

CREUTZFELDT-JAKOB DISEASE AND HEMOPHILIA: ASSESSMENT OF RISK

Revised Edition

Bruce Evatt

National Centers for Infectious Diseases
Centers for Disease Control and Prevention
Georgia, U.S.A.

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World Federation of Hemophilia
1425 René Lévesque Boulevard West, Suite 1010
Montréal, Québec H3G 1T7
CANADA
Tel. : (514) 875-7944
Fax : (514) 875-8916
E-mail: wfh@wfh.org
Internet: www.wfh.org

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Dr. Sam Schulman

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Creutzfeldt-Jakob Disease and Hemophilia: Assessment of Risk

Bruce Evatt

Introduction

For people with hemophilia, access to blood products is the key to a normal, active life. Without them they face a life of chronic pain and increasing disability. Unfortunately, although these products keep people alive, they are not without risk. This became clear during the 1980s when many people were infected with HIV from blood products. Because of the AIDS epidemic, governments around the world, companies that make blood products, and people with hemophilia have done much to improve the safety of blood products over the last 10 years. In many countries blood is now screened and treated to kill viruses.

Despite steps to keep the blood supply safe, there is still the remote possibility that blood and blood products contain infectious substances. This is an important concern for people with hemophilia who worry that they might get sick if they keep using blood products. The types of infections people worry about fall into three groups: infections caused by substances that can be screened for and inactivated, infections that can be screened for but not completely inactivated, and infections that cannot be tested for or inactivated. The first two groups do not cause much worry because infected blood can be thrown out. However, the third group makes people who receive blood products fearful because there is not enough information to know if the blood products can cause serious health problems.

Diseases such as Creutzfeldt-Jakob Disease (CJD) and variant CJD (vCJD) fall into the third group. CJD and vCJD are forms of transmissible spongiform encephalopathies (TSEs). They destroy the central nervous system and eventually result in death. TSEs have long incubation periods, which means that symptoms show up many years after being infected. However, once they appear, it is too late to treat the disease and deterioration is quick. TSEs are

thought to be caused by a substance known as a prion, which is a protein found in cells. It is believed that the infectious agent of TSEs is an abnormal variation of a normal prion. Normal prions are found in many parts of the body but are most often found in the brain and nervous system.

TSEs affect both animals and humans; vCJD is thought to be the human form of Bovine Spongiform Encephalopathy (BSE) or "mad-cow" disease [1-4]. Scientists believe that BSE is related to another TSE, known as scrapie, which is found in sheep. TSEs such as BSE, which infect other animal species and humans, may be more infectious than the TSE agent linked with traditional CJD. This possibility has led public health officials in the United Kingdom to be very concerned about vCJD and its influence on the blood supply [5].

People with hemophilia and physicians are faced daily with articles and press releases about CJD and vCJD that include phrases such as "unknown agent," "found in blood," "long incubation period," and "unknown risk to blood recipients." Because of the AIDS epidemic, these phrases create anxiety as well as demands that any danger be determined. For all groups concerned with hemophilia, the question to be answered is "Do human CJD and vCJD present a risk for people receiving blood or blood products and, if so, what is the level of risk?" This article looks at some of the major factors that determine the level of risk and tries to put them in perspective based on current knowledge.

Route of Infection

One factor in determining the level of risk is the route of infection, or how the infectious agent was transmitted. The most common type of transmission is by eating infected tissue. Kuru, a TSE affecting humans, was transmitted among a cannibalistic tribe in New Guinea who ate the brains of dead relatives, and scrapie may be

transmitted among sheep by eating infected afterbirth [6-8]. BSE was transmitted to herds of cattle through feed that contained meat or organs from sheep and cattle infected with TSE [9-10]. The cases of vCJD that were seen later in humans were most likely caused by people eating products from these BSE-infected cattle [11-14]. People can be exposed to TSE if infected tissue is introduced directly into the brain by organ or tissue transplants near the brain or by unclean surgical instruments. Infected material can also be injected directly into muscle. However, only about 100 cases of TSE in humans that have been transmitted in ways other than eating have been found anywhere in the world [15].

To determine which route of infection is most dangerous, we need to look at the effect that the route has on the disease's incubation time, that is the length of time between infection and visible signs of the disease. The introduction of infected tissue directly into the brain produces the shortest incubation time, ranging from 15 to 20 months. Injection of infected material into muscle produces incubation times ranging from 5 to 30 years, maybe even longer. Table 1 shows the different types of exposure and the length of time before disease can be seen.

