

# **variant Creutzfeldt-Jakob disease and Hemophilia – Further guidance on assessing the risks of plasma-derived products for treating hemophilia**

Prepared by Albert Farrugia, BSc, PhD, on behalf of the WFH Task Force on TSEs

## **Introduction**

In 2001, the WFH Task Force on Transmissible Spongiform Encephalopathies (TSEs) published a risk assessment of plasma-derived products ([http://www.wfh.org/Content\\_Documents/Blood\\_Safety/vCJD\\_Bulletin2\\_revised.pdf](http://www.wfh.org/Content_Documents/Blood_Safety/vCJD_Bulletin2_revised.pdf)). Since that time, a number of developments have occurred that have increased our knowledge and understanding of the implications of TSEs for blood safety. The following document reviews these developments, and should be read in conjunction with the 2001 document and the recent bulletins (<http://www.wfh.org/ShowDoc.asp?Rubrique=30&Document=37>) put out by the WFH Task Force.

## **Blood safety and TSEs**

Animal experiments have continued to show that the transfusion of blood is an efficient vehicle for transmitting TSEs. The particular strain of TSE does not appear to influence blood infectivity. In studies using mice, both classical and variant forms of Creutzfeldt-Jakob disease (CJD) were transmitted with equal frequency<sup>1</sup>. In sheep, whole blood transfusions transmitted both BSE and scrapie<sup>2</sup> at frequencies suggesting a high rate of infectivity. In humans, classical CJD (cCJD) has not been associated with blood transfusion. However, the initial description of variant CJD (vCJD) showed that it differed from the classical form in that large parts of the lymphoreticular system (LRS) - consisting of lymphoid tissue such as the lymph glands, the spleen, the tonsils, etc - showed extensive infiltration by the abnormal prion protein, which is thought to be the infectious agent<sup>3</sup>. Since the LRS has extensive blood supply the possibility that the level of infectious prion in blood from individuals infected with vCJD may be higher than that in cCJD.

Therefore, the first reported case of vCJD transmission by blood transfusion in late 2003<sup>4</sup> does not come as a surprise. Subsequently, a second case has been described, in which a patient asymptomatic for vCJD was found to have vCJD prions in lymphoid tissue following autopsy; this patient's history of transfusion with blood from a patient who subsequently developed vCJD provided the causal link<sup>5</sup>.

In contrast, cCJD has never been demonstrated to be transmitted in humans by blood, despite indications that this is the case in animals. Surveying a series of five studies and 2479 patients, in 2000 the authors concluded that case-control studies do not suggest a risk of developing Creutzfeldt-Jakob disease from blood transfusion<sup>6</sup>. However, they wisely pointed out the limitations arising from the choice of control population and the difficulty of accurately recalling past transfusions. Given the longer history of cCJD (first described in 1920) compared to vCJD (first described in 1995), its presence as a blood safety risk should have appeared, as all blood infectious agents have, in the hemophilia population. Evatt et al<sup>7</sup> have surveyed a small series of brains from autopsied people with hemophilia who died from neurological conditions mostly associated with AIDS, and have found none of the spongiform changes associated with cCJD. Again, the authors point to the limitations from the small sample size, but the findings are encouraging none the less.



However, Vamvakas has pointed out that, because of the limitations imposed by low case numbers and prolonged incubation periods, "*it is impossible to conclude at this time that this risk (of cCJD transmission through blood transfusion) does not exist*"<sup>8</sup>. He demonstrates that the number of patients requiring assessment to exclude a 1 in 1000 risk of acquiring cCJD from blood transfusion with 95% confidence is 40000 (Table 1).

