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Directorate C - Public Health and Risk Assessment
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# SCIENTIFIC COMMITTEE ON EMERGING AND NEWLY IDENTIFIED HEALTH RISKS

# (SCENIHR)

## Opinion on

# The Safety of Human-derived Products with regard to Variant Creutzfeldt-Jakob Disease

Adopted by the SCENIHR
during the 11th plenary meeting of 11-12 May 2006
after public consultation

Synthesis Report : http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_vcjd_synth.pdf
Stakeholder comments : http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_vcjd_comments.zip

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## EXECUTIVE SUMMARY

In 2004 two instances were reported indicating the possible transmission of variant Creutzfeldt-Jakob disease (vCJD) by blood transfusion. In February 2006 a third instance of vCJD infection by blood transfusion was reported. This prompted a review of the current state of knowledge and practice of vCJD infection in relation to the safety of blood and blood components, including the evaluation of previous scientific Opinions of the Scientific Committee for Medicinal Products and Medical Devices (SCMPMD). The general conclusions and recommendations of the previous Opinions on the safety of human derived products including blood and blood components are still valid. However, two aspects do need our attention: 1) the possibility of transmission of vCJD by blood and blood components, and 2), the presence of asymptomatic vCJD infected individuals in the population who may be responsible for secondary transmission of the disease by blood/blood components or surgery.

While neither of the three transfusion-related vCJD infections reported is definitively proven to be caused by the preceding blood transfusion this is the most likely explanation. These three instances raise serious concern over the possibility of infection by blood transfusion (or through surgery and dental procedures) from asymptomatic preclinically or subclinically infected donors. Therefore, it is assumed that vCJD infectivity is likely to be present in human peripheral blood which is in accord with the results of recent work in experimental animals on transmission by transfusion. The present risk assessment of exposure to vCJD infectivity in whole blood and in blood components allows a rationale to define precautionary measures to reduce vCJD transmission within the human species by the intravenous or other routes.

While all the clinical cases of vCJD so far have been homozygous for methionine, (MM) at codon 129 of the prion protein (*PRNP*) gene, one of the three reported transfusion-related instances was heterozygous (MV). In addition, the results of the UK study evaluating anonymised appendix and tonsil surgical specimens, showed that vCJD infection might be more common than is suggested by the numbers of actual cases of vCJD to date. Two of the three positive samples could be evaluated for their genotype at codon 129 and were found to be of the VV genotype. It is therefore possible that following exposure to BSE, vCJD infectivity is present in a considerable number of individuals in the UK in an asymptomatic phase of the disease, including individuals with MV and VV genotypes. This poses an additional threat to the use of blood and other products of human origin as a potential source of secondary transmission. The potential transmission by blood raises concern, especially in view of the fact that routine screening with respect to vCJD is not (yet) possible. A possible iatrogenic transmission through surgical instruments used in invasive procedures also has important implications.

Considerable advances in test methodologies for prion diseases have been made in recent years. However, no diagnostic system has yet emerged with the level of sensitivity and specificity required for routine screening of blood or urine. It is essential that confirmatory assays are available for any assay proposed for large scale screening of donated blood. In addition, prior to introduction into routine practice, such assays should be independently assessed and validated for their analytical performance. Validation of any new methodology should be mandatory prior to introduction, and it is recommended

that the EU adopts a procedure similar to that used for the BSE testing. For validation, carefully controlled vCJD reference materials should be used. The issue of false positives needs especially careful consideration. Even minute percentages of false positives may actually involve a large number of individuals if the tests are performed on a large scale in the EU population, with a varying "prevalence" of asymptomatic carriers. The ethical implications of testing and informing an individual of a positive test result, without providing any certainty as to the likelihood of progression to clinical disease, should not be considered lightly.

Based on conservative assumptions made for the purposes of this risk assessment, there is a considerable risk that an asymptomatic donor infected with vCJD could cause infective material to be passed on to one or more recipients of blood or blood components. In the worst case scenario each therapeutic unit of blood from an asymptomatic infected donor could contain as much as 4500iv ID50. This amount of infectivity is deemed sufficient to cause transmission of the infection, with or without development of the disease. Considering that the donor population is much younger than the recipient population, with only a small overlap in age, preliminary data from one mathematical model indicate that blood transfusion alone will not be sufficient to maintain vCJD in the human population at large.

Taking into account the eligible blood donor population, and using the data of the UK appendix-tonsils study, the number of donations and the percentage of the population actually donating blood, up to 1250 infected donations may occur per year. As donations are typically split between 3 recipients, 3750 new infections would occur each year in the UK as a result of these infected donations in the worst case scenario. Transfusion statistics show that in general only about 50 % of blood recipients survive more than 3 years. Accordingly half of the blood recipients will not live long enough after transfusion to develop vCJD. If all the surviving recipients do develop disease, 1875 new individuals per year could develop vCJD in the UK population

The current decline in the onset of clinical vCJD in the UK and the general low number of cases in the older age groups who comprise the majority of blood recipients, indicate that this worst case scenario considerably overestimates transfusion-related vCJD disease development.

There are several possible explanations for this. It is possible that most infections have a very long incubation period so that the individual dies before disease develops, or that infections in some groups such as the MV heterozygotes or VV homozygotes are not associated with blood infectivity, or that different genotypes do not transmit efficiently to each other even if the unit is infectious. There are analogies in animal models for these scenarios, and they reflect the difficulty in making realistic estimates of the number of cases expected from blood transfusion. Taking the lower limit of the confidence interval of the prevalence from the UK appendix-tonsils study and assuming that only ten percent of infectious donations actually transmit the infectious agent, the number of infected donations resulting would be 9 per year in contrast to the 1250 predicted by the worst case scenario. Independent of the method of calculation transmission by blood transfusion may occur. Based on current data, the frequency cannot be reliably estimated, but even in the UK it is probably low. The frequency is largely dependent on the number of asymptomatic vCJD infected individuals in the general population which is likely to differ from one Member State to another.

Epidemiological studies, similar to the UK appendix-tonsils study, are needed to collect data on the presence of infection ($PrP^{Res}$) in the general population before estimations can be made on the possible frequency of contaminated blood donations in countries other than the UK. However, due to the rather low vCJD prevalence in other Member States there are considerable difficulties in the collection of such data, and alternative approaches for possible estimations of vCJD prevalence may therefore be used such as calculations based on BSE exposure.

There is no evidence that individuals working in hospital settings have developed vCJD by virtue of their profession. Transmission of TSEs during surgical or dental procedures remains a concern, but to date there is no evidence that it has actually occurred in relation to vCJD. To minimize the risk of transmission of vCJD by surgical instruments cleaning and inactivation procedures are recommended based on the probability of the patient under investigation/treatment being infected with vCJD (or any other TSE).

There are no proven instances of vertical transmission of any human prion disease. The available animal and human data are inadequate to allow firm conclusions concerning vertical transmission to be drawn. It is recommended that there is a follow-up for children that are born to mothers who had or developed clinical vCJD. There are no data indicating that breast milk transmits human prion disease.

In the absence of evidence on vertical transmission in man, the risk posed by the use of cord blood which is of fetal origin can be considered to be negligible. However, contamination with maternal blood during collection remains a possibility.

In conclusion, as long as there is a risk that infectious prion protein is present in blood and blood components, there will be a risk of transmission of vCJD disease by transfusion. Blood transfusion appears the most likely route for inter-human transmission of vCJD, although other routes of transmission should also be considered like surgery and organ or cell transplants.. The Committee does not consider that additional specific measures are needed to reduce the risk from vCJD infectivity in blood. In the UK and some other countries measures have already been taken including donor exclusion of blood transfusion recipients, leucodepletion, import of fresh frozen plasma, and reduction of amounts of plasma in blood components for transfusion. When there is a concern for spreading of vCJD by blood transfusion, donor exclusion of blood transfusion recipients is the appropriate measure. In addition, there are good practices to reduce any risk for transmission of infectious diseases such as optimal use of the transfusion to reduce the number of patients exposed, and optimal blood donation techniques and blood transfusion practices which minimize the number of blood donors to which an individual patient is exposed. The Committee recognises that it is important that Member States maintain the principle of regional blood supply structures, national surveillance systems and international information exchange at the EU level.

## 1. BACKGROUND

In July 2004, the United Kingdom announced that a second instance of probable vCJD transmission via blood transfusion had been identified. The patient received the blood donated by an individual who was confirmed in 2001 as a definitive vCJD case. Although, the receiver died of unrelated causes, and showed no clinical signs of vCJD at the time of death, abnormal prion protein was found in spleen tissue and a cervical lymph node by autopsy. This patient was found to be heterozygous for codon 129 of the *PRNP* gene and differed thus from the genotype found so far in patients who have developed the disease.

As a safeguard against the possible transmission of vCJD and in order to protect the blood supply the British Government had previously introduced a number of precautionary measures. This second instance has led to the introduction of further precautionary measures 'because of a small but unquantifiable risk', according to the Minister of Health, tightening the donor exclusion criteria.

The significance of the second instance of vCJD infection transmission by blood is that while all previous clinical cases of vCJD were genotyped as homozygous (MM) at codon 129 of the prion protein gene, this new instance was heterozygous (MET-VAL, MV) at codon 129. It is known, from studies of other prion diseases, that *PRNP* codon 129 genotype affects the susceptibility of individuals to disease and longer incubation periods are seen in acquired prion disease in non-MM genotypes.

The following considerations should be taken into account:

- A second human instance with evidence for vCJD infection gives further confirmation that vCJD can be transmitted by blood transfusion. The finding was consistent with the existence of a substantial risk associated with the receipt of non-leucodepleted blood from a donor incubating vCJD. The extent to which leucodepletion reduces that risk is not known. Published studies confirm that intra-species infection by blood transfusion is possible in sheep and non-human primates.
- This instance provides evidence that vCJD infection can be transmitted to heterozygotes, and is not just restricted to homozygotes. It is unknown whether vCJD disease develops in only MM homozygous individuals or if clinical cases will emerge in the other genotypes at a later time because of a longer incubation period.
- Although it cannot be known whether this individual would have eventually developed the disease, the finding of $PrP^{Res}$ is significant as it indicates that iatrogenic transmission by blood in the incubation phase (or in genuinely subclinical disease), through surgical instruments or by instruments used in invasive procedures can occur.

According to the UK Spongiform Encephalopathy Advisory Committee (SEAC) Statement of 7 August 2004, the Committee agreed inter alia that careful post-mortem examination of all recipients of blood (leucodepleted or not) from donors incubating vCJD would help to quantify the nature and magnitude of the risks of transmission of the vCJD agent through blood donated by preclinical cases of vCJD.

The Scientific Committee for Medicinal Products and Medical Devices (SCMPMD) issued its last Opinion on 'the Risk quantification of CJD transmission via substances of human origin' in February 2000 (SANCO/SCMPMD/2000/0005) and on the 'Safety of Human-derived Products with regards to TSEs' in January 2002 (SANCO/SCMPMD/2002/0001). The latter did not cover exclusion criteria for donors (covered in the opinion adopted 21.10.98) and 16.02.2000) nor the substitution with alternative products and is not up to date with regards to recent developments in the science e.g. in the infectivity in blood and transmissibility via blood.

In the light of the new findings, a request for an updated Opinion on the risk quantification of vCJD transmission by blood including an update with regard to recent scientific developments has been sought.

It should also be noticed that all previous opinions issued were related to the use of certain products and did not address other possible paths of transmission such as vertical transmission or the infectivity of umbilical cord cells stored in cell banks. Considering that there is a relatively high possibility that the onset of clinical signs of vCJD occur during the fertile period of humans, it is desirable to extend the scope of the request to include the two items.

The Committee for Medicinal Products for Human Use (CHMP) of the EMEA has recently issued a review of its 'Position Statement on Creutzfeldt-Jakob disease and Plasma-derived and Urine-derived Medicinal Products' (EMEA/CPMP/BWP/2879/02/rev1). Several recommendations and proposals related to vCJD were issued.

## 2. TERMS OF REFERENCE

In light of the recent evidence mentioned above, the Scientific Committee on Emerging and Newly Identified Health Risk (SCENIHR) is requested:

- to review the previous scientific SCMPMD Opinion on the 'Safety of Human-Derived Products with regard to TSEs' adopted on 18 January 2002, and the SCMPMD Opinion on 'Quality and Safety of Blood' adopted on 16 February 2000 in order to quantify, if possible, the nature and magnitude of the risks of transmission of the vCJD agent through blood donated by preclinical cases of vCJD, through surgical instruments or by instruments used in invasive procedures,

- to evaluate the risk of vertical transmission of vCJD in pregnant women and

- to evaluate the risk of vCJD transmission by the tissues stored in umbilical cord cell banks.