Animal experiments support what has been discovered about the human disease. In animals, injections of infected material into the brain caused disease 10 to 100 times more often than injections of the same type of material into the bloodstream [16].

Concentration of Infectious Agent in Different Types of Tissue

Another factor to consider when determining risk is the type of infectious material a person is exposed to. Animal studies have shown that some types of tissue are more infectious than others. For example, they indicate that infected tissue from the nervous system is more infectious than infected blood or blood products. Table 2 shows the results of some of these studies [16-22]. (Please note that while results are positive, an equal number or more studies were unable to show these effects.)

In October 1997, scientists presented some important work at a meeting at the US Food and Drug Administration. In these studies, scientists looked at the ability of blood from mice infected with CJD to cause CJD in healthy mice. They also separated blood from infected mice into the products used to treat hemophilia and injected these into healthy mice to see if CJD would develop [23]. The scientists discovered that the

Table 1: Influence of Route of Infection on Incubation Time

Route of infection	Number of cases	Incubation time (range)
Direct introduction into the brain by		
1. contaminated surgical instruments	4	20 months (15 - 28 months)
2. contaminated EEG probes	2	18 months (16 - 20 months)
Tissues transplanted near the brain		
1. corneal transplants	2	17 months (16 - 18 months)
2. dura mater transplants	25	5.5 years (1.5 - 12 years)
Introduction into muscle		
1. human growth hormone	76	12 years (5 - 30 years)
2. gonadotropin	4	13 years (12 - 16 years)

Table 2: Infectiousness of Human and Animal Blood Components

Material	Source of Inoculum	Assay Animal	Route of Infection	# Positive/ # Donors
Human Material sporadic CJD	buffy coat	Guinea pig/ hamster	brain	2/2
sporadic CJD	whole blood	mouse	brain	1/3
sporadic CJD	plasma (3x conc.)	mouse	brain	1/1
Animal Material				
scrapie	sheep serum	rat	brain	1/1
scrapie	rat serum	mouse	brain	1/1 (pool)
scrapie	mouse serum	mouse	brain	1/1 (pool)
scrapie	mouse whole blood	mouse	brain	3/13
CJD	Guinea pig buffy coat	Guinea pig	brain, under the skin, muscle, peritoneum	10/28 (pairs)
CJD	mouse buffy coat	mouse	peritoneum	4/7 (pools)

CJD agent seemed to be present in low quantities in the blood and in some of the blood products. They found that cryoprecipitate (the starting material for producing factor VIII) and Cohn fractions I, II (the starting fractions for immune globulins), and III could transmit the infection to healthy mice. Fractions IV and V (the starting material for factor IX and albumin) did not transmit the infection in these experiments. Cryoprecipitate had the highest level of CJD agent of the plasma fractions. Red and white cells had the highest level of CJD agent among the different blood components, 10 to 100 times higher than the level in plasma or other blood fractions.

While the results from these studies need to be confirmed, they do suggest that the starting material for the manufacture of factor VIII concentrates may contain low quantities of the CJD agent. Each batch of concentrate is made from the blood of 20,000 to 60,000 donors. Although CJD is quite rare, scientists have estimated that as many as 1 donor in 60,000 may be infected and not know that they are infected because of the long incubation period. If this is true then most individuals receiving long-term treatment with factor VIII concentrates have probably been exposed to the disease [24].

This is a scary thought, but it is important to remember that animal experiments have shown

that blood is a substance with low-level infectiousness for CJD, and injection of infectious material into the bloodstream is a less efficient transmission route, making it unlikely that any infection would result. Also, there is probably a level of CJD agent below which no infections occur. It is possible that further processing of cryoprecipitate into factor VIII concentrate further reduces the level of infectiousness and the risk of transmission.

Genetic Factors

People's genes also affect how easily they can be infected with TSEs. Genes control how an animal or human develops. Genetic information is found in chromosomes, which exist in pairs. Studies show that people whose genetic information for normal prion protein is identical on both chromosomes are much more likely to develop CJD and vCJD [25-27]. Genetic differences may be related to susceptibility and the length of the disease's incubation.