Table 1 Approximate risk of developing Creutzfeldt-Jakob disease (following the transfusion of a blood component from a donor who subsequently developed the disease) that cannot be excluded with 95% or 99.9% confidence

<b>Patients in the National Blood Data Resource Centre database</b>	<b>Cases of Creutzfeldt-Jakob Disease developing among the database patients</b>	<b>Approximate risk of developing Creutzfeldt-Jakob Disease not excluded with 95% confidence</b>	<b>Approximate risk of developing Creutzfeldt-Jakob Disease not excluded with 99.9% confidence</b>
250	0	1.51%	3.19%
500	0	0.74%	1.54%
1000	0	0.37%	0.77%
2000	0	0.18%	0.38%
4000	0	0.09%	0.19%
20000	0	0.02%	0.04%
40000	0	0.01%	0.02%

**While it is wise to maintain a cautionary approach in relation to the possibility of blood transfusion being a vehicle for cCJD, it is unlikely that concrete evidence would not have arisen in the eighty years since the disease's description.**

### **Prions and plasma fractionation**

In rodent models of TSEs, blood infectivity is found both within the cellular (primarily leucocyte) components and the plasma<sup>9</sup>. Plasma infectivity is found in both the pre-clinical and clinical phases of the disease, although the infectivity titre measured through intravenous, as opposed to intracerebral, inoculation of the indicator animals is low<sup>1</sup>. Plasma infectivity cannot be removed by high-speed centrifugation or by leucocyte-filtration<sup>9</sup>, suggesting that it is not associated with aggregated or cell-bound forms of the prion protein. Therefore, the possibility that this infectivity will collect with the therapeutic proteins (such as factor VIII) in the course of plasma fractionation cannot be discounted. As the prion protein is highly resistant to the processes used to eliminate infectivity from other infectious agents, such as solvent-detergent and heat treatment, any decrease in this potential infectivity has to be achieved through clearance during the purification of the therapeutic proteins during fractionation.

Manufacturers of plasma products have therefore carried out investigations on the capacity of their processes to partition prions away from therapeutic proteins over the course of fractionation. These studies have taken two broad forms. In studies of *endogenous* (or internal) infectivity, blood from animals which are sick or harbouring TSEs is fractionated and infectivity is tracked by injecting the resulting fractions into other animals and checking for the development of disease. In studies of *exogenous* (or external) infectivity, a tissue fraction, generally purified from brain from an animal with TSE, is introduced into one or more stages of the fractionation process, and the resulting fractions are similarly tested in animals. For the result to have any use, both these approaches depend heavily on the ability of the investigators to replicate in the laboratory the plasma fractionation process as carried out on an industrial scale, a process fraught with difficulty.

Most studies performed by manufacturers have used *exogenous* approaches. This in itself limits the extent to which these studies can provide useful data, as there is no guarantee that the form of infectivity introduced to track the fractionation is related to the infectivity in blood. However, *endogenous* infectivity is found in very low amounts, so that a useful impression of how much a manufacturing method is capable of clearing infectivity is difficult to achieve. Nevertheless some such studies have been done, and, while broadly agreeing with the *exogenous* studies, have stimulated the European Medicines Agency (EMA) to comment that, "*Preliminary information reported to the EMA Workshops in 2002 and 2004 suggests that endogenous infectivity might persist further through the fractionation process than would be expected from spiking studies*"<sup>10</sup>. While most of the studies on which this cautious statement is based emanate from one group and have not been subject to the same level of peer review that the *exogenous* studies have been subjected, the possibility that the forms of infectivity used in most of the manufacturers' studies may not reflect the situation in blood is worrisome. Investigations using *exogenous* infectivity have noted that the form in which the prion protein is presented to the fractionation system affects the distribution into different fractions<sup>11</sup>. **We agree with the EMA that, "There is a need for further research in this area to investigate the partition and removal of endogenous infectivity and the extent to which this is comparable to data from spiking studies."**<sup>10</sup>

Nevertheless, the spiking (*exogenous*) studies have yielded useful information. For the mainstream products of plasma fractionation - immunoglobulin and albumin - several widely used fractionation steps clear prions away from therapeutic fractions to considerable levels (reviewed by Foster 2004<sup>12</sup>). Furthermore, when studying *strains* of prions from different TSEs, partitioning during fractionation appears to be similar and is not affected by the strain of prion<sup>13</sup>. This finding indicates that studies on currently known prion diseases should allow reasonable predictions on any new emerging diseases, the emergence of which should not be discounted.