# 3. SCIENTIFIC RATIONALE

## 3.1 Introduction

The Directorate General Health and Consumer Protection of the European Commission has published several Opinions of the Scientific Committee on Medicinal Products and Medical Devices (SCMPMD) dealing with the safety of human-derived products with regard to variant Creutzfeldt - Jakob disease (vCJD) (SCMPMD 1999, 2000a, b, 2002). Concern over the possible transmission of variant Creutzfeldt - Jakob disease (vCJD) by blood products was raised by publications indicating the transmission of Transmissible Spongiform Encephalopathy (TSE) in animal models. While exposure to other tissues is possible, the largest exposure to products of human origin involves blood, either through transfusion of whole blood or components, through proteins derived from blood such as immunoglobulins, albumin or clotting factors, or by contamination of surgical instruments. This opinion relates to blood and blood components and not to plasma derived products.

The Opinions stated there was at that time no proof or disproof for vCJD infectivity in human blood. Also in animal studies TSE infectivity could not be reproducibly detected in blood. Thus the presence of vCJD infectivity in human blood could neither be excluded nor confirmed (SCMPMD 2002). Bioassays in laboratory animals are currently the only way to investigate the presence of TSE infectivity.

The risk was considered minimal, so no additional measures were proposed. On an individual basis, however, several Member States instigated donor deferral for people who had stayed for some time in the UK, while others did not.

Whereas the previous Opinions solely concerned themselves with blood, the current Opinion covers a wider area including possible transmission of vCJD by tissue and cells stored in cell banks and by the use of surgical instruments.

In 2004 two reports were published indicating the possible transmission of vCJD by blood transfusion (Llewelyn et al2004, Peden et al 2004).

Llewelyn et al (2004) reported on a survey including forty-eight recipients who were identified as having received blood from a total of 15 donors who developed vCJD. One of the recipients developed symptoms of vCJD 6.5 years after transfusion, although infection due to past dietary exposure could not be excluded.

Peden et al (2004) reported an individual who died from a non-neurological disorder 5 years after transfusion from a donor who subsequently developed vCJD. Protease resistant protein ($PrP^{Res}$) was detected in this patient by immunohistochemistry in the spleen and in a cervical lymph node. Interestingly, this patient was heterozygous at (MV) at the prion protein (*PRNP*) gene codon 129. All tested clinical cases of vCJD to date have been homozygous for methionine at codon 129 (MM).

Although both instances could not be definitively proven to be caused by the preceding blood transfusion, these two instances raise serious concern about the possibility of infection by blood transfusion from preclinically or subclinically infected donors especially in view of the fact that routine screening for vCJD is not (yet) possible. This concern was increased by the report of transmission of BSE in sheep blood transfusion experiments (Hunter et al 2002, Houston et al 2000).

This may have important implications for possible iatrogenic transmission by blood and/or cells and organs from donors in the incubation phase, and transmission through surgical instruments used in invasive procedures.

The evidence that potential vCJD infectivity may be present in heterozygotes at codon 129 poses an additional serious threat to the use of blood and other products of human origin, especially in view of the fact that routine screening for vCJD is not (yet) possible. This may have important implications for possible iatrogenic transmission by blood and/or cells and organs from donors in the incubation phase, and transmission through surgical instruments used in invasive procedures.

These observations resulted in the formulation of several questions (section 2) for the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) regarding the safety of blood and other human tissues in relation to the possible transmission of vCJD.

In addition, a third instance of possible transmission of vCJD infection by blood transfusion was reported on February 9th 2006 by the Health Protection Agency of the UK (HPA 2006).

## 3.2 Current status of knowledge on vCJD – overview

Variant Creutzfeldt-Jakob disease (vCJD) is a neurodegenerative disorder and belongs to the group of TSEs. The histological features of TSEs include vacuoles in the brain of the infected species, neuronal loss and gliosis, leading to dementing disease and death. The distinctive feature of TSEs is the accumulation of a particular abnormal (prion) protein in the brain. vCJD was first described as an independent disease entity, different from other forms of Creutzfeldt-Jakob disease (CJD) in 1996 (Will et al 1996). The great majority of yearly notified CJD cases arise without identifiable cause (sporadic CJD, sCJD). Another form of CJD (genetic CJD, gCJD, or familial CJD, fCJD) is associated with mutations of the prion protein gene (*PRNP)*. In addition, CJD can result from medical exposure to infectious material (iatrogenic CJD, iCJD). Different forms of CJD occur at different frequencies in different countries (especially vCJD) but sCJD affects approximately 1.5 to 2 persons per million/ population per year in all studied countries.

Current evidence indicates that vCJD is caused by the agent responsible for bovine spongiform encephalopathy (BSE). The ingestion of BSE contaminated food is the most likely transmission route and disease-induction mechanism of human vCJD (Hill et al 1997, Bruce et al 1997 ).

As of September 1st, 2005, 182 cases of vCJD have been reported, there of 157 cases in the United Kingdom, 3 cases in Republic of Ireland, 14 cases in France, 1 case in Italy, 1 case in Saudi-Arabia, 1 case in Portugal, 1 case in the Netherlands, 1 case in Spain, 1 case in the United States of America, 1 case in Canada and 1 case in Japan. The understanding of the nature of the infectious agent of vCJD and of the pathogenesis of vCJD is critical to the question of vCJD transmission *within* the human species by the intravenous or other routes.

### *3.2.1 The infectious agent*

The nature of the infectious agent causing TSEs has been discussed controversially for a long time (Soto and Castilla 2004). There is substantial scientific evidence in favour of the “protein

only" hypothesis. In 1967, Alper and colleagues proposed for the first time that TSEs might be caused by particles replicating without nucleic acid (Alper et al 1967). In 1982, Prusiner introduced the "protein only" hypothesis (Prusiner 1982): A protein that purifies with the scrapie infectivity was characterized in 1982 (Bolton et al 1982). The gene encoding the prion protein (PrP) was cloned in 1985. In 1988, it was shown that infectivity of the prion can be neutralized by anti-PrP antibodies. The first mutation in the *PRNP* gene associated with familial TSE was identified in 1989. In 1990, transgenic animals overexpressing mutant PrP were shown spontaneously to develop clinical and pathological signs of TSE. In 1993, PrP knockout mice were observed to be resistant to scrapie. Conformational differences between the physiological prion protein, $PrP^{C}$, and its pathological form, $PrP^{Sc}$, were reported in 1993 (for detailed references on the historical overview, see (Soto and Castilla 2004). The *in vitro* conversion of the physiological prion protein, $PrP^{C}$, into its pathological form, $PrP^{Sc}$, was reported in 2001 (Saborio et al 2001). The generation of *de novo* infectious prions from protein *in vitro* was reported in 2004 (Legname et al 2004), and in 2005 (Castilla et al 2005a), although in these cases alternative explanations of the origin of infectivity can not be excluded. In addition, the current protein only hypothesis fails to explain adequately all features of prion diseases, for example strain variation.

Different models currently exist for the conversion of $PrP^{C}$ to $PrP^{Sc}$ (Soto 2004). The infectious $PrP^{Sc}$ may occur as a small oligomer acting as a seed for recruiting, converting and stabilizing the misfolding of the physiological prion protein $PrP^{C}$ (nucleation-polymerization model). According to the template-associated conversion model, the main step of formation of the pathological prion protein is the formation of an intermediate state on binding to a molecular chaperone. The intermediate is proposed to interact with monomeric or oligomeric $PrP^{Sc}$, which acts as a template for its conversion. Consistent with both models, conformational transition of the prion protein from an alpha-helical isoform to a beta-sheet-rich isoform is now widely considered to underlie the pathogenesis of prion-related diseases at the structural level, making TSEs similar to other protein conformational disorders such as Alzheimer's disease (Baskakov et al 2004, Bucciantini et al 2002, Lundmark et al 2002, Soto 2001).

Barriers for prion transmission between different species exist, and different prion strains characterized by different biological properties exist (Bruce et al 1997, Tanaka et al 2005, Jones and Surewicz 2005). For example, prion proteins from BSE case can be distinguished from those from scrapie cases (Scott et al 2005).

In summary, the pathogenesis of prion-related diseases is currently widely considered to be related to abnormal protein folding. Normal cellular protein is converted into an insoluble, aggregated, beta-sheet rich form which is deposited in the brain. The mechanism of prion-related diseases is believed to involve disease transmission by replication of protein conformation. The accumulation of protease resistant prion protein in tissues is a characteristic feature of prion disease. vCJD is a typical prion disease in this respect.

#### *3.2.2 vCJD pathogenesis and the immune system*

*PRNP* polymorphism is a determinant of vCJD in humans. All tested clinical cases of vCJD to date have been homozygous for methionine at codon 129 of the prion gene. Wadsworth et al (2004) demonstrated that the generation of the vCJD phenotype in a particular transgenic mouse model required expression of human prion protein with methionine in position 129. Expression of human PrP with valine 129 resulted in a distinct phenotype that was associated with the persistence of a barrier to transmission of BSE-derived prions. In this mouse model, the polymorphic residue 129 of human PrP dictated propagation of distinct prion strains after BSE prion infection, suggesting that human infection with BSE-derived prions could result in

a variety of phenotypes of vCJD, depending on the genotype of the prion source and the recipient (Wadsworth et al 2004). Such complexity of phenotypic determination in human prion diseases has recently been confirmed by Pan and colleagues (Pan et al 2005). Whether certain MHC genotypes are associated with vCJD remains controversial (Jackson et al 2001, Pepys et al 2003, Laplanche et al 2003).

Besides a genetic influence on vCJD susceptibility, the cellular environment seems to be an important modifier of vCJD: vCJD prions typically accumulate in nervous and lymphoid tissues (Hilton et al 2004). In animal models of scrapie, proinflammatory cytokines and immune cells are required for lymphoid prion replication, and chronic lymphocyte inflammation enables prion accumulation in otherwise prion-free organs such as kidney, pancreas, or liver. Thus, chronic inflammatory conditions may act as modifiers of natural and iatrogenic prion transmission (Aguzzi and Heikenwalder 2005, Heikenwalder et al 2005). Urinary prion excretion was recently demonstrated in presymptomatic and in sick animals in a murine model for scrapie (Seeger et al 2005). It was dependent on specific intrarenal inflammatory conditions (i.e. presence of lymphofollicular foci). Prion excretion due to renal inflammation may be an explanation for the horizontal transmission of TSEs among various animal species like sheep, deer and elk (Seeger et al 2005).

Acquired disease pathogenesis is a dynamic process including infection, peripheral prion replication, and prion transmigration from the periphery to the central nervous system. Although the mechanisms of prion lymphoinvasion and neuroinvasion are not yet completely understood, many molecular details have been elucidated (Aguzzi and Heikenwalder 2005). The cellular basis for prion transmigration from the gut into the lymphoid system seems to depend on membranous epithelial cells (M cells) that may represent a site of prion entry. Evidence for such mechanisms, so far, comes from coculture models (Heppner et al 2001).

Within the lymphoid system, B cells are important for peripheral prion spread and neuroinvasion (Klein et al 1997). B cell effects on prion replication seem to derive primarily from the expression of cytokines such as the proinflammatory cytokine lymphotoxin α (= TNF beta). Within lymphoid organs, B cells contribute to the maturation and maintenance of pre-existing mature follicular dendritic cells (FDCs). FDCs accumulate $PrP^{Sc}$ after scrapie infection, and prion replication in spleen depends on $PrP^{C}$ expressing FDCs (for detailed literature, see (Aguzzi and Heikenwalder (2005). Defects in haematopoietic compartments, such as impaired B-cell maturation, or in stromal compartments, such as abrogation of FDCs, can delay or prevent lymphoreticular prion accumulation. Kaeser and colleagues studied the distribution of infectivity in splenic fractions of mice expressing the gene that encodes $PrP^{C}$ solely on haematopoietic or on stromal cells following intraperitoneal challenge with prions, and observed that optimal prion replication requires $PrP^{C}$ expression by both stromal and haematopoietic compartments (Kaeser et al 2001).

Under physiological conditions, FDCs contribute essentially to antigen presentation by antigen trapping via capturing of immune complexes by Fcγ receptors, and via the binding of opsonized antigens to the CD21/CD35 complement receptors, suggesting a role for complement components in prion-related diseases. Indeed, mice genetically engineered to lack complement factors (Klein et al 2001) exhibited enhanced resistance to peripheral prion inoculation. Consistent with this observation, Mabbott and colleagues observed that temporary depletion of the complement component C3 or genetic deficiency of C1q significantly delayed the onset of scrapie (Mabbott et al 2001). It is still unclear how prions reach FDCs, how they disseminate throughout the lymphoid system, and how they travel from FDCs to nerve endings, resulting in neuroinvasion.

Peripheral blood may be involved in propagation and transport of vCJD prions, although $PrP^{Sc}$ has not yet been detected in peripheral blood of vCJD patients (Wadsworth et al 2001). Currently, evidence of blood-borne infectivity in other forms of TSE exists and implies that mobile cells contribute to the spread of infectivity (Houston et al 2000, Llewelyn et al 2004, Peden et al 2004). Thus, lymphocytes were shown to acquire prion infectivity when residing in lymphoid organs (Raeber et al 1999), and may contribute to the prion load in blood. Consistent with this observation, blood infectivity in experimental systems could be related to the white cell content of blood components (Brown et al 1999, Brown et al 1998), and leucodepletion of cellular blood components for haemotherapeutic purposes is considered to reduce peripheral blood infectivity (Turner 1998, Turner 2000). However, it has been reported that 50% of infectivity in blood is not cell associated but is transmitted by the plasma (Gregori et al 2004). Dendritic cells may represent another type of immune cells that facilitate transport of prions from the periphery to sites of prion replication (Aucouturier et al 2001, Huang et al 2002). Direct penetration into the brain across the blood-brain barrier may occur (Soto 2004).