Studies on CJD Transmission by Blood

Studies of CJD transmission in humans through blood transfusions or blood products have been limited because of the long incubation period of the disease. There are four types of data

available on transfusions and CJD donors: case reports, surveillance programs, case-control studies, and cohort studies. To date, none has uncovered any cases of CJD caused by blood transfusion. However, it is important to understand the strengths and weaknesses of each type of data.

Case reports, which are descriptions of a patient's medical case, have linked both growth hormone and transplants of dura mater, which covers the spinal cord and brain, with CJD. The large number of cases and the fact that this treatment is uncommon contributed to the discovery of the link. Blood transfusions, on the other hand, are very common, which makes it much more difficult to establish a link with CJD. Only three reports of possible cases of CJD following transfusion have been identified. One patient developed CJD after he had received a liver transplant; four people in Australia developed CJD after blood transfusions; and one person in Canada developed CJD eight months after receiving albumin that had been prepared from a plasma pool including a donor with CJD [28-31]. In none of these cases was there enough information to associate the disease with the blood or blood product. We can expect to see a certain number of cases of CJD in people who receive blood transfusions because of the very large number of transfusions that are given. Without more information, scientists cannot establish a link between the use of blood products and CJD.

Surveillance programs track cases of CJD among the population using information from death certificates and examining autopsy tissues from people who have used blood products for a number of years. Surveillance reports have provided some information on the transmission of CJD by blood or blood products. The use of blood and blood products has increased substantially in the last 40 years. 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. However, the number of cases has remained constant throughout the world. Most surveillance programs for CJD were started only 10 to 20 years ago. This means that a small change in the number of CJD cases from 40 years ago cannot be easily detected. Also death certificates are about 80% to 85% effective in

identifying CJD cases [32]. Unless patients have unusual clinical characteristics, such as onset at a young age or unusual neurological symptoms, it may be very difficult to find a link. Since the mid-1980s many people with hemophilia have died with central nervous system problems, which were thought to be secondary to HIV. If physicians confuse CJD illness for AIDS-related problems, cases of CJD might be missed.

One of the largest surveillance programs is in the United States. From 1979 to 1994 the total number of cases of CJD and the number occurring in young people have not increased and there have been no cases reported in people with hemophilia or other blood disorders [33]. Since 1983, the Centers for Disease Control (CDC) has examined the bodies of 30 people who died with symptoms of a nervous system disease. Most of them had had severe hemophilia and had received blood products for more than 15 years [34]. No cases of CJD have been uncovered. Similar findings were found by investigators in the UK [35]. The numbers are still too low to draw any final conclusions. However, the information suggests that if CJD is transmitted through blood products, it is uncommon and/or has an extremely long incubation time.

In case control studies, people who received blood products and who developed CJD are compared with people who received blood products but who did not develop CJD. Six studies of this sort have been carried out and none has detected a link between blood transfusions or blood products and CJD [36-41]. These studies suggest that transmission by transfusion, if it occurs, is not common. One group of researchers combined data from three of the studies, consisting of 178 cases of CJD and 333 cases in which CJD did not develop, and did not find any link between CJD and blood transfusions [42]. The major weakness in these studies is that CJD is very rare in the donor population; only 0.0016 % of units of blood transfused might carry the CJD agent. The lack of any link between blood transfusion and CJD may be due to the fact that very few people receiving blood transfusions are exposed to the CJD agent.

Cohort studies look at the number of patients who develop CJD after receiving blood from

donors infected with CJD. So far, no cases of CJD have been found, but some results stand out. In two studies, no recipients developed CJD but less than 30% lived longer than five years following the transfusion [43]. These studies support the theory that transmission by blood transfusion is uncommon. However, the incubation periods are not long enough to make the findings definitive [31, 44]. In another study, 76 out of 101 people who received cryoprecipitate between 1979 and 1985 have lived at least 11 to 17 years after receiving infected blood products and none has developed CJD [44].