### **The implications for hemophilia concentrates - assessing the risks**

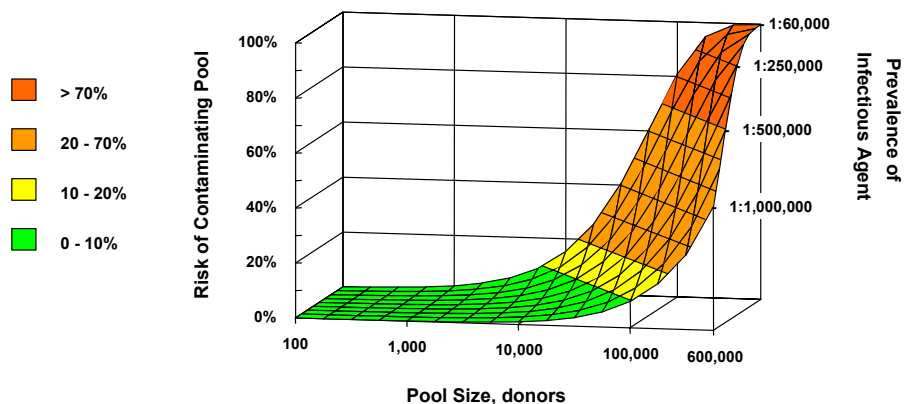
A number of regulatory agencies have developed risk assessment models for plasma products, and have attempted to include some quantification in order to calculate relative risks. Examples of the type of model may be found in the references<sup>14,15,16</sup>. Essentially, all these approaches draw upon a number of parameters in assessing the risk of TSE infection for an individual recipient of plasma derivatives:

- The number of blood / plasma donations pooled in the production process
- The rate of TSE infection in blood donors
- The volume of blood / plasma donation
- The concentration of TSE infectivity in plasma
- The number of units of product from the production process
- The amount of clearance of TSE infectious units achieved during the production process
- The amount of product to which the patient is exposed

We will discuss some of these parameters in more detail:

***The number of blood / plasma donations pooled in the production process*** The risk of including an infectious agent in a plasma pool depends on the prevalence of the agent and the size of the pool (Fig 1). Using these considerations, Lynch et al (1996)<sup>17</sup> concluded that decreasing the pool size decreases the risk of exposure to an infectious agent in the donor population. However, this only assumes significance when agents have a low prevalence and/or pool sizes are lower than is practically feasible for plasma product manufacture. They pointed out that chronically transfused patients such as people with hemophilia are ultimately exposed even with small pool sizes and low prevalence agents. Brown (1998)<sup>18</sup> has used similar principles to show the effect on the risk of exposure to classical CJD, at the prevalence rates that this is found in the normal population. He calculates that increasing pool sizes of 10,000 and 100,000 units, respectively, led to an increase from 1% to 13% contamination assuming one year of pre-clinical blood infectivity, and an increase of 13% to 74% contamination assuming 10 years of pre-clinical blood infectivity.

Fig 1 Probability map of the risk of contaminating plasma pools of different sizes From Lynch (2002) on [http://www.fda.gov/OHRMS/DOCKETS/ac/02/slides/3868S1\\_6.ppt](http://www.fda.gov/OHRMS/DOCKETS/ac/02/slides/3868S1_6.ppt)