A completely different mechanism of prion transport in the peripheral circulation has been proposed by Fevrier and colleagues (Fevrier et al, 2004): they observed that both $PrP^{C}$ and $PrP^{Sc}$ were actively released into the extra cellular environment by PrP expressing cells before and after infection with sheep prions, respectively. $PrP^{C}$ and $PrP^{Sc}$ in the medium were associated with exosomes, membranous vesicles that are secreted upon fusion of multivesicular endosomes with the plasma membrane. Exosomes bearing $PrP^{Sc}$ were found to be infectious, suggesting that exosomes may contribute to intercellular membrane exchange and to the spread of prions throughout the organism.

Vaccination against the abnormal prion protein is challenging. Strategies for immunization against prion diseases represent a field of sincere efforts (Aguzzi and Sigurdson 2004). $PrP^{C}$ is a normal protein with yet unknown function and is present at the surface of a variety of cells including immune cells under physiological conditions (Cashman 2001). In addition, prion-specific antibodies are not generated during the course of prion infections. However, passive immunization with antibodies directed against the normal $PrP^{C}$ can prevent prion disease, and induction of humoral immune responses to $PrP^{C}$ or $PrP^{Sc}$ might be protective. Future research in the prion immunology field is likely to focus on the establishment of efficient diagnostic, prophylactic and therapeutic anti-prion concepts (Aguzzi et al 2004, Aguzzi and Sigurdson 2004, Heppner and Aguzzi 2004, Soto 2004, Weissmann and Aguzzi 2005). A successful vaccination should result in an immune response to either the normal or abnormal prion protein, protecting against disease without adversely affecting the functionality of normal $PrP^{c}$.

Taken together, current evidence on the pathogenesis of vCJD indicates that orally ingested prions enter mainly via the gut lymphoid tissue, and are transported to the other lymphoid organs. Prions are replicated in the periphery in lymphoid tissues such as the spleen, the appendix and tonsils, and are then transported to the brain mainly by peripheral nerves. Direct penetration into the brain across the blood-brain barrier may occur. Thus, peripheral blood may be involved in propagation and transport of vCJD prions, although $PrP^{Sc}$ has not yet been detected in peripheral blood of vCJD patients.

### *3.2.3 Distribution of infectivity and $PrP^{Sc}$ in CJD*

The broad picture of the distribution of infectivity and $PrP^{Sc}$ in cases of TSEs is that in general the highest titres in clinical cases are found in the brain and other nervous tissues while the major other types of tissue of concern are the lymphoid organs, such as the spleen. However the details vary depending on the species, the strain of agent, the individual case and its clinical status and possibly pathophysiological parameters (see

above on the effect of inflammatory cytokines). Specifically large amounts of $PrP^{Sc}$ may be found in organs such as the spleen, appendix and tonsils in cases of variant CJD but not in sporadic CJD. It has been shown by assays of infectivity in a mouse model that spleen from vCJD cases was infectious at a level about one thousand fold less than in brain (Bruce et al 1997). While it is possible that some infectivity or $PrP^{Sc}$ can be found in lymphoid tissues from cases of sporadic CJD, the scale is very different. It is also suspected that lymphoid tissue involvement in sCJD occurs relatively late in the illness, whereas it occurs preclinically in vCJD. Similarly in scrapie infected sheep more infectivity and $PrP^{Sc}$ can be found in the peripheral non-neurological tissues of certain genotypes than of others. There may be specific combinations of circumstances in which a tissue is more or less infectious than others.

Table 1 presents the reported distribution of infectivity and/or $PrP^{Sc}$ in vCJD and includes the other forms of CJD for comparison. It is consistent with data for other TSEs from both natural and model animal systems. Tissues are classified into high, medium and low infectivity, but it should be born in mind that detection is mostly based on the detection of $PrP^{Sc}$, and the quantitative relationship between $PrP^{Sc}$ and infectivity is not precisely established. A tissue with very little $PrP^{Sc}$ could therefore in theory be infectious.

**Table 1. Presence of abnormal Prion Protein and infectivity in human tissues**

| **Tissue** | **Presence of abnormal Prion Protein and level of infectivity** | | | |
|---|---|---|---|---|
| | **CJD other than vCJD** | | **vCJD** | |
| | $PrP^{Res}$ detected | Assumed level of Infectivity | $PrP^{Res}$ detected | Assumed level of Infectivity |
| Brain | + | High ***** | + | High ***** |
| Spinal cord | + | High ***** | + | High |
| Spinal ganglia | + | High | + | High |
| *Dura mater* | NT | High | NT | High |
| Cranial nerves | + | High | + | High |
| Cranial ganglia | + | High | + | High |
| Posterior eye | + | High ***** | + | High |
| Anterior eye and cornea | - | Medium | - | Medium |
| Olfactory epithelium | + | Medium | NT | Medium |
| Tonsil | - | Low | + | Medium * |
| Appendix | - | Low | + | Medium |
| Spleen and thymus | + | Low ***** | + | Medium ***** |
| Other lymphoid tissues | - | Low ***** | + | Medium ***** |
| Peripheral nerve | - | Low | - | Low |
| Dental Pulp | - | Low | - | Low |
| Gingival Tissue | - | Low | - | Low |
| Blood and bone marrow | NT | Low | NT | Low |
| CSF | - | Low * | - | Low |
| Placenta | NT | Low | NT | Low |
| Urine | NT | Low | NT | Low |
| Other tissues | NT | Low ***** | NT | Low |

**Key**: + = tested positive - = tested negative NT = not tested
***** = infectivity proven in experimental transmission studies

### *3.2.4 Evidence for the presence of vCJD infectivity in peripheral blood*

In February 2004, Llewelyn et al reported on the first instance of possible transmission of vCJD by blood transfusion in humans (Llewelyn et al 2004). The instance had been identified by the efforts of the UK National Blood Authorities and the National CJD Surveillance Unit (NCJDSU). Forty-eight individuals were identified by the NCJDSU as having received a labile blood component from a total of 15 donors who later became vCJD cases and appeared on the surveillance unit's register. One of these recipients was identified as developing symptoms of vCJD 6.5 years after receiving a transfusion of red cells donated by an individual 3.5 years before the donor developed symptoms of vCJD. A second instance of possible transmission of vCJD infection by blood transfusion in humans was reported in August 2004 (Peden et al 2004). Pre- or sub-clinical vCJD was reported in a patient who died from a non-neurological disorder 5 years after receiving a blood transfusion from a donor who had subsequently developed vCJD. Protease-resistant prion protein, the typical feature of prion disease, could be detected in the spleen and in a cervical lymph node, but not in the brain. The patient was heterozygous (MV) at codon 129 of the *PRNP* gene providing the first evidence in humans that susceptibility to vCJD infection is not confined to the methionine homozygous *PRNP* genotype (MM).

A third instance of transmission of vCJD infection by blood transfusion was reported on February 9th 2006 by the Health Protection Agency of the UK, providing further evidence that vCJD can be transmitted between humans by blood transfusion (HPA 2006). This third instance makes it almost certain that transmission of vCJD via blood transfusion occurs in humans.

These indications of the presence of vCJD infectivity in human peripheral blood are in line with experimental data proving blood borne transmission of TSEs in animals. In 2000, preliminary evidence indicated the sheep-to-sheep transfusion transmission of BSE (Houston et al 2000). Such evidence was confirmed in preclinically infected and clinically affected animals in 2002 (Hunter et al 2002). In 2004, BSE transmission in primates was demonstrated to be at least as efficient by the intravenous route as by the intracerebral route (Herzog et al 2004).

### *3.2.5 Conclusions*

The current evidence indicates that infectivity is present in the peripheral blood of humans infected with vCJD. The risk assessment of exposure to vCJD infectivity in whole blood and in blood products allows a rationale to define precautionary measures to reduce the risk of vCJD transmission within the human species by the intravenous or other routes.

## 3.3 Review of the TSE Detection Methods

A detailed review of potential screening methods for the detection of vCJD or other TSE diseases was not included in previous opinions of the EU Scientific Steering Committee. This opinion will therefore provide a summary of the current state of the art as it relates to TSE detection.

Given the continuing debate around the nature of TSE agents, for the purposes of this review it will be assumed that the Prion Protein (PrP) is essential for infectivity, and that the infectious aggregated form of this protein ($PrP^{Sc}$ for $PrP^{Scrapie}$) is closely associated with the principal agent responsible for transmissibility, and therefore serves as a suitable marker for infectivity. It should be noted that methods for $PrP^{Sc}$ detection usually involve detection of $PrP^{Res}$, the protease resistant core. For the purposes of this review, $PrP^{Res}$ and $PrP^{Sc}$ are often used as equivalent terms. To date $PrP^{Sc}$ remains the only disease specific marker, and this assumption underpins most of the development efforts aimed at developing diagnostic tests for the detection of TSE agents.
A distinction should be made between detection methods amenable for rapid large scale screening, and tests that are useful in confirming initial diagnoses. A considerable effort has been expended in attempts to develop ante-mortem tests for prion agents as such tests could provide a possible risk reduction measure for screening purposes. Other less rapid detection methodologies remain important, and still play an important role in confirming diagnosis, characterising strain differences and estimating levels of infectivity.

Rapid and effective diagnostic procedures for large-scale screening rely on the availability of easily collectable samples for analysis (i.e. blood or urine) as well as a screening assay with the required level of sensitivity combined with a high level of specificity. In the UK, examination of routinely removed lymphoid tissues has found $PrP^{Res}$ positivity typical of that found in vCJD in three out of 12,674 appendices and tonsils that could be evaluated (Hilton et al 2004), but such a survey was performed at some considerable effort, and using samples not suitable for general routine screening (since it requires appendicectomy). Low levels of sensitivity will lead to false negative results, whereas a low specificity will result in large numbers of false positive results, with ensuing ethical difficulties associated with how such results are handled. Ethical issues are apparent even if a specific and sensitive assay would be available as there is no cure for the disease.

The levels of TSE infectivity likely to be present in the blood of individuals with vCJD are likely to be very low (section 3.2.3). Ante-mortem screening tests, to be useful as a risk reduction measure, must be capable of identifying individuals prior to the onset of clinical disease, at a time where potential TSE infectivity will be lower than during the clinical phase of disease. Where infected brain contains levels of infectivity during the clinical phase of disease in the order of $10^6$-$10^7$ IU/ml (an infectious unit is defined as the lowest concentration of agent required to initiate infection when inoculated using the optimal bioassay system; i.e. intra-cerebral inoculation in the same species), sensitivities in the range of 1-10 infectious units per ml will be required for testing of blood. The low limit of detection required represents a formidable challenge, especially in a complex matrix such as blood.

### *3.3.1 Biochemical assay methods for the detection of $PrP^{Sc}$*

All commercial test kits currently available for the detection of the $PrP^{Sc}$ require proteolytic digestion of the sample prior to detection of any residual $PrP^{Sc}$ (which has a partial resistance to proteolytic digestion). The digestion is required to remove any normal PrP, which might otherwise interfere with the subsequent immunological detection of $PrP^{Sc}$. Assays have also been developed which do not require proteolytic digestion (e.g. the Conformational Dependent Immunoassay, or CDI assay, Safar et al

2002). The CDI assay relies on substantial enhancement of immuno-reactivity of $PrP^{Sc}$ following protein denaturation, mediated through the exposure of "buried" epitopes.

Assays, which do not rely on proteolytic digestion prior to detection of $PrP^{Sc}$, are likely to demonstrate a higher level of sensitivity, although exceptions to this rule have been reported (Bellon et al 2003). The $PrP^{Sc}$ protein is not completely resistant to proteolytic digestion, and the degree of resistance is strain specific (Kuczius and Groschup 1999). There may also be $PrP^{Sc}$ forms within the same sample with varying resistances to proteolytic digestion (Minor 2004, Yakovleva et al 2004). The relevance of these various $PrP^{Sc}$ forms to infectivity is not clear, and has only further complicated the development of any diagnostic test that relies on proteolytic digestion prior to detection. An additional complication is that assumptions derived from observations in animal systems, may not be applicable to disease in humans.

To circumvent the issue of potential loss of signal following proteolytic digestion, attempts have been made to generate monoclonal antibodies that detect conformational epitopes present only on $PrP^{Sc}$ (Korth et al 1999) or antibodies that are targeted at epitopes expressed on the surface of $PrP^{Sc}$ but not on the normal form of the PrP protein (Paramithiotis et al 2003). The applicability of such antibodies in routine diagnostic testing is still under investigation, and test systems have been reported utilising such approaches.