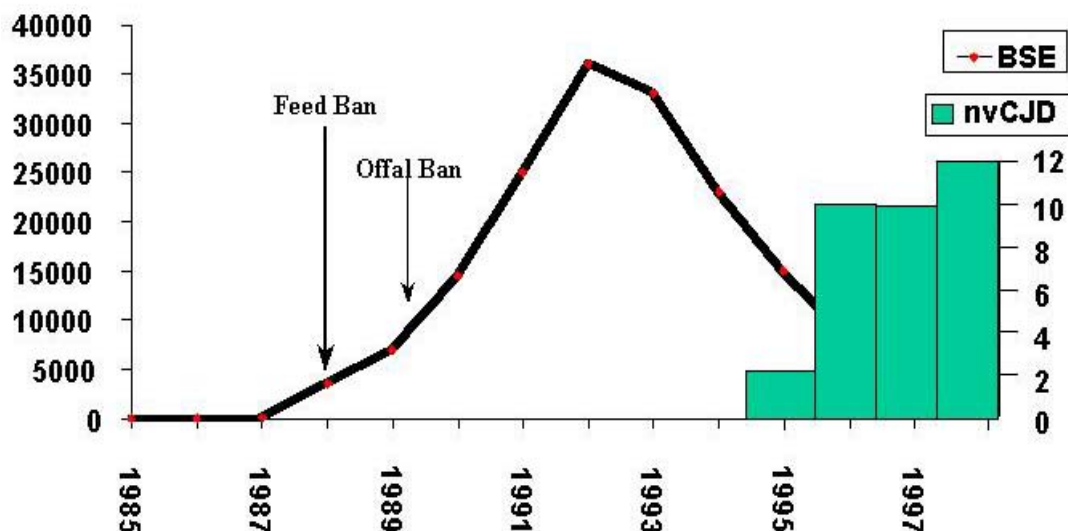
All these studies support the idea that transmission of CJD by blood transfusions or blood products is very rare if it exists. However, because of the short incubation times seen in the studies and the very small number of cases, no one can definitely say that transmission is non-existent.

Bovine Spongiform Encephalopathy and 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 [9-10]. 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. Cattle that had died 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 vCJD 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

Figure 1: BSE and vCJD in the U.K.



observations that no cases had appeared before 1993; only one case had appeared outside the U.K.; the cases followed the BSE epidemic; the vCJD prion resembles the BSE prion; and the pathologic patterns seen in vCJD 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 vCJD 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 vCJD by blood or blood products. Subsequently, tonsils and spleen of animals infected with BSE adapted to various species and individuals infected with vCJD (but not sporadic CJD) have been found to contain prion protein, in concentrations as high as 10 per cent of that found in the brain. [46] In addition, several studies have shown that vCJD can be transmitted by blood transfusion in different experimental animals. The most notable transmission was reported in 2002 when blood obtained from sheep was experimentally infected with BSE and blood obtained from sheep with naturally acquired scrapie was transfused into healthy sheep that had not previously been exposed to these agents. Two of 24 (8%) of the healthy sheep that received the blood contracted BSE and four of 21 (20%) developed scrapie. [47]

Recently, a suspected human transfusion-acquired case of vCJD was reported. [48] This patient was one of 48 patients who received various blood components from 15 donors who later developed vCJD in the United Kingdom. This patient, over the age of 60, developed symptoms of vCJD 6.5 years after receiving five units of red cells, one of which was donated by a donor 3.5 years before developing the symptoms of vCJD. Although it is possible that this infection was acquired by eating infected beef in Britain, several observations support the hypothesis that this was a transfusion-acquired case:

- Statistical analysis of cases in Britain suggests the chance of acquiring a case in the absence of transfusion to be 1 in 15,000 to 1 in 30,000.
- The cellular components of blood contain the highest concentration of suspected infectious agent for vCJD and, therefore,

are more likely to transmit the disease as compared with the other components or derivatives.

- The possible incubation period, 6 years, was appropriate for vCJD transmission via a peripheral route of infection (blood).
- Nearly all the vCJD cases have been considerably below the age of 55 (15-40)
- In light of the accumulating experimental animal data, it would not be unreasonable for the transmission to have occurred.

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 transmissible 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 infectious 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 be a rare or nonexistent cause of current and past cases of classical CJD in humans. Variant CJD, on the other hand, must be considered differently.

Although not proven, it is reasonable to assume that transmission of vCJD by blood transfusion is possible. For patients with hemophilia, two important issues need to be considered while estimating risk:

- the risk of various blood products; and
- the geographic location of the risk.