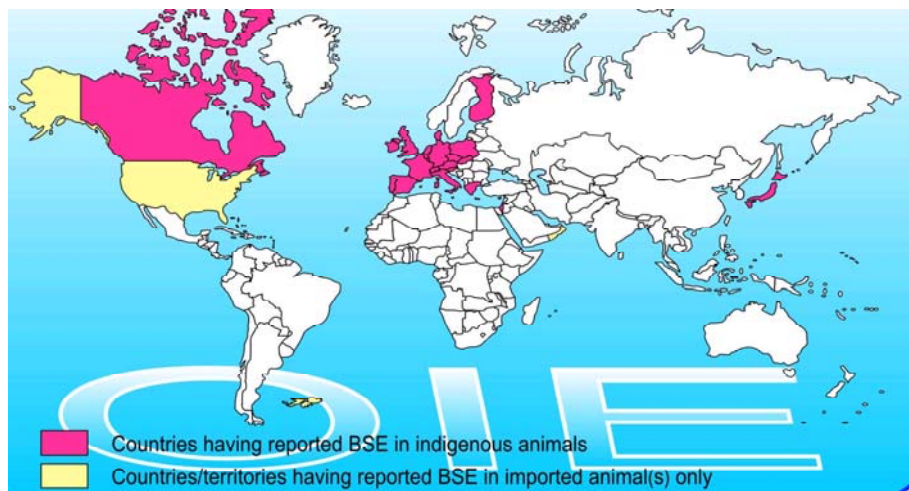


While it is correct that individuals receiving plasma products on a continuing basis will eventually be exposed to a potentially contaminated pool no matter what its size, there remains the unknown possibility that with large pool sizes the dilution effect will nullify transmission of disease while it is assumed that an infectious dose will by definition be infectious no matter how much the dilution, this point has never been examined experimentally.

**A plasma pool constructed from well-accredited apheresis donors, selected for low risk of exposure to specific agents, would provide a practical route to minimizing exposure to emerging agents which cannot be tested and/or removed.** For example, excluding donors potentially exposed to BSE from the plasma pool for coagulation factors could significantly decrease, if not obviate, the risk of vCJD. This is because the specific risk factor can be identified, although in some regions restricting the donor pool in this way would affect supply to unsustainable levels in the absence of other measures such as the recruitment of dedicated aphaeresis donors and the use of recombinant products when possible.

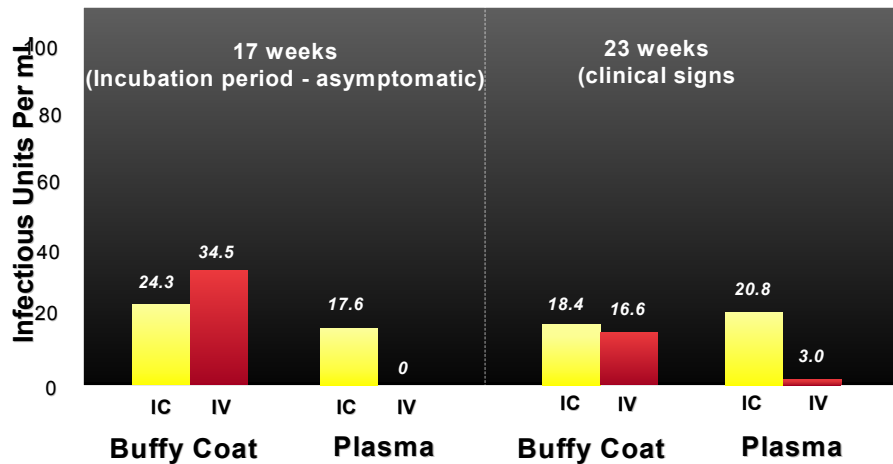
***The rate of TSE infection in blood donors*** The prevalence of vCJD in a population must be presumed to be reflective of the extent to which the population has been exposed to BSE through eating meat products from cows fed contaminated meat and bone meal from the UK. In terms of assuring the safety of the blood supply, this has been mostly addressed through the deferral of blood donors who have been potentially exposed to BSE through residence in countries with BSE. While the focus of concern has been the UK, a number of other countries have now reported indigenous BSE (Fig 2). The extent to which blood donors can be excluded from the manufacturing pool for factor concentrates depends on other aspects of the blood system and in particular, the extent to which it is possible to build up a donor pool of, preferably, apheresis donors who can be used solely for this purpose (see above). **The susceptibility of certain genotypes to developing vCJD and other TSEs may lend itself to some refinement of such donor selection processes. However, the recent finding that a second (asymptomatic) case of vCJD transmission through blood occurred in an individual with a genotype previously unassociated with vCJD raises the possibility that all the population exposed to BSE may, with varying incubation periods, be susceptible to vCJD<sup>19</sup>.**