The sensitivity of most immunological assays for the detection of $PrP^{Sc}$ is such that most would not be suitable for screening of blood. An improved CDI system has reported levels of sensitivity approaching that necessary for the detection of $PrP^{Sc}$ in blood, and is comparable to the detection limit of vCJD and CJD in transgenic mice carrying the human prion gene (Bellon et al 2003, Safar et al 2002). The sensitivity of detection in transgenic mice, however, cannot be correlated to human infectious units, and thus this level of sensitivity may not be sufficient for routine screening. An evaluation of the limit of detection of this test using WHO vCJD reference materials is currently ongoing.

The concentration of $PrP^{Sc}$ by techniques such as phosphotungstic acid precipitation (Wadsworth et al 2001) may be useful in any diagnostic test (Bellon et al 2003). The initial report that concentrations of $PrP^{Sc}$ can be "amplified" through a process of repeated protein denaturation and subsequent refolding (Protein Misfolding Cyclic Amplification: PMCA) (Saborio et al 2001, Saa et al 2005) has been repeated in other laboratories, and although initial attempts to show a corresponding increase in infectivity were unsuccessful, the *in vitro* generation of infectivity has recently been claimed (Castilla et al 2005a).

Shaked et al (2001) reported the detection of $PrP^{Sc}$ in the urine of hamsters, bovines and humans in the clinical stages of TSE infection although intra-cerebral inoculation of the urine associated $PrP^{Sc}$ in hamsters did not result in disease transmission. The possibility that the $PrP^{Sc}$ signal observed in urine may originate from cross-reacting protease resistant enterobacterial proteins has been raised (Furukawa et al 2004). A recent report provides evidence that this signal is in fact due to immunoglobulin light chain fragments rather than $PrP^{Res}$ and the test proposed by Shaked et al (2001) did not discriminate between cases and controls in a blinded study (Head et al 2005). As indicated in section 3.2.2 it was reported very recently that urinary prion excretion could take place in a murine model of scrapie under conditions of lymphofollicular renal inflammation

(Seeger et al 2005). However, the authors were unable to detect $PrP^{Sc}$ by biochemical methods although the urinary proteins were able to induce scrapie in indicator mice.

### *3.3.2 Infectivity methods for the detection of TSE agents*

Animal infectivity assays remain the definitive standard for the measurement of TSE agents, but the long incubation times, cost and use of large numbers of animals precludes their use in routine screening. Confirmation of TSE infection in the animal bioassay is by histopathology and the identification of characteristic spongiform changes or amyloid deposition. In addition, confirmation by detection of $PrP^{Sc}$ is commonly used. This may be done by immunohistochemistry or immunological assays such as immunoblotting. Recent data have indicated that using a sensitive immunoassay provides a higher level of sensitivity for disease confirmation than immunohistochemistry or standard histology (Safar et al 2005).

Most animal bioassay systems are only optimally sensitive for prions originating from the same species. The species barrier that exists when transmitting prion agents from another species (Hill and Collinge 2002) further limits the sensitivity of the test for screening human samples. Transgenic mice engineered to express the human prion gene have resulted in increased sensitivity for the detection of human prion agents (Korth et al 2003), but such systems are not amenable for use in routine screening, and have sometime yielded unexpected results (e.g. increased susceptibility to sCJD and decreased susceptibility to vCJD in the same model) (Taguchi et al 2003).

Animal infectivity assays remain an important tool in a number of areas of prion biology:

- In the characterisation of new strains of agent. Strain differences manifest themselves in different incubation times, different neuropathological lesion profiles (Bruce et al 1997) or different protein/glycosylation profiles (Collinge et al 1996). To date there is one particular protein/glycosylation type that has been uniquely associated with BSE and the associated infections of the animals and humans (Head et al 2004). Such assays can therefore provide important information when analysing unknown or newly emerging strains of TSE agents.

- In the characterisation of reference materials. Such reference materials are used in assessing new diagnostic technologies e.g. titres of vCJD reference materials in the human PrP transgenic mouse model will enable a determination of the limits of detection for biochemical assays (Safar et al 2002).

- In the confirmation of biochemical in vitro assays. For example when prion reduction studies are performed using only biochemical determinations of $PrP^{Sc}$.

The route of inoculation plays an important role in transmission in animal bio-assay systems, and in general intracerebral inoculation is the most efficient route followed by intraperitoneal, intravenous and oral routes respectively. Recent studies in primates have cast some doubt on the view that intravenous inoculation is less sensitive than intracerebral inoculation (Herzog et al 2004), but additional work is needed to determine if this effect is consistently observed in the animal system used in this study.

*In vitro* tissue culture systems for the detection of TSE infectivity have been described, and for levels of sensitivity comparable to that observed in the animal bioassay, the selection of specific sub-populations of cells sensitive to infection and replication appears necessary (Klohn et al 2003). Other publications have suggested that such selection may not be necessary (Vorberg et al 2004), and that indeed a wide selection of cell lines is susceptible to TSE infection, but a detailed analysis of the limit of detection was not performed in these studies. Tissue culture systems have the advantage of speed (~3 week assay time) and could be potentially automated. The systems to date have only been described for the detection of rodent adapted scrapie strains, but hold out hope that comparable systems could also be developed for the detection of human TSE agents.

### *3.3.3 Surrogate markers of TSE disease*

Efforts have been expended to identify markers other than $PrP^{Sc}$ that are associated with progression to clinical disease or found during clinical disease. A number of candidate surrogate markers have been proposed, including blood levels of endothelium derived relaxing factor (EDRF) or erythrocyte associated relaxing factor (EARF) (Miele et al 2001).

Elevated concentrations of 14-3-3, a cytosolic protein present in high concentrations in mammalian brains, are found in the cerebrospinal fluid (CSF) of patients with sporadic Creutzfeldt-Jakob Disease (sCJD) (Hsich et al 1996). In the appropriate clinical setting, the detection of elevated CFS 14-3-3 has a high degree of accuracy for the diagnosis of sCJD (Zerr et al 1998). Other proteins such as tau protein, a microtubular protein, are also elevated in sCJD and may also be a useful surrogate marker (Otto et al 1997). CSF 14-3-3 is less useful in vCJD, where only half the patients have elevated levels, however elevated concentrations of tau protein have been found to have a high sensitivity and specificity for this condition (Green et al 2001). Phosphorylated tau protein is also raised in both vCJD and sCJD, but the diagnostic accuracy of phosphorylated tau is no better than that of total tau protein for either condition (Goodall et al 2005).

Continued research is needed with such surrogate markers, using demographically defined population groups, to determine the predictive diagnostic value in the general population. Care also needs to be exercised when translating results observed in animals to humans, as pathophysiological responses may differ between species.

### *3.3.4 Other methods for the detection of TSE agents*

TSE diseases result in spongiform changes in the brain, with the subsequent alteration of brain function, brain activity and visible signs that manifest during the clinical phase of disease. The assessment of clinical symptoms remains an extremely important tool in the diagnosis of vCJD, and in distinguishing vCJD from other forms of TSE. In support of clinical assessments, Magnetic Resonance Imaging (MRI) of the brain is now the principal test in the diagnosis of vCJD (Zeidler et al 2000, Zeidler et al 2001), and different MRI appearances usually distinguish between sCJD and vCJD. The characteristic MRI changes may occur early in disease (Shiga et al 2004). Such methods currently provide strong support for the diagnosis but are not in themselves diagnostic tests. Tonsil biopsy is a helpful test in suspected cases of vCJD where diagnostic doubt exists. It rests on the involvement of the lymphoreticular system and the detection of

disease-associated PrP, however it is an invasive procedure and useful only as a supportive clinical diagnostic test (Hilton et al 2004ab, Hill et al 1997*)*

### *3.3.5 Tests in Development for Blood*

The development of assays for TSEs based on blood samples has been a focus of activity for commercial and other groups for some time and is apparently making real progress (Brown 2005). Currently the most promising approaches aim to detect $PrP^{Sc}$. Few of the relevant studies have appeared in the peer reviewed literature because of commercial interests although some of the enabling technology has been published. The applications of the technology have been presented at meetings and in general the level of detail available is less than would be required for a scientific publication. It seems probable that in the very near future detection of infected individuals in the preclinical and subclinical phases through testing blood samples will be possible. For the purpose of this opinion it was decided to review the methods, bearing in mind that the list may be incomplete and the supporting information inadequate.

Early approaches to assays of very low amounts of $PrP^{Sc}$ included immunocapillary electrophoresis (ICE) (Schmerr et al 1999) in which samples were digested with proteinase and residual PrP was detected by competition with a synthetic peptide for binding to a specific antibody. The key features were the concentration of the blood sample and resolution of the complexes by capillary electrophoresis. The method was technically complex and proved difficult to reproduce on human and chimpanzee samples (Cervenakova et al 2003). A modified method has been published, but is not yet validated (Yang at el 2005). SIFT (Screening for Intensely Fluorescent Targets) is based on the fact that because $PrP^{Sc}$ aggregates, it presents a higher number of antibody binding sites than the unaggregated normal form, so that fluorescent intensity in immunoassays is more intense for $PrP^{Sc}$ (Bieschke et al 2000). While this is a sensitive method in the detection of known infectious material such as brain samples, the current state of development is not known and it has not apparently been applied to blood.

As already discussed above, CDI under the right conditions is very sensitive (Safar et al 1998, Safar et al 2005, Bellon et al 2003), and its suitability for the assay of blood is currently being explored.

A palindromic ligand has been developed by Adlyfe which converts from the alpha helical to beta sheet form in response to interactions with $PrP^{Sc}$. The transition is detected by fluorescence, and the method is sensitive (Grosset et al 2005). It has been reported to distinguish samples from infected and uninfected laboratory animals, sheep and cows (Pan et al 2004). A ligand (Seprion) has been developed by Microsens which is reported to bind specifically to $PrP^{Sc}$ of any species and to distinguish blood from infected and uninfected sheep (Lane et al 2003). It has also been reported to distinguish blood from an iatrogenic case of CJD from controls (Wilson 2004).

An antibody, 15B3, reported to be specific for $PrP^{Sc}$ (Korth et al 1999) has been used in the development of methods for the detection of $PrP^{Sc}$ in blood (Zwald et al 2004). Finally, the cyclic amplification of $PrP^{Sc}$ *in vitro* (PMCA) has been reported to be sufficiently sensitive to detect prions in blood from the preclinical phase (Castilla et al 2005b, Soto et al 2005).

#### *3.3.6 Conclusions*

Considerable advances in test methodologies for prion diseases have been made in recent years, and the application of these advances in the diagnosis of vCJD has, in particular, been fruitful. However, no diagnostic system has yet emerged with the high level of sensitivity and specificity required for routine screening of blood or urine. Care must be exercised in applying conclusions derived from animal experiments to the situation in humans.

As with screening tests approved within the EU for BSE, test validation of any new methodology should be mandatory prior to implementation. Given the scarcity of vCJD material, a stepwise approach should be followed, where only those tests which have attained a prescribed level of sensitivity and specificity for example in carefully controlled animal experiments, are allowed to proceed into testing with vCJD reference materials. This approach will ensure that any test implemented will generate meaningful and interpretable data suitable for risk management decisions in relation to vCJD. Options have been presented using a combinatorial approach, where individuals identified as positive using a low specificity test could be subsequently screened using other tests intended to either validate or invalidate the first positive result. The use of such approaches warrants extreme caution, and the ethical issues of informing an individual of such results, without providing any certainty as to the likelihood of progression to clinical disease, should not be considered lightly. Quite aside from the likely emotional impact on individuals with a positive test, decisions will be needed as to what level general public health protection measures should be taken (e.g. if such individuals require dental treatment or surgical operations).

It is essential that the assays are independently validated, confirmatory assays are available and all ethical implications are considered and carefully taken into account before implementing testing. It is recommended that assays are approved by EU similar to the situation for BSE testing.

### 3.4 Evaluation of current epidemiological data

The epidemiological data reviewed here include the incidence/prevalence of vCJD, the basic case characteristics, studies of risk factors and observational data related to possible instances of secondary transmission. This necessitates a review of disease surveillance systems as well as specific research approaches such as case-control studies.

#### *3.4.1 Surveillance Systems & Methodologies*

Epidemiological data on vCJD depend fundamentally on case ascertainment and, therefore, on the nature of CJD surveillance systems and their methodologies. Efficient and organised disease surveillance is vital to proper risk evaluation and risk management of CJD. Complete case ascertainment for vCJD requires surveillance of all forms of human prion disease, different forms of CJD may be sometimes clinically similar and require careful evaluation.

**Background**

The present UK surveillance system was established in 1990 and first described vCJD in 1996. Aside from identifying CJD cases and collecting detailed relevant data, the

NCJDSU (National CJD Surveillance Unit) undertakes a variety of research projects including a case-control study. Suspect cases of all forms of CJD, including vCJD, are referred to the NCJDSU. Wherever possible, cases are visited in life and all clinical and investigation data are collected. Cases are also identified through neuropathology and death certificates. There is a separate paediatric surveillance system, established to identify vCJD cases in children. Cases are classified according to the accepted diagnostic criteria (WHO 2002). A detailed face-to-face interview of the family along with an examination of hospital and General Practice (Primary Care) notes allows recording of relevant data including surgical operations and blood donations/treatments. The relatives of all vCJD cases and appropriate controls are interviewed using a standard protocol.