Risk to patients receiving other blood products

The causative agent of vCJD has been shown to be present in low concentration in the blood of experimental animal models of the disease. White blood cells (which are present in packed red cell transfusions) have a much greater concentration of infectivity than plasma). Plasma derivatives, such as clotting factor concentrates, are manufactured from plasma pools. The lower risk of infection from such concentrates is partly due to two factors: 1) the dilution of a contaminated donation by thousands of uncontaminated donations in the same plasma pool, and 2) the manufacturing process, which includes steps that are known from extensive laboratory studies to remove the infectious agent (e.g., precipitation, filtration, and column chromatography). Based upon animal experiments, the total reduction of infectious material in contaminated plasma has been calculated to be more than a million-fold. As a result, health authorities have considered plasma derivatives to be among the lowest risk blood products for vCJD.

Geographic localization of risk

The risk of blood-borne transmission of vCJD is, for practical purposes, restricted to the U.K., where 145 people have died from the disease, and where by far the largest number of individuals have been potentially exposed to “mad cow disease,” the cause of the vCJD outbreak. Outside the U.K., only Europe and Japan have had cases of BSE, and the number of affected cattle is extremely small compared with the U.K. Plasma collected from donors in the U.K. is not currently used for manufacturing plasma clotting factor concentrates. In addition, blood donor screening procedures used around the world have restrictions on donors who have resided in the U.K. and other countries where vCJD has been identified.

Exposure of hemophilia patients in the U.K. to vCJD

Plasma from a number of donors incubating vCJD was used in the U.K. to manufacture plasma-derived blood products prior to policy changes with regard to the use of U.K. plasma. As a result, several hundred hemophilia patients have used these products. These patients have been counseled and are under health

surveillance for signs of developing vCJD. Reports indicate that, currently, none have developed apparent vCJD.

Summary

Finally, the most reasonable assessment of risk at this time would be to accept possible blood transmission of vCJD as a reality.

- The risk of such occurrences appears to be predominately a risk for recipients of blood components collected in the U.K.
- Donor screening procedures and blood banking policies initiated in countries throughout the world appear to be appropriate to reduce such transmissions to a minimum.
- Currently, clotting factor concentrates manufactured from plasma obtained outside the U.K. appear to carry a very low risk of transmission of vCJD because of the exclusion of potential donors incubating vCJD and the reduction of potential agents by the manufacturing processes. Continued vigilance is important to identify any changes in the level of suspected risk.

The WFH continues to monitor this situation and will keep the hemophilia community informed.