Fig 2 Geographical distribution of countries reporting endogenous BSE from 1989 to January 2004  
 On [http://www.oie.int/Cartes/BSE/a\\_Monde\\_BSE.htm](http://www.oie.int/Cartes/BSE/a_Monde_BSE.htm)



***The concentration of TSE infectivity in plasma*** This parameter is highly contentious and, in the absence of a quick and reliable method to measure endogenous blood infectivity, is likely to remain uncertain. A survey of the literature indicates a concentration of 1 to 100 infectious units per millilitre in the blood of animals infected with TSEs transmits disease. This concentration is dependent on the route of infection and the blood component - cells or plasma - being measured. In studies on mice infected with different human TSEs, measurement of infectivity in plasma showed infectivity in the pre-symptomatic stage only when the indicator test animals were inoculated intracranially (Fig 3). While the intravenous route is acknowledged as being less efficient than the intracranial route, transfusion transmission in primates occurs with shorter incubation periods in the intravenous route compared to the established oral route for BSE<sup>20</sup>. This has prompted the EMEA to recommend that, "For the purpose of risk assessments, it is recommended that the worst case assumption that the relative efficiency of the intravenous and intracerebral routes is 1:1 should be used"<sup>10</sup>. On this basis, and until more data accrues, we would consider an infectious concentration of approximately 20 infectious units/ml in the plasma to be a reasonable assumption.

**Fig 3 Infectivity Levels in Buffy Coat and Plasma of vCJD-Infected mice  
Cervenakova 2003**



***The amount of clearance of TSE infectious units achieved during the production process***

In the area of viral safety for factor concentrates, the donor selection procedures discussed above have had, compared to the effect of viral elimination steps, a marginal effect on the safety for recipients<sup>22</sup>. As discussed, such steps are of limited efficacy with prions, but fortuitously, the purification steps for products, including concentrates, have the potential to partition prions away from the therapeutic protein. As a general rule, studies on factor concentrates indicate that the higher the purity achieved for the coagulation protein, the better the level of prion clearance.

Thus, ion-exchange and immuno-affinity chromatographic<sup>23,24</sup> steps in the manufacture of FVIII have been shown to eliminate significant amounts of TSE infectivity. Precipitation of impurities while leaving FVIII in the supernatant generally achieves useful partitioning of FVIII away from the prion protein<sup>11,13</sup>, but the clearances achieved for individual steps are relatively small. (See Table 2)

This clearly raises the question of "How much clearance is required?" While high clearance levels are desirable and can have, depending on the type of mathematical modelling, a profound effect on a risk assessment, the final risk assessment is the result of a combination of all the parameters discussed in this paper. For example, for a well-defined TSE like vCJD where the infection is limited to a specific behavioural factor (exposure through BSE determined by the surrogate of geographic residence), risk can be significantly lowered through the kind of donor selection measures discussed above, allowing a higher tolerance for low clearance factors.