Other EU member states have established CJD surveillance systems; while these vary in structure, there are good grounds for expecting that cases of vCJD would be promptly identified. There are established collaborations within the EU with harmonization of methodologies. In particular three collaborative groups have received EU-funding: EUROCJD began in 1993, NEUROCJD began in 1996, and SEEC in 2001. Collaborative research is now continuing under the umbrella of Neuroprion (a Framework VI, DG Research funded project). There is an established system of reporting of cases from countries of European Union to DG Health and Consumer Protection.

**Clinical identification**

The core of any system is the clinical identification of cases of vCJD. The absence of a specific, clinical, non-invasive test requires the involvement of clinical neurologists in case identification and classification. There are established clinical diagnostic criteria that are both sensitive and specific. (WHO 2002, Will et al 2000).

**Neuropathological identification**

A definite diagnosis of vCJD requires neuropathology, usually via autopsy, but sometimes via brain biopsy. Neuropathological expertise and a high post mortem examination rate are, therefore, important, especially in clinically doubtful cases. However, overall autopsy rates are low in many countries (in the UK, for hospital deaths, around 5%) and especially in neurodegenerative/dementing illnesses in the older age groups. In suspected prion disease, the rates have been substantially higher, but, in the UK, they have declined in the last few years. Some CJD autopsy data in the UK are given in Figure 1.

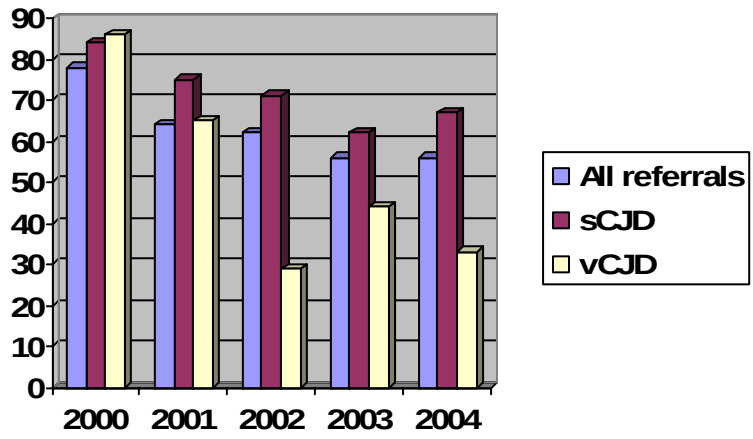


**Figure 1. Autopsy rates (% of cases) in the UK 2000-2004 for all referrals of suspect CJD cases, definite & probable sCJD cases, definite & probable vCJD cases**

Clearly, relatively low and declining autopsy rates are a concern in relation to complete case ascertainment, especially in relation to atypical cases, atypical age groups and the identification of any newly emerging disease phenotypes.

**Lymphoreticular surveillance**

There is involvement of lymphoreticular system (LRS) tissue in vCJD and this can be (indeed, perhaps, must be) pre-clinical. There are two reported instances of definitive pre-clinical LRS involvement. In one, vCJD symptoms developed about 8 months, and in the other, 2 years after a routine appendectomy. In both, the appendix specimen was later obtained and found to be positive on PrP immunohistochemistry. In a further case, an appendix removed 9 years prior to vCJD onset was negative for $PrP^{Sc}$ (Hilton et al 2004a, Hilton 1998, Ironside 2000). Appendices and tonsils are fairly frequently surgically removed and their analysis for vCJD-PrP allows a form of lymphoreticular surveillance. There is one published study, reporting on anonymised routine appendicectomy and tonsillectomy samples (details below) and results from a tonsillectomy study are pending (Ironside 2000, Hilton et al 2002, Hilton et al 2004b). In addition, there was a recently reported individual in whom $PrP^{Res}$ was found in the spleen and a cervical lymph node (Peden et al 2004).

**Case-control studies**

Case-control studies allow an analysis of potential risk factors and there is one current study of vCJD (being undertaken in the UK). The study has analysed data from over 100 cases. To date (September 2005) there are provisional data to support the dietary theory of vCJD and no other risk factors (including surgery and blood transfusion) have been identified in the case-control study (Ward et al 2006).

**Retrospective Reviews**

There have been retrospective clinical and pathological reviews aimed at determining whether cases of vCJD were missed prior to 1996. The UK National Retrospective Review undertook a pathology-based review from 1970 onwards without, to date, any reported previously missed cases of vCJD but the final report is awaited. A death-

certificate based retrospective study covering England between 1979 and 1996 and a similar study covering Wales between 1985 and 1995, did not identify any previously missed vCJD cases (Majeed et al 2000, Hillier et al 2002).

### *3.4.2 Current Variant CJD Figures*

**UK data**

As of September 2005 157 cases of definite and probable vCJD were identified in the UK . It is always possible that cases have been missed but it is very unlikely that large numbers have been overlooked. The UK has a comprehensive state-based health system, an established, active, well-funded surveillance system and there has been intense interest in vCJD with a high level of awareness of the condition in both medical services and the general population. The retrospective reviews discussed above have not identified previously 'missed' cases. Figures from the UK are reviewed on a quarterly basis and the analyses placed on the NCJDSU's website (www.cjd.ed.ac.uk). Since 2003 the analyses have shown a statistically significant decline in cases; the current best fit model to the data is a quadratic one with a peak in the number of deaths in 2000 (see Figure 2).

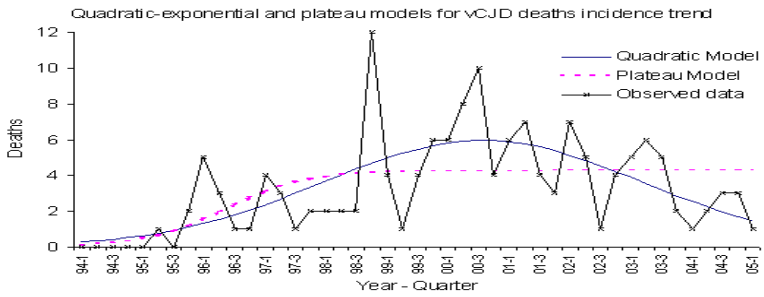


**Figure 2. UK May 2005 Quarterly Analysis**

The current incidence in the UK is estimated at 1.5 deaths/quarter and a total of 5 deaths is predicted in the twelve months from March 2005 (95% prediction interval of 1 to 11). (NJ Andrews, www.cjd.ed.ac.uk).

The age-distribution of the UK vCJD cases is notable for three reasons. Firstly, the relative youth of cases needs explanation. The UK mean age at death is 30 (median: 28, range: 14-74) and at onset is 28 (median: 26, range: 12-74). The details are given in Figure 3.

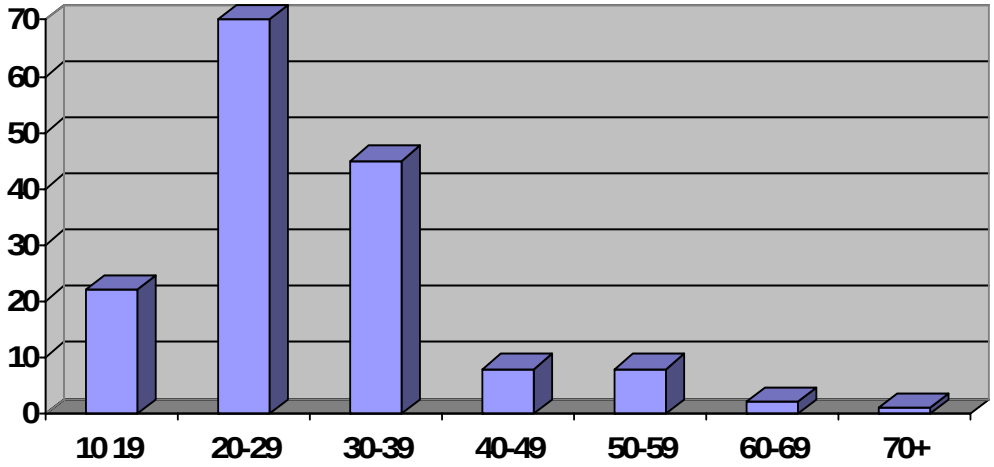


**Figure 3. Age Distribution of deaths due to vCJD in the UK (first 156 cases)**

Secondly, the mean age of cases has not changed significantly over the course of the epidemic and this also needs explanation.

Thirdly, vCJD occurs in an age group that is likely to contain most blood donors.

The age-data suggest the possibilities of age-related exposure to infection, age-related incubation periods and age-related susceptibility. There is no definitive explanation at present but age-related exposure is not thought likely to be the complete explanation. Age-related susceptibility is plausible; for example, gut-associated lymphoid tissue decreases with age and is a likely portal of infection entry.

**Data from other countries**

In other countries, the figures at 1 September 2005 are: France 14, Republic of Ireland 3, Italy 1, Netherlands 1, Portugal 1 and Spain 1. Outside Europe, one case has occurred in each of the following countries: USA, Canada, Japan and Saudi Arabia. It is important to understand that cases are attributed to countries on the basis of their country of residence at the time of their developing symptoms and this does not necessarily mean that the infection was acquired in these countries; on present evidence, one of the Irish cases, the American, Canadian and Japanese cases are thought to have acquired infection whilst in the UK. It is impossible to provide a definitive statement about the completeness of case ascertainment in all countries. It is highly probable that cases occurring in EU countries would be identified, if they presented to neurological services, given the long-standing collaborative systems described above.

Appropriate surveillance systems are dependent on the continued funding within Member States. The declining incidence in the UK and the lower number of cases (to date) in many EU countries might lead to reduced interest and support in some countries. This would be premature particularly considering the increasing numbers of vCJD cases outside the UK and would clearly undermine confidence in future epidemiological data. Autopsy rates are also of importance as discussed above.

### *3.4.3 Modelling & Predictions of vCJD figures*

There have been several publications attempting to estimate future numbers of cases of vCJD on the basis of past cases; these are discussed below.

The UK appendicectomy and tonsillectomy study was a different approach to the prediction of future numbers of cases and produced a result potentially at variance with those from the quarterly case data analysis. In this study, 3 appendices out of 12,674 lymphoreticular tissue samples (mostly appendices, but also included tonsils) examined were found to be positive for disease-related PrP. This equates to a LRS positive prevalence of 237 per million of the UK population (95% confidence interval (CI) of 49-692). Therefore the worst case scenario assumes there are 41 520 affected individuals, based on a population of 60 million. 83% of the appendicectomies were carried out in the 10-30 age group. The study results equate to 3,808 LRS positive individuals in this age group in the UK (95% CI: 785-11,128). (Hilton et al 2004a). Subsequently, two of these individuals were identified as having PRNP 129 VV genotype while it was not possible to identify the genotype of the third individual (Ironside et al 2006).REF. Given the performance of the currently available assays these data should be treated with caution: the results may be under or overestimates. However, as immunohistochemistry is not the most sensitive method for the detection of $PrP^{Sc}$ an underestimate is the most likely.
Considerations of what may happen in other countries have usually been based on levels of imports of potentially relevant materials (from the UK) and the distribution of cases of BSE.

An essential underlying assumption of these predictive studies has been that vCJD in humans is due to exposure to BSE infectivity in the diet. There is a considerable body of data to support this assumption (Will 1989, Hill et al 1997, Bruce et al 1997, Knight 1999). Figure 4 shows the temporal relationship between the BSE and vCJD epidemics in the UK.

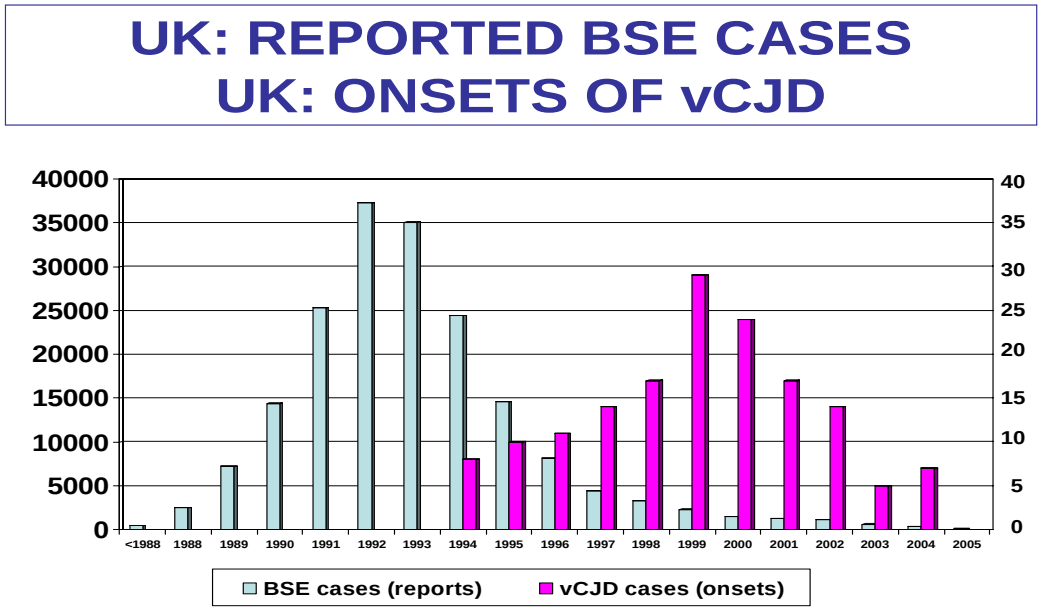


**Figure 4. Reported BSE cases (DEFRA) and vCJD onsets in the UK 1980-2005 (NCJDSU, June 2005)**

## General Review of possible future of vCJD

The published prediction papers have produced a variety of figures, reflecting differing assumptions and methods, given the many uncertainties and unknowns. Over time, however, the estimated maximum number of cases has tended to fall and the range tended to narrow (Figure 5).