References

- Masters CL, Harris JO, Gajdusek C, Gibbs CJ, Bernoulli C, Asher DM. Creutzfeldt-Jakob disease: patterns of worldwide occurrence and the significance of familial and sporadic clustering. *Ann Neurol* 1979; **5**: 177-88.
- Brown P, Cathala F, Raubertas RF, Gajdusek DC, Castaigne P. The epidemiology of Creutzfeldt-Jakob disease: conclusion of a 15 year investigation in France and review of the world literature. *Neurology* 1987; **37**: 895-904.
- Will, RG. Incidence of Creutzfeldt-Jakob disease in the European Community. In: *Bovine Spongiform Encephalopathy: The BSE Dilemma*. Gibbs CJ Jr, ed. New York: Springer-Verlag, 1996; 364-74.
- World Health Organization. Consultation on clinical and neuropathological characteristics of the new variant of CJD and other human and animal TSEs. Geneva: WHO, 1996.
- Cousens SN, Bynnycky E, Zeidler M, Will RG, Smith PG. Predicting the CJD epidemic in humans. *Nature* 1997; **385(6613)**: 197-8.
- Gajdusek DC. Unconventional viruses and the origin and disappearance of kuru. *Science* 1977; **197(307)**: 943-60.
- Gibbs CJ Jr, Amyx HL, Bacote A, Masters CL, Gajdusek DC. Oral transmission of kuru, Creutzfeldt-Jakob disease, and scrapie to nonhuman primates. *J Infectious Dis* 1980; **142(2)**: 205-8.
- Hunter N. *Natural Transmission and Genetic Control of Susceptibility of Sheep to Scrapie*. *Current Topics in Microbiology and Immunology*, Vol 172. Berlin-Heidelberg: Springer-Verlag, 1991.
- Wilesmith JW, Hoinville LJ, Ryan JB, Sayers AR. Bovine spongiform encephalopathy: aspects of the clinical picture and analyses of possible changes 1986-1990. *Veterinary Record* 1992; **130(10)**: 197-201.
- Anderson RM, Donnelly CA, Ferguson NM, Woolhouse ME, Watt CJ, Udy HJ, *et al*. Transmission dynamics and epidemiology of BSE in British cattle. *Nature* 1996; **382(6594)**: 779-88.
- Bruce MR, Will RG, Ironside JW, McConnell I, Drummond D, Suttie A, *et al*. Transmission to mice indicate that "new variant" CJD is caused by the BSE agent. *Nature* 1997; **389**: 498-501.
- Hill AF, Desbruslais M, Joiner S, Sidle KCL, Gowland I, Collinge J. The same prion strain causes vCJD and BSE. *Nature* 1997; **389**: 448-50.
- Wilesmith JW, Wells GAH, Cranwell MP, Ryan JBM. Bovine spongiform encephalopathy; epidemiologic studies. *Vet Rec* 1988; **123**: 638-44.
- Collinge J, Sidle KC, Meads J, Ironside J, Hill AF. Molecular analysis of prion strain variation and the aetiology of 'new variant' CJD. *Nature* 1996; **383(6602)**: 685-90.
- Brown P, Gajdusek DC. *The Human Spongiform Encephalopathies: Kuru, Creutzfeldt-Jakob Disease, and the Gerstmann-Straussler-Scheinker Syndrome*. *Current Topics in Microbiology and Immunology*, Vol. 172. Berlin-Heidelberg: Springer-Verlag, 1991.
- Kuroda Y, Gibbs CJ Jr, Amyx HL, Gajdusek DC. Creutzfeldt-Jakob disease in mice: persistent viremia and preferential replication of virus in low-density lymphocytes. *Infection Immunity* 1983; **41(1)**: 154-61.
- Manuelidis EE, Kim JH, Mericangas JR, Manuelidis L. Transmission to animals of Creutzfeldt-Jakob disease from human blood. *Lancet* 1985; **2(8460)**: 896-97.
- Tateishi J. Transmission of Creutzfeldt-Jakob disease from human blood and urine into mice. *Lancet* 1985; **2(8463)**: 1074.
- Tamai Y, Kojima H, Kitajima R, Taguchi F, Ohtani Y, Kawaguchi T, Miura S, Sato M, Ishihara Y. Demonstration of the transmissible agent in tissues from a pregnant woman with Creutzfeldt-Jakob disease [letter]. *N Engl J Med* 1992; **327(9)**: 649.
- Clarke MC, Haig DA. Presence of the transmissible agent of scrapie in the serum of affected mice and rats. *Vet Rec* 1967; **80(16)**: 504.