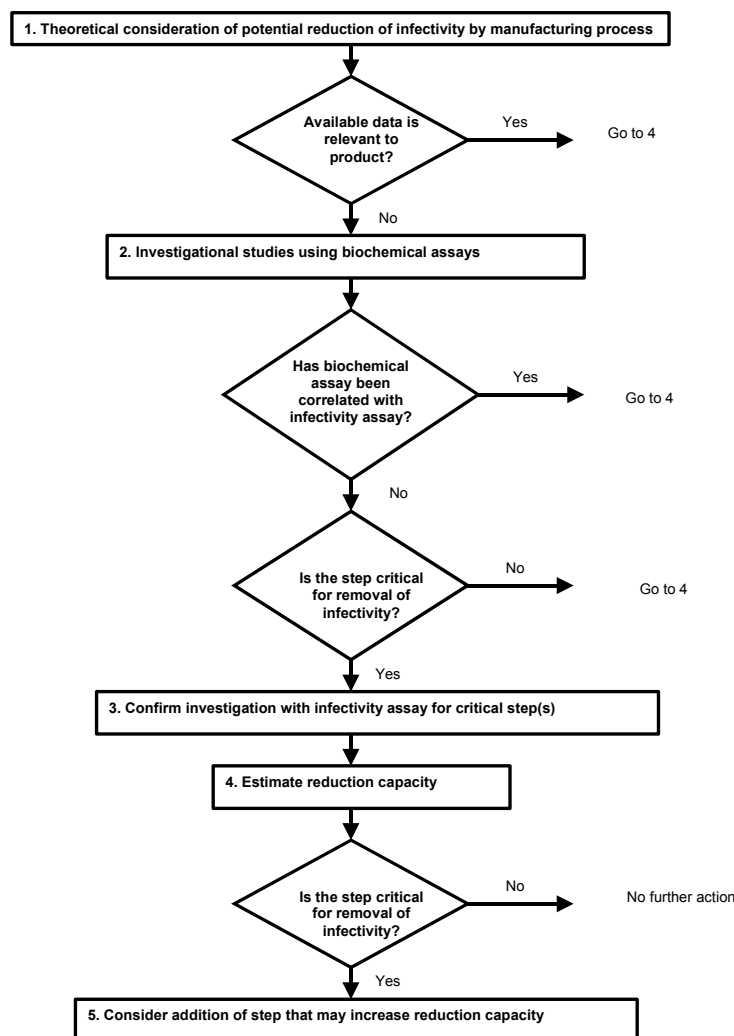
**Table 2**

Validated manufacture steps	Hamster scrapie <sup>1</sup> : Spiking material	Detection Method	PrP or infectivity reduction factor	Company
Cryoprecipitation, precipitation and adsorption, SD treatment and ion-exchange chromatography, membrane filtration	263K: Microsomal fraction	Western blot	6.8 <sup>3</sup> log <sub>10</sub>	SNBTS Protein Fractionation Center
Cryoprecipitation and Cryoprecipitate/PEG separation	263K: Brain homogenate	Western blot Bioassay	2.2 <sup>3</sup> log <sub>10</sub> /ml 3.0 log LD <sub>50</sub> /ml	Bayer
Cryoprecipitate  Ethanol precipitation 8%	Sc237: Brain homogenate, microsomal fraction, CLD <sup>2</sup>  Sc237: Purified PrP <sup>Sc</sup>  Sc237: Brain homogenate, microsomal fraction, CLD <sup>2</sup>  Sc237: Purified PrP <sup>Sc</sup>	Conformation dependent immunoassay	<1 log <sub>10</sub>  2.4 log <sub>10</sub>  <1 log <sub>10</sub>  3.1 log <sub>10</sub>	Aventis Behring
FactorVIII immunoaffinity column Ion exchange chromatography	263K: Brain homogenate	Bioassay	4.6 log LD <sub>50</sub> /ml 3.5 log LD <sub>50</sub> /ml	American Red Cross/Baxter

<sup>1</sup>Hamster-adapted scrapie (263K or Sc237); <sup>2</sup>CLD – caveola-like domains; <sup>3</sup>Sum of all steps tested

In addition, the similar behaviour of different prion strains in fractionation suggests a process's capacity to eliminate emerging prions before their actual recognition through disease, and is very important in the absence of a screening test. Therefore, we support the path for manufacturers and regulatory authorities proposed by the European Medicines Agency (EMA) for the assessment of the capacity of processes to clear prions<sup>10</sup> (Fig 4).