Future predictions are obviously at least partly based on the assumption that there is no continuing extensive exposure to BSE.

As detailed above, the UK quarterly analysis estimates the current vCJD incidence at 1.5 deaths/quarter and a total of 5 deaths is predicted in the twelve months from March 2005 (95% CI of 1 to 11). (NJ Andrews, www.cjd.ed.ac.uk).

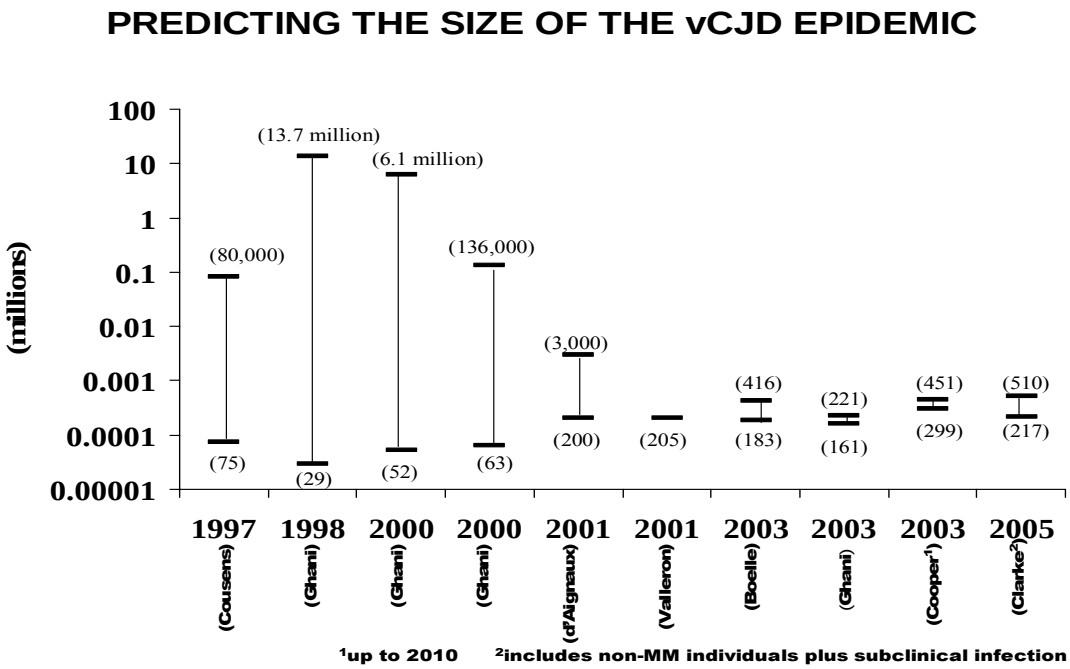


**Figure 5. Published estimates for the prediction of the size of the UK vCJD epidemic.**

*Note: The different predictions have often used different methodologies and different assumptions. The different figures and ranges above are not necessarily directly comparable. The graph is therefore simply to illustrate the general fall in the predicted numbers over time and the general approximate agreement of recent estimates.*

A further publication (Ghani et al 2003) gave the best estimate of future cases as 40 (95% CI: 9-540) based on the number of clinical cases at that time.

However, while these data are relatively reassuring, there are a number of caveats:

- All cases, to date, have been in *PRNP*-129 MM individuals (133 tested)
- Other *PRNP* 129 genotypes may be affected with different incubation periods (the report of lymphoreticular prion involvement in an MV blood recipient, and the two VV genotypes in the appendix-tonsil study are relevant to this consideration).
- There may be other, presently unrecognised, genetic susceptibility/incubation period determinants
- Secondary transmission by blood or surgery may occur (there are three instances of vCJD infection after transfusion, making it virtually certain that transfusion associated transmission occurs).
- The incubation period (and its variability) of vCJD is unknown and this has implications for possible future numbers. There are publications attempting to estimate the incubation period of dietary-vCJD and the effects of this on future numbers of cases.

In addition, the figures from the UK appendix-tonsils study are perhaps less reassuring. The paper of Ghani et al (2003) gave a best estimate of future cases of 100 (rather than 40) if the appendix-tonsils population data are included in the analysis (and the 95% prediction interval becomes 10-2,600 rather than 9-540).

However, there are a number of considerations:

- The confidence intervals in the appendix study are wide
- The *PRNP*-129 genotype of 2 of the 3 positive individuals has been determined VV. VV homozygotes comprise approximately 11 percent of the UK population
- There may be subclinical cases of human BSE infection (i.e. infected, with lymphoreticular positivity, but not developed into clinical vCJD). This would have an impact on the number of cases of vCJD itself, but would potentially lead to an increased risk of inadvertent secondary transmission (in the absence of a reliable pre-clinical/sub-clinical test).

As far as other countries are concerned, any predictions are yet more problematic. Cases reported to date have not exactly mirrored BSE cases. The occurrence of cases in certain places such as Sicily, Saudi Arabia and Japan are potentially concerning.

### *3.4.4 Epidemiological approaches to secondary transmission*

#### 3.4.4.1 General approaches

Any assessment of possible secondary transmission must take into account non-epidemiological data (such as laboratory experimental studies of infectivity and transmission). However, it can be difficult to know how much one can extrapolate from such data to the human population situation and, therefore, epidemiological approaches are potentially valuable. Obviously there are possible problems including ethical considerations and the long incubation periods associated with such events.

The number of individuals with potentially infective tissues is an important background for secondary infection and this issue has been addressed above. Clearly, there are greater opportunities for secondary transmission from preclinical or subclinical cases than from clinical cases.

#### 3.4.4.2 Identification of possible instances of exposure

Surveillance systems should identify instances of possible risk of secondary transmission from recognised cases of vCJD, but currently there is no means of identifying pre-clinical or subclinical cases. Incidents relating to preclinical cases may be identified retrospectively, after the clinical development of disease. Investigation of such cases is conducted in the UK surveillance system, covering all possible considerations including blood and surgery.

During organised surveillance, vCJD cases are identified and detailed medical histories may be obtained. Such data can give indications of likely problems, both specific and general. For example, if the infected individual had an appendicectomy or donated blood shortly prior to vCJD onset, then others specifically at risk may be identified. Alternatively, to gauge the general nature of the problem, it is useful to know how many vCJD cases did undergo surgery or donate blood during a relevant risk period. However, these data need further refinement as indicated below. The data that are available will

depend on the particular surveillance methodology. In the UK, detailed past medical history, including blood donations and blood treatments are recorded in each case.

**The follow up of potentially exposed individuals**

Direct follow-up of individuals who have been exposed to a potential risk requires them to be notified of the exposure and their consent to be reviewed with the attendant ethical issues.
An alternative is the recording of names of those potentially exposed and passing on these data to surveillance systems that then wait to see if these individuals are later identified as cases. There are ethical issues here as well. The UK TMER study (see below) initially followed the second course, but potentially exposed individuals may now be informed of the incident, depending, to some degree, on the nature of the exposure.

**Case-control studies of risk factors**

Case-control studies can evaluate risk factors such as exposure to infection via surgery and blood. There are no current data from the UK vCJD case-control study to implicate either of these (Ward et al 2006). The reported findings were consistent with dietary exposure to contaminated beef products being the main route of infection of vCJD. However, the potential incubation period of vCJD must be kept in mind.

**Clustering analysis**

If cases of vCJD result from point exposures to infection via surgery, then clustering of cases would be expected. Thus use of affected labile blood components might also give rise to clustering of cases, but the distribution of blood-derived products is potentially wide. The analysis of spatiotemporal clustering of cases is a very complex matter. Presently, there are only three verified specific concentrations of cases of vCJD:

- The particular involvement of certain countries (essentially UK & France), thought to reflect BSE and not secondary transmission.
- The higher incidence of cases in the north of the UK compared to the south. The basis of this is uncertain, with no convincing data to implicate factors such as dietary differences. There is no evidence to suggest it is based on secondary transmission factors.
- The ‘Leicester cluster’. This is the only statistically significant cluster of cases around a UK city. The basis is uncertain, but it has been attributed to local butchering practices and there is no evidence to suggest it is based on secondary transmission factors.

Continuing surveillance and analyses may provide further data in the future.

3.4.4.3 Vertical Transmission

Vertical transmission is a particular concern, especially given the relatively young age group affected by vCJD. Again, any considerations must take into account experimental evidence, but also again, there are issues concerning the valid extrapolation of such data to the human situation and epidemiological approaches are also needed. The issue of

possible vertical transmission in cattle BSE is discussed elsewhere in this report (section 3.6.3) but, currently, there are no data to support an important role for such transmission.

**Current epidemiological evidence for vertical transmission**

Children have been born to mothers with prion disease (including vCJD), both within any expected incubation period and during clinical illness. In the UK, 114 children have been born to vCJD cases (in 60% the affected parent was the mother). Currently there is no reported instance of prion disease resulting in the offspring. (Knight, personal communication, NCJDSU 2005).

**Follow up of children born to affected mothers**

There are four ways of following children who have been born to affected mothers: they could be formally reviewed on a regular basis; their medical records could be 'flagged' in some way; their names could be kept by a surveillance system in case they later present as a case; there could be no formal system but instead a reliance on an established surveillance system to identify them and their family history if they developed prion disease.

3.4.4.4 Surgery

There are two aspects to possible exposure to infectivity by surgery: accidental exposure related to recognised vCJD cases and the general risk from unrecognised cases (missed diagnoses, pre-clinical and subclinical cases). Only the first aspect is considered here.

**Follow up of individual incidents**

There are ethical considerations concerning the identification and follow-up of individual potential exposures, as discussed above. In the UK, the surveillance system should successfully identify any exposure instances and the development of vCJD in the exposed. There are no reported instances of surgical transmission at present, but the likely long incubation period of vCJD should be kept in mind.

The practical management of any specific incidents is managed by the CJD Incidents Panel (part of the Health Protection Agency) in the UK.

**Case-control studies**

The UK case control study considers surgery as a potential risk factor; so far there is no evidence to implicate surgery.

**Organized studies**

Organized studies trying to identify specific surgical procedures and their possible spatio-temporal relationship to other individuals and their procedures are logistically (and, potentially, ethically) complex. There are no such studies in the UK concerning vCJD.

3.4.4.5 Blood

The potential risks of blood (labile components) and of (fractionated) blood components are dealt with in section 3.6.

**Blood data from routine vCJD surveillance**

Routine disease surveillance can provide some general data; this is of limited value but can be helpful. For example, some general evaluation of potential risk can be made from simple data such as the number of blood donations made by vCJD cases and the timing of such donations with respect to disease onset.

**Blood as a risk factor in case-control studies**

Blood and blood components are potential risk factors considered in the UK case-control studies. There is, currently, no evidence that they are risk factors for vCJD (Ward et al 2006).

**UK Transfusion Medicine Epidemiological Review (TMER)**

The UK Transfusion Medicine Epidemiological Review (TMER) study was set up in 1997 as collaboration between the NCJDSU and the UK National Blood Authorities (NBAs). Essentially, the NCJDSU identifies cases of vCJD, obtains a detailed blood donation history and passes names of relevant cases to the appropriate NBA(s). The relevant NBA traces the donations and their fates and provides the NCJDSU with names of the recipients which then checks to see if any have developed or develop vCJD (Tables 2 and 3). There is also a 'reverse study' concerning vCJD patients who have a history of receiving blood. This study concerns labile components.

**Table 2. vCJD donor summary** ("www.cjd.ed.ac.uk/TMER, September 2005)

| **vCJD DONOR SUMMARY** | **157 vCJD cases** |
|---|---|
| Eligible to donate | 147 |
| Reported by relatives to have donated | 31 |
| Numbers where donor records traced* | 23 |
| Numbers from whom components actually issued | 18 |
| Numbers of recipients identified | 66 |

** donor records were traced on three individuals where the relatives had reported the case had not been a donor*

**Table 3. Use of blood from vCJD donors** ("www.cjd.ed.ac.uk/TMER, September 2005)

| **USE OF BLOOD FROM vCJD DONORS** | **157 vCJD cases** |
|---|---|
| Red Cells | 27 |
| Leucodepleted red cells | 25 |
| Buffy-coat reduced red cells | 2 |
| Fresh frozen plasma | 3 |
| Fresh Frozen Plasma (leucodepleted) | 2 |
| Whole blood | 2 |
| Cryo-depleted plasma | 1 |
| Cryoprecipitate | 1 |
| Platelets (pooled) | 1 |
| Platelets (pooled, leucopdepleted) | 2 |

In addition, 9 vCJD donors contributed plasma to 23 plasma pools identified by UK fractionators (BPL & PFC) as having been used for the manufacture of plasma products prior to 1999.