21. Dickinson AG, Fraser H. Modification of the pathogenesis of scrapie in mice in treatment of the agent. *Nature* 1969; **222(196)**: 892-3.
22. Manuelidis EE, Gorgacs EJ, Manuelidis L. Viremia in experimental Creutzfeldt-Jakob disease. *Science* 1978; **200(4345)**: 1069-71.
23. Meeting of the Transmissible Spongiform Encephalopathies (TSE) Advisory Committee of the Federal Food and Drug Administration, Washington, DC, October 7, 1997. (Presentation of Robert F. Rohwer, Ph.D., University of Baltimore VA Medical Center, Baltimore, MD, and Paul Brown, M.D., National Institutes of Health, Bethesda, MD.)
24. Lynch TJ, Weistein MJ, Tankersley DL, Fratantoni JC, Finlayson JS. Considerations of pool size in the manufacture of plasma derivatives. *Transfusion* 1996; **36(9)**: 770-5.
25. Parchi P, Castellani R, Capellari S, Ghetti B, Young K, Chen SG, *et al.* Molecular bias of phenotypic variability in sporadic Creutzfeldt-Jakob disease. *Ann Neurol* 1996; **39(6)**: 767-78.
26. Brown P, Cervenakova L, Goldfarb LG, McCombie WR, Rubenstein R, Will RG, *et al.* Iatrogenic Creutzfeldt-Jakob disease: an example of the interplay between ancient genes and modern medicine. *Neurology* 1994; **44(2)**: 291-3.
27. Collinge J, Palmer MS, Dryden AJ. Genetic predisposition to iatrogenic Creutzfeldt-Jakob disease. *Lancet* 1991; **337**: 1441-2.
28. Creange A, Gary F, Cesaro, Adle-Biasette H, Duvoux C, Cherqui D, *et al.* Creutzfeldt-Jakob disease after liver transplantation. *Ann Neurol* 1995; **38**: 269-72.
29. Klein R, Dumble LF. Transmission of Creutzfeldt-Jakob disease by blood transfusion. *Lancet* 1993; **341**: 768.
30. Collins S, Master CL. Iatrogenic and zoonotic Creutzfeldt-Jakob disease: The Australian perspective. *Med J Aust* 1996; **164**: 598-602.
31. Ricketts MN, Cahsman NR, Stratton EE, Eisaadany S. Is Creutzfeldt-Jakob disease transmitted in blood? *Emerging Infectious Diseases* 1997; **3**: 155-63.
32. Davanipour Z, Smoak C, Bohr T, Sobel E, Liwnicz B, Chang S. Death Certificates: An efficient source of ascertainment of Creutzfeldt-Jakob disease cases. *Neuroepidemiology* 1995; **14**: 1-6.
33. Holman RC, Kahn AS, Kent J, Strine TW, Schonberger LB. Epidemiology of Creutzfeldt-Jakob disease in the United States, 1979-1990: analysis of national mortality data. *Neuroepidemiology* 1995; **14**: 174-81.
34. Evatt B, Austin H, Barhart E. *et al.* Surveillance of Creutzfeldt-Jakob disease among persons with hemophilia. *Transfusion* 1998; **38**: 817-20
35. Lee CA, Ironside JW, Bell JE, *et al.* Retrospective neuropathological review of prion disease in UK haemophilic patients. *Thrombos Haemost* 1998. **80**: 909-11.
36. Davanipour Z, Alter M, Sobel E, Asher D, Gajdusek DC. Creutzfeldt-Jakob disease: possible medical risk factors. *Neurology* 1985; **35**: 1483-86.
37. Will RG. Epidemiological surveillance of Creutzfeldt-Jakob disease in the United Kingdom. *Eur J Epidemiol* 1991; **7**: 460-65.
38. Esmonde TFG, Will RG, Slattery JM, Knight R, Harries-Jones R, de Silva R, *et al.* Creutzfeldt-Jakob disease and blood transfusion. *Lancet* 1993; **341**: 205-7.
39. Harries-Jones R, Knight R, Will RG, Cousens S, Smith PG, Matthews WB. Creutzfeldt-Jakob disease in England and Wales, 1980-1984: a case-control study of potential risk factors. *J. Neurol Neurosurg Psychiatry* 1988; **51**: 1113-19.
40. Esmonde TFG, Ireland BN, Will RG, Ironside J. Creutzfeldt-Jakob disease: A case-control study. *Neurology* 1994; **44**: A193.
41. Kondo K, Kuroiwa Y. A case control study of Creutzfeldt-Jakob disease: association with physical injuries. *Ann Neurol* 1982; **11**: 37-81.
42. Wientjens DPWM, Davanipour Z, Hofman A, Kondo K, Matthews WB, Will RG, Vanduijn CM. Risk factors for Creutzfeldt-Jakob disease - a reanalysis of case-control studies. *Neurology* 1996; **46**: 1287-91.

43. Heye N, Hensen S, Muller N. Creutzfeldt-Jakob disease and blood transfusion. *Lancet* 1994; **343**: 298-9.
44. Hearing Before the House Committee on Government Reform and Oversight, Subcommittee on Human Resources, 105th Congress, July 31, 1997. (Testimony of David Satcher, M.D., Ph.D., Director, The Centers for Disease Control and Prevention.)
45. Cousens SN, Zeidler M, Esmonde TF, De Silva R, Wilesmith JW, Smith PG, Will RG. Sporadic Creutzfeldt-Jakob disease in the United Kingdom: analysis of epidemiological surveillance data for 1970-96. *BMJ*. 1997; 315(7105); 389-395.
46. Herzog C, Sales N, Etchegaray N, Charbonnier A, Freire S, Dormont D, Deslys JP, *et al*. Tissue distribution of bovine spongiform encephalopathy agent in primates after intravenous or oral infection. *Lancet* 2004; 363(9407):422-28.
47. Hunter N, Foster J, Chong A, McCutcheon S, Parnham D, Eaton S, MacKenzie C, *et al*. Transmission of prion diseases by blood transfusion. *J Gen Virol* 2002; 83(Pt 11):2897-905.
48. Llewelyn CA, Hewitt PE, Knight RS, Amar K, Cousens S, Mackenzie J, Will RG. Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. *Lancet* 2004; 363(9407):417-21.