibbs CJ, NEJM 1998;339:1994-2004)

transmittable agent which is probably contained in low titer in the blood of infected persons and animals. The agent possibly partitions in cryoprecipitate and immune globulin fractions of plasma during Cohn fraction procedures but little or none can be found in fractions that eventually are used to manufacture albumin and Factor IX concentrates. As has been noted with other infections agents the losses of infectious titer found with initial fractionation procedures are possibly duplicated by further fractionation procedures used in the manufacture. Transfusion or intravenous injection may be such an inefficient route for CJD transmission that the low titer of any infectious material that may be in blood or blood products may be below the threshold for causing disease in humans. The inefficient routes of inoculation and the low titer of infectious material probably cause longer incubation times before clinical disease; thus, with any transmission by blood or blood products, incubation times longer than 30 years may be a possibility. From the present clinical and epidemiologic studies, transmission by blood or blood products appears to a rare or nonexistent cause of current and past cases of classical CJD in humans. To date, the occurrence of nvCJD remains localized to the UK, and no transfusion transmissions have been identified. Because so little data is available on this agent, it will continue to be a concern for public health policy makers. Since blood products are necessary to prevent the immediate risk of death or significant morbidity in many clinical conditions, therapeutic decisions should be made after consideration of the known risk in these clinical situations versus the theoretical long-term risk of the rare occurrence of CJD. In most cases, the answer is obvious. Because of the difficulty in studying this disease with the present level of scientific assay methods and the drawbacks of the present epidemiologic methods, the best defense against this disease and other TSE remains continued vigilance, involving surveillance, cohort studies, and case investigations. It is also important that new information concerning investigations into CJD be made available as rapidly as possible to both medical personnel and to patients receiving blood products.

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