There have been three individual instances of note:
1. Two possible clinical cases of vCJD transmission by blood.
2. One possible instance of transmission of vCJD-infection (but not vCJD)

CASE 1
A 62 year old patient was transfused with 5 units of red cells during surgery in 1996. One unit had been donated by a 24 year old who developed vCJD symptoms 3 years and 4 months following donation (later dying of neuropathologically confirmed vCJD). In 2002, 6.5 years after the transfusion, the recipient developed symptoms of vCJD and the diagnosis was later neuropathologically confirmed (*PRNP* codon 129 MM). The recipient had lived in the UK during the identified dietary risk period but a statistical analysis determined that the chance of this being a dietary case was between 1:15 ,000-1:30 ,0000.

The affected donor had donated another unit of blood, the red cells of which were transfused to a patient who died of cancer only 5 months after the transfusion. The platelets from this donation were included in a platelet pool that has not been traced to a recipient. Plasma from both donations was included in two different plasma pools for the production of fractionated plasma products (Llewelyn et al 2004)

CASE 2
In 1999, an elderly patient received a unit of non-leucodepleted red blood cells from a donor who had developed symptoms of vCJD (later, neuropathologically confirmed) 18 months after donation. The recipient died 5 years after the transfusion with no evidence of any neurological disorder. As the recipient had been identified as a recipient of potentially vCJD-infected blood, an autopsy was undertaken with specific analyses to detect vCJD. The brain showed no evidence of vCJD, but the spleen was positive for $PrP^{Res}$ with the protein type being typical for vCJD and distinct from the types associated with sporadic CJD. Positivity was found also in a cervical lymph node, but not in tonsil, appendix nor dorsal root ganglion. This therefore represents an instance of either preclinical or subclinical vCJD in a recipient of potentially infected blood. The individual was found to have the *PRNP* codon 129-MV genotype (Peden et al 2004).

CASE 3
On February 9$^{th}$ 2006, the HPA reported on a clinical case of vCJD approximately 8 years after receiving a blood transfusion from a donor who later developed vCJD. This person had been previously identified as one of the group of people who are known to have received blood from infected donors (HPA 2006).

**Mathematical modelling**

Data on the presence and treatment of an infection in the population may be used for modelling disease development (Duerr et al 2005, UK Collaborative Group on HIV

Drug Resistence 2005). Such an approach was used for estimation of the fate of vCJD in the population and the risk whether the infection could establish itself (Dietz, personal communication, 2005). In this model data on the blood donors and blood recipients were taken into consideration including the age distribution of both populations. In addition, some assumptions were made on the behaviour of vCJD itself. The model describes the spread of an infection by blood donation after it was introduced into the population via the alimentary route. It predicts that an infection could not become endemic by transfusion alone and that only few cases could be avoided by excluding donors with a transfusion history. The most likely explanation for this is the age dependent effects in the model, i.e. the difference in age distribution between the blood donors, who are generally younger, and the blood recipients, who are generally older (Dietz, personal communication 2005).

However, the SCENIHR is aware that other unpublished models have reached different conclusions (Minor, personal communication 2006).

#### *3.4.5 Problems & Possible Deficiencies in Current Systems*

The complete ascertainment of all cases of vCJD depends on good clinical neurology services and good national surveillance systems. These are mainly dependent on factors internal to each country, but EU-funded collaborative projects have been of enormous value. The declining UK vCJD figures, the lower prediction estimates in recent studies and the non-involvement of many individual countries may lead to lessened interest, reduction of funding and, therefore, less efficient surveillance.

It is important to note that the clinico-pathological phenotype of human prion disease reflects not only the nature of the relevant agent (in this case BSE) but also the genotype of the individual and, potentially, other factors (such as mode of acquisition). Therefore, surveillance systems must be aware of the possibility of new phenotypic expressions of human BSE infection. Current clinical diagnostic criteria are based on the characteristics of previously described cases. Neuropathological studies are of critical importance; relatively low and declining autopsy rates are a potential concern.

Predictions of likely numbers of future cases are limited by the uncertainties in the data that underlie these predictions, such as the incubation period of vCJD and the nature of possible disease susceptibility factors.

#### *3.4.6 Conclusions*

The incidence of vCJD in the UK is decreasing but there remain considerable uncertainties and concerns over future numbers of cases.

While other countries have not been involved to the same degree, France continues to identify new cases and new countries have been affected.

With the control of BSE in cattle and the precautions taken to prevent BSE infected material to enter the human food chain, there remains the issue of controlling secondary human-to-human transmission.

There is recent evidence to implicate blood as a potential means of secondary transmission.

Transmission via surgery remains a concern, but to date there is no evidence that it has actually occurred.

Vertical transmission has not yet been shown to be a means of secondary transmission of vCJD. The monitoring of vCJD cases, vigilance for any new phenotypes of human BSE infection and the identification of any actual secondary transmission events are the necessary underpinnings for all predictions and policies; laboratory experiments are crucial but these cannot replace observation and epidemiological studies of human populations.

Predictions of one mathematical model shows that it is unlikely that a vCJD infection could establish itself in the population by blood transfusion only.

### 3.5 Evaluation of prion decontamination procedures for surgical instruments

The countermeasures implemented in response to the BSE epidemic are expected to be effective in preventing further spread of this disease to humans from cattle, thereby minimizing the risk of new primary vCJD infections. However, additional challenges for public health in the context of TSEs arise from the hypothetical as well as the established risks of human-to-human transmission of vCJD and other forms of CJD, respectively (Brown et al 2001, Taylor 2003, Beekes et al 2004, Llewelyn et al 2004). The experience with iatrogenic CJD, of which 267 cases were reported until July 2000 (Brown et al 2000), and the detection of infectivity or $PrP^{Sc}$ in a variety of tissues from vCJD patients in addition to the brain and spinal cord (e.g. lymphatic system and peripheral nervous system, Table 1) (Bruce et al 2001, Wadsworth et al 2001, Hilton et al 2002, Haik et al 2003, Ramasamy et al 2003) have led to the formulation of national and international recommendations and guidelines aimed at the prevention of iatrogenic transmission of these diseases (Simon & Pauli 1998, World Health Organization 1999, Abschlussbericht der Task Force vCJD 2002, ACDP/SEAC 2003).

In hospitals, it is of the utmost importance to avoid the spread of TSE infectivity via surgical instruments (e.g. by single use or cleaning, followed by chemical disinfection, or sterilization (Beekes et al 2004, Sehulster 2004). The potential risks are increased by the fact that $PrP^{Sc}$ has also been detected in skeletal muscles of patients with sCJD, albeit at low levels (Glatzel et al 2003). Infectivity is present in lymphoid tissues, and possibly blood, during the preclinical phase of vCJD (Hilton et al 1998 and 2002, Llewelyn et al 2004, Peden et al 2004). A model for testing of cleaning and disinfection effectiveness of prion-contaminated instruments was described by Fichet et al. (2004).

#### *3.5.1 Cleaning*

Instruments are cleaned before decontamination to remove tissues and fluids. Cleaning of surgical and diagnostic instruments may be difficult because of the shapes and construction of surgical instruments, where the first priority is obviously suitability for surgical use, not ease of cleaning. There may therefore be parts of the instruments which are hard to reach. Instruments that can be disassembled for cleaning, or are designed to have fewer inaccessible parts may help in the future. Studies have implied that in the UK at least, contamination remaining after cleaning is common; an estimate used in risk

assessments has been that 10 mg of tissue may remain on a single instrument after routine cleaning (UK Department of Health 2005). For instance in a recent study lymphocytes were observed on 30% of the investigated single use laryngoscope blades (Hirsch 2005). In addition, proteinaceous material may remain on instruments after standard cleaning practices (Miller 2001) and this is of particular importance in prion diseases. At this moment there is no validated cleaning process available for instruments that might be contaminated with TSE agents like vCJD.

Cleaning or sterilisation procedures may not be consistently applied to high standards. Studies in the UK indicated that in an initial survey only 9% of hospitals investigated reached the appropriate standards in cleaning and 58% in sterilisation. Following extensive investigations, investment and audit, these figures rose to 68% and 93% respectively (UK Department of Health 2005). It is not known how general these findings are, but they suggest that the issue of implementation may be important. One solution has been to propose that single use instruments be used. For complex instruments this is impractical, and the expense involved for many simpler instruments is likely to be prohibitive.

### *3.5.2 Decontamination and sterilisation*

The high resistance of TSE agents to conventional methods of chemical or thermal inactivation and to UV or ionizing radiation makes decontamination difficult. (Alper et al 1966 and 1967, Brown et al 1982 and 1986, Kimberlin et al 1983, Taguchi et al 1991, Tateishi et al 1991, Ernst & Race 1993, Taylor et al 1994, Manuelidis 1997, Taylor 1999, for review see Taylor 2000). Following exposure to high titre TSE preparations, a considerable residual infectivity can be detected bound to the surface of the steel (Zobeley et al 1999, Flechsig et al 2001), which may affect inactivation procedures so that specific consideration must be given to decontamination procedures in the reprocessing of surgical instruments (Rutala & Weber 2001). The transmission of CJD by neurological probes despite repeated cycles of cleaning and sterilisation is well known.

Solution studies on the inactivation of prion agents by a variety of agents demonstrate a biphasic inactivation kinetics, with a rapid inactivation phase followed by a slower second phase (Brown et al 1986, Ernst & Race 1993, Tateishi et al 1991, Taylor et al 1994, Taylor et al 1999). Most inactivation occurs during the initial rapid phase, and it was proposed that the slower second phase resulted from the presence of a subpopulation of prion agent more resistant to inactivation. Additional studies have since demonstrated that qualities of the spike preparation can have a dramatic effect on the capacity of a given inactivation solution to inactivate prions. Procedures which result in ”fixing” of the prion agent, such as drying, treatment with organic solvents or crosslinking with aldehyde based disinfectants results in an increase in the proportion of prion protein demonstrating resistance to inactivation (Taylor 1999). The fixation of the agent probably modulates resistance to inactivation through either preventing access to the inactivation solution (i.e. drying) or by preventing denaturation of the aggregated prion protein (i.e. aldehyde fixation).

The observation of increased resistance to inactivation following certain procedures has implications for the disinfection of surgical instruments suspected of CJD contamination. Allowing instruments to dry before or after the cleaning process, or

treatment with organic solvents or fixing agents prior to disinfection is likely to increase the proportion of prion agent resistant to disinfection. On the other hand, procedures capable of reducing protein load at equipment surfaces are likely to considerably increase accessibility of any remaining infectivity to the inactivating agent. In a recent study, bone surfaces devoid of any visible protein or fat deposits, yet containing still 6 logs of prion infectivity, when treated with 0.3M NaOH at ambient temperature resulted in complete inactivation of the remaining infectivity (Taylor 2003). In this instance and in contrast to solution studies, no resistant prion sub-population was observed. Similar results have also been observed with steel wires exposed to prions and washed prior to exposure to inactivating agents (Flechsig et al 2001), reinforcing the importance of cleaning combined with decontamination. Gas plasma treatments used in cleaning metal surfaces industrially have shown promise (Baxter et al 2005). However it must be stressed that the validation of processes for the removal of infectivity from solid surfaces is extremely difficult. While some of the approaches proposed seem promising, no process has yet been validated in accordance with accepted scientific procedures.

Treatments that are considered to result in complete decontamination include use of 1-2 M NaOH solution (for 1h), NaOCl solution (20,000 ppm available Cl, for 1h) as well as 3, 4 or 6 M GdnSCN solution (for 24h, 1h or 15 min, respectively), or steam sterilization at 134°C for 18 min to 1h with the material immersed in water (Simon & Pauli 1998, World Health Organization 1999, Hörnlimann et al 2001, Fichet et al 2004, ACDP/SEAC 2003). Combinations could also be effective such as autoclaving with NaOH or enzymatic pretreatment (ACDP/SEAC 2003, Taylor et al 1999, Yan et al 2004).

Such stringent conditions are hazardous to both equipment and operators. While they are mandatory for the reprocessing of instruments used in patients with or at risk from CJD they do not offer an option for the routine maintenance of surgical instruments used on patients without a recognizable risk of human TSE. Here, generally applicable decontamination strategies that take into account the theoretical risk of CJD and vCJD transmission from asymptomatic carriers on the one hand, without compromising the conventional processes for cleaning, disinfection and sterilization on the other, are required.

In routine situations it is necessary to use an effective yet instrument-friendly procedure. Some experiments were performed using tests on animals implanted with contaminated steel wires as originally described by Zobeley et al (1999) and Flechsig et al (2001). The use of various decontaminating reagents was investigated showing a decrease of infectivity (Beekes et al 2004). However this correlation needs to be carefully validated as proposed by McLeod et al (2004) who obtained slightly different results with the same agents.