Fig 4 Proposed investigational path for TSE reduction during the manufacture of plasma products. From ref 10



***The amount of product to which the patient is exposed***

Clearly, experience with other infections has shown that the more exposed a patient is to product, the higher the risk, for example the difference between the HIV infection rates between severe and mild hemophilia in some communities is well recognized. The level of exposure will also have an effect on the risk of infection by TSEs. For example, the risk of exposure for a patient with relatively mild Type I von Willebrand disease is clearly lower than for a patient with hemophilia. This is important as, while in some communities the risk of TSEs and other blood-borne pathogens for people with hemophilia is steadily decreasing with the use of recombinant products, this option is not available for patients with von Willebrand's disease. Therefore, a risk assessment for TSE exposure will be different for these two groups of patients. Similarly, the use of prothrombin complex concentrates for patients with hemophilia B must be viewed with concern, as we stated in the 2001 risk assessment, since the TSE clearing capacity for these products is inferior to that of single FIX concentrates. However, their use in reversing warfarin overdose<sup>25</sup> gives these products an ongoing role. Recognizing that the use of these products will be very infrequent in particular patients will alter the risk assessment and allow a more reassuring

outcome than may seem immediately apparent given the relatively modest prion clearance factors achieved in the manufacture of prothrombin complex concentrates.

### **Approaching a risk assessment**

The use of structured assessments for evaluating the risks of plasma products transmitting TSEs is a new field. As discussed in this paper, the parameters, which contribute to risk, are relatively easy to identify. Their use in a risk assessment is also straightforward, and reference is made to the published assessments referred to above. While the international regulatory framework is still developing in this area, authorities are starting to use these assessments to influence policy. In Australia, the assessments of an independent committee advising the government on TSEs<sup>26</sup> led it to conclude that

*Although the theoretical risks from plasma derived AHF (anti-haemophilic factor) are very small, they cannot be said to be totally negligible. It is prudent to recommend that, as soon as feasible, AHF be made available in recombinant form, or a product of a purification process that is proven to reduce prion content by at least 7 logs.<sup>27</sup>*

While such advice may merit consideration in Australia, the WFH advises authorities, users, and all those involved in the delivery of hemophilia care to assess each environment on its own merits, and take careful note of all the issues outlined in this risk assessment. It is perfectly feasible to counter the emerging threat of TSEs in hemophilia products, if appropriate notice is given to good science and balanced measures. It is important to avoid the complacency which characterized previous blood-borne epidemics in people with hemophilia, while retaining a sense of proportion to this particular issue, and maintain a constant watch on the balance between safety and supply.

*Albert Farrugia PhD  
August-September 2004*

Acknowledgement: The helpful and expert comments of Dr. Paul Brown are gratefully recognized.

---

<sup>1</sup> Cervenakova L et al (2003) Similar levels of infectivity in the blood of mice infected with human-derived vCJD and GSS strains of transmissible spongiform encephalopathy. *Transfusion* 43:1687-1694

<sup>2</sup> Hunter N et al (2002) Transmission of prion diseases by blood transfusion. *J Gen Virol* 83 :267-71

<sup>3</sup> Hill AF et al (1997) Diagnosis of new variant Creutzfeldt-Jakob disease by tonsil biopsy. *Lancet* 349: 99-100.

<sup>4</sup> Llewelyn et al (2003) Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion *Lancet* 363:417-412

<sup>5</sup> Peden A et al (2004) Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient *Lancet* 264: 527-29

<sup>6</sup> Wilson K et al (2000) Risk of acquiring Creutzfeldt-Jakob disease from blood transfusions: systematic review of case-control studies. *BMJ* 321:17-19

<sup>7</sup> Evatt B et al (1998) Surveillance for Creutzfeldt-Jakob disease among persons with hemophilia. *Transfusion*.38:817-20.



- 
- <sup>8</sup> Vamvakas EC (1998) The risk of transmission of Creutzfeldt-Jakob disease by transfusion of blood, plasma and plasma derivatives. *J Clin Apheresis* 14: 135-143
- <sup>9</sup> Brown P et al (1999) Further studies of blood infectivity in an experimental model of transmissible spongiform encephalopathy, with an explanation of why blood components do not transmit Creutzfeldt-Jakob disease in humans. *Transfusion*; **39** :1169–78
- <sup>10</sup> European Medicines Agency (2004) CHMP position statement on Creutzfeldt-Jakob disease and plasma-derived and urine-derived medicinal products. EMEA/CPMP/BWP/2879/02/rev 1. On <http://www.emea.eu.int/pdfs/human/press/pos/287902rev1.pdf> Last accessed on 5 October 2004
- <sup>11</sup> Vey M et al (2002) Purity of spiking agent affects partitioning of prions in plasma protein purification. *Biologicals*; **30** :187–96
- <sup>12</sup> Foster P (2004) Removal of TSE agents from blood products *Vox Sanguinis* 87 (Suppl. 2) , S7–S10
- <sup>13</sup> Stenland CJ (2002) Partitioning of human and sheep forms of the pathogenic prion protein during the purification of therapeutic proteins from human plasma. *Transfusion* ; **42** :1497–1500
- <sup>14</sup> Anderson S (2003) Risk analysis for TSE and plasma products. Presented at the Transmissible Spongiform Encephalopathy Advisory Committee of the Food and Drug Administration, 20 February 2003. On [http://www.fda.gov/ohrms/dockets/ac/03/slides/3923S1\\_11.ppt](http://www.fda.gov/ohrms/dockets/ac/03/slides/3923S1_11.ppt) Last accessed on 5 October 2004
- <sup>15</sup> Agence Française de Sécurité Sanitaire des Produits de Santé (Afssaps) (2000) Risk analysis of new variant Creutzfeldt-Jakob disease transmission by blood and blood products. On <http://agmed.sante.gouv.fr/ang/pdf/mcj02.pdf> Last accessed on 5 October 2004
- <sup>16</sup> Det Norske Veritas (2003) Risk Assessment of Exposure to vCJD Infectivity in Blood and Blood Products for Department of Health . On [http://www.dnv.com/binaries/vCJD\\_Update\\_Report\\_tcm4-74414.pdf](http://www.dnv.com/binaries/vCJD_Update_Report_tcm4-74414.pdf) Last accessed on 5 October 2004
- <sup>17</sup> Lynch TJ et al (1996) Considerations of pool size in the manufacture of plasma derivatives. *Transfusion*; **36**:770-5.
- <sup>18</sup> Brown P (1998) Donor pool size and the risk of blood-borne Creutzfeldt-Jakob disease. *Transfusion*. **38**:312-5.
- <sup>19</sup> Peden et al (2004) Preclinical vCJD after blood transfusion in a *PRNP* codon 129 heterozygous patient *Lancet*; **264**: 527–29
- <sup>20</sup> Herzog C et al (2004) Tissue distribution of bovine spongiform encephalopathy agent in primates after intravenous or oral infection *Lancet*; **363** : 422-428
- <sup>21</sup> Herzog C et al (2004) Tissue distribution of bovine spongiform encephalopathy agent in primates after intravenous or oral infection *Lancet*; **363** : 422-428
- <sup>22</sup> Farrugia A (2004) Plasma for fractionation: safety and quality issues *Haemophilia*, **10**, 334–340
- <sup>23</sup> Drohan WN. Transmissible spongiform encephalopathies: needs perceived by the blood fractionation industry. International Workshop on Clearance of TSE Agents from Blood Products and Implanted Tissues, Gaithersburg MD, USA, September 13-14, 1999

---

<sup>24</sup> Foster PR (2004) Distribution of a bovine spongiform encephalopathy-derived agent over ion-exchange chromatography used in the preparation of concentrates of fibrinogen and factor VIII *Vox Sanguinis* 86 , 92-99

<sup>25</sup> Yasaka M et al (2003) Effect of prothrombin complex concentrate on INR and blood coagulation system in emergency patients treated with warfarin overdose *Ann Hematol.*;82:121-3.

<sup>26</sup> On <http://www.health.gov.au/nhmrc/sectse/contents.htm>

<sup>27</sup> Australian Health Ministers' Advisory Council Blood and Blood Products Committee (2003) Report of the working party on the supply and use of Factor VIII and Factor IX in Australia. On [http://www.nba.gov.au/pdf/factor\\_VIII\\_IX.pdf](http://www.nba.gov.au/pdf/factor_VIII_IX.pdf) p 62