The following conclusions are currently considered justified:

- NaOH (1M for 1 h) and NaOCl (20,000 ppm for 1h) have a strong decontaminating activity as previously established by comprehensive infectivity studies (Kimberlin et al 1983, Brown et al 1986, Taylor et al 1984, Taylor 2000, Flechsig et al 2001, Rutala & Weber 2001, Fichet et al 2004).
- Certain alkaline cleaners, supplemented with some minor additional components have shown promising activity. This requires further study and a careful

examination of the formulation and effect of commercial products (Lemmer et al 2004, Baier et al 2004, Fichet et al 2004).
- The efficacy of 4 M GdnSCN and 5% SDS is confirmed (Flechsig et al 2001, Taylor 2004, Lemmer et al 2004).
- Combinations of treatments could also be effective such as autoclaving with NaOH or a combination of enzymatic cleaning and autoclaving (ACDP/SEAC 2003, Taylor et al 1999, Yan et al 2004).)
- The inefficacy of 4 M urea is confirmed (Brown et al 1986, Prusiner et al 1993, Lemmer et al 2004). Other chemicals/methods listed as ineffective are: alcohols, ammonia, β-propiolactone, chlorine dioxide, dry heat, ethylene oxide, formaldehyde, formalin, glutaraldehyde and related compounds, hydrochloric acid, hydrogen peroxide, iodophors, peracetic acid, phenolics, sodium dichloroisocyanurate, 10,000 ppm sodium hypochlorite, and ionising/UV/microwave radiation (ACDP/SEAC 2003).

Thus associations of SDS (sodium dodecyl sulphate) & NaOH, or peracetic acid & NaOH may be of interest, but more tests both *in vitro* and *in vivo* must be performed. (Baxter et al 2005)

*3.5.3 Conclusions*

- No procedure for the decontamination of surgical instruments has yet been adequately validated to the extent that its universal introduction can be recommended. However, state of the art cleaning should be used as it is a prerequisite to ensure subsequent inactivation methods.
- Disinfectants with fixative properties such as those containing aldehydes must not be used for decontamination of instruments suspected to be contaminated with TSEs as they tend to stabilise rather than inactivate prions.
- Drying of instruments before cleaning or decontamination is likely to reduce the effectiveness of the decontamination procedure
- The implementation of whatever procedures are introduced should be monitored.

## 3.6 Risk assessment on transmission of vCJD

Conventionally a risk assessment comprises the following stages:
- exposure assessment
- hazard identification
- hazard characterization
- risk characterization

This is then followed by an evaluation by the risk manager of the acceptability or otherwise of the risk.

This framework has been followed here while particular emphasis is given to exposure assessment. Although the nature of the hazard is already largely known, there are still a number of uncertainties which remain.

Where fairly reliable quantitative data are available they have been used. However for many of the risk factors discussed above no suitable quantitative information exists. The normal approach in a risk assessment for public health purposes is to make a

conservative/worst case assumption in such circumstances. It must be recognized that if a series of worst case assumptions are adopted the final conclusion may be far removed from the real risk. Since the hazards have been well addressed elsewhere, the risk assessment will focus on the possibilities for exposure to the infectious agent by various routes and treatments.

*3.6.1 Risk assessment for transmission of vCJD by blood or blood components*

3.6.1.1 Identification of risk factors

Before carrying out a risk assessment it is necessary to identify the risk factors involved in the transfer of blood from a blood donor infected with vCJD to a recipient(s). These risk factors include:

A: EXPOSURE FACTORS

i) Number of blood donors with vCJD as a proportion of all blood donors
ii) Likelihood that that the vCJD donor will not be identified prior to their blood entering the blood bank
iii) Level of vCJD material in the donors blood (degree of infectivity)
iv) Dilution/partition factors for the vCJD blood prior to being given to an individual/ group of individuals
v) Number of times blood or blood components are administered to a sensitive individual
vi) Potential for bioaccumulation of the infectious agent
vii) Route of exposure

B: HAZARD FACTORS

viii) Susceptibility of individuals to vCJD by blood or blood components
ix) Presence of other factors favouring the development of vCJD

A: EXPOSURE

**Number of blood donors with vCJD as a proportion of all blood donors**

Blood and blood components tend to be used in the same geographical area in which they are donated. The number of blood donors with vCJD is likely to vary from country to country over a range of two or more orders of magnitude. The highest proportion of blood donors with vCJD is likely to be in the UK.

A distinction needs to be made between the number of individuals in a country with vCJD infection and those with a clinical disease. The number of those with the frank disease appears to be very small even in the UK. It is possible that the prevalence of those with vCJD infection is substantially higher than those with overt disease.

If we base our estimate of the number of infected individuals on the number of clinical cases to date, then the final number is unlikely to be much greater than several hundreds (if we base our estimate of the number of infected people on the results of the UK appendix-tonsils study, then the total number might be as high as several thousands)

(Hilton et al 2004a). About 4 % of the eligible population in UK donates blood in any one year (DNV 2003).

**Likelihood that that the vCJD donor will not be identified prior to their blood entering the blood bank**

The donors of concern are unlikely to be expressing the symptoms of vCJD and will therefore not be identified by routine assessment. Currently there are no available validated laboratory tests to screen potential donors for blood borne CJD prions. At present, therefore such donors can not be identified.

In some countries individuals are excluded from giving blood if they have had a previous transfusion in the UK or have even visited the country for a period during the BSE epidemic. Also people who have ever received blood are now excluded in several Member States. Although the approach may be considered a reasonable one, it is difficult to estimate the risk reduction achieved by these exclusions. The results of a recently developed mathematical model indicate that the exclusion of blood recipients from donation does not result in a considerable reduction of the predicted number of vCJD cases in the model employed (Dietz, personal communication 2005).

**Level of CJD material in the donors' blood (degree of infectivity)**

The level of TSE infectivity in the blood of animals and humans is likely to be many folds lower than that in brain. Levels of infectivity of vCJD probably vary from individual to individual depending, among other factors, on how long they have been incubating the disease. The amount of prion that needs to be transferred to a susceptible patient in order to initiate CJD is uncertain. It is assumed that there is a threshold dose below which no infection will occur. This is not proven for man.

Rodent experiments suggest that the level of infectivity in blood in the late preclinical stage is about ten infectious units per ml when measured by intracerebral inoculation (Cervenakova et al 2003a, Brown et al 1999, Brown et al 1998).This figure is consistent with transmissions of scrapie and BSE by transfusion experiments in sheep. Transmission has also been reported in primates, consistent with infectivity at a level of the same order. These experiments are subject to the reservation that infectivity may be different by different routes.

A unit of blood is about 450ml and could therefore contain 4500 infectious units (see 3.6.1.2). Even if the infectivity were 100 fold less a recipient of a whole unit would be exposed to an infectious dose.

In principle a vCJD donor could give whole blood every three months over several years, although in practice for the UK the average number of donations per year is only 1.2 per person (DNV 2003) There is no reason to believe that a preclinical or subclinical vCJD donor would donate blood less frequently than a non-vCJD donor. It is unknown how infectivity changes during the incubation period. In rodent models it gradually increases from about one third through the incubation period, and in the sheep transfusion experiments infection was transmitted by donations taken about half way through the incubation period.

**Number of times an individual may receive contaminated blood or blood components**

It remains uncertain whether in humans there is a cumulative effect of repeated exposure and if so whether there is a critical interval beyond which a second dose of prions will not supplement the effects of the initial exposure. This issue is not considered relevant because a single donation from an infected individual is thought to contain more than one infectious unit.

However, for certain blood components within one transfusion exposure to multiple donors may occur. As the number of donors is a risk factor the number of donors should be limited. An example is platelet transfusion in which apheresis techniques should be used, allowing harvesting of a sufficient dose of platelets from one donor to be transfused to one individual patient.

B: HAZARD

**Susceptibility of individuals to vCJD via blood and blood components**

As relatively few individuals have contracted vCJD it is not surprising that the individual risk factors are not well characterized. Key considerations from a risk assessment point of view are:

- Is there such a thing as a susceptible individual i.e. one who for genetic or environmental reasons is substantially more vulnerable to contracting vCJD than the population at large. And if so is the extent of susceptibility fixed or does it vary substantially with time?

All individuals who have developed disease so far have been homozygous for methionine at codon 129 of the prion gene *PRNP*. If MM homozygosity at codon 129 is the single determinant for vCJD and exposure leads inevitably to disease, about one third of the UK population would theoretically be at risk. The frequency of MM homozygosity within the EU is generally comparable to that observed in the UK (Nurmi et al 2003). However, an individual with codon 129 MV genotype was found to have vCJD infection (Peden et al 2004, section 3.2.4, 3.4.4), and two individuals with vCJD infection were identified as VV homozygotes at codon 129 in the appendix-tonsils study (Ironside et al 2006, section 3.4.3). vCJD has affected relatively young people, the reason for this is unknown, but various factors (section 3.4.2) have been suggested. However, these do not appear to be relevant for blood transfusion.

A large proportion of the blood used is given to older patients and people with serious illness. Most recipients of blood or blood components are therefore unlikely to fulfil criteria to donate blood in the future for reasons of age or the nature of the disorder for which they are treated. Because of this use of the blood, it seems unlikely that a transmission chain by blood components will be sustained. In this opinion, an overall survival rate of blood recipients is estimated to be 50% over three to five years after transfusion (Wallis et al 2004, Dietz, personal communication, 2005).

3.6.1.2 Quantitative risk assessment for transmission of vCJD by blood or blood components

The term "blood donation" as used throughout this risk assessment means whole blood and blood components collected from an individual and intended for transfusion to another individual. The current state-of-the art in hemotherapy requires transfusing blood components instead of whole blood. When blood is donated as whole blood, it is separated into the different components *in vitro*. Alternatively, the blood components may be obtained from the donor using apheresis techniques. 'Apheresis' is the method of obtaining one or more blood components by machine processing of whole blood in which the residual components of the blood are returned to the donor during or at the end of the process (Commission Directive 2004/33/EC). It should be realised that after every separation technique residual material of other components are present.

Blood components for haemotherapy are

1. red blood cells,
2. platelets and
3. plasma.

`Red cells' means the red cells obtained from a single blood donor, with a large proportion of the plasma from the donation removed. 'Plasma' means the liquid portion of the blood in which the cells are suspended. In the context of this opinion we refer only to the use of plasma as single unit, non-pooled fresh frozen plasma. 'Platelets` means a concentrate of blood platelets, suspended in 200-250 ml plasma. A therapeutic unit of platelets contains 2-4 x $10^{11}$ platelets. A therapeutic unit of platelets may be obtained either by pooling 4-6 platelet preparations obtained from whole blood donations, or by a single aphaeresis procedure. Thus, pooled platelet concentrates contain platelets and plasma from 4-6 different blood donors, whereas a single donor is sufficient to obtain a therapeutic unit of platelets by the apheresis technique.

The risk assessment is in two parts;

- Identification of potential for transfer of infection from a blood donor with vCJD infection
- The relative risk for blood transfusion at the population level

**Identification of potential for transfer of infection from a blood donor with vCJD infection**

**Donor factors**

It is highly likely that transmission of vCJD via blood or blood components is possible (Llewelyn et al 2004, Peden et al 2004, HPA 2006). From animal studies the estimated range of infectivity in blood ranges from 0.1-310 i/c ID50/ml (Cervenakova et al 2003a, Brown et al 1999, Brown et al 1998). The evidence indicates that the lower end of this range is more likely to be realistic for preclinical or sub-clinical donors. The assumed value for calculation purposes is 10 i/c ID50/ml.

These units are expressed as infectivity as measured by the intracerebral route (i/c). By the intravenous route based on animal studies, the infectivity is considered to be less, however, there are no relevant human data. Administration via the intravenous route

may result in a 1-5 fold lower transmission rate than by the intracerebral route (Brown et al 1998). The worst case scenario would result in an estimated infectivity of 10 iv ID50/ml.

Conclusion on the assumed infectivity levels in whole blood. The typical volume of blood taken from a donor is 450+/-45ml. Thus the total infectivity of a whole blood unit from an infected donor could be of the order of 4500 iv ID50.

**Processing factors**

a) Distribution of infectivity in blood and blood components

In blood there are a number of estimates of the partitioning of vCJD between the various blood components. However, these estimates are extrapolations from non-vCJD studies (Brown et al 1998). For the purposes of risk assessment it is assumed that:

- 50% (10-60%) of the infectivity is considered to be in plasma, (therefore the infectivity is taken to be 2250 iv ID50) per therapeutic unit.
- 25% is found in packed red blood cells, estimated infectivity 1125iv ID50 per therapeutic unit.
- 25% is associated with the buffy coat (white blood cells and platelets), estimated infectivity 1125iv ID50 per therapeutic unit.

There are theoretical estimates of the level of reduction of infectivity in whole blood achieved by leucodepletion which have suggested that removal of TSE infectivity may be as great as 200-fold (DNV report). However, experimentally leucoreduction has failed to demonstrate any removal of plasma borne infectivity (Brown et al 2005) or of spiked model TSE agents (Prowse and Bailey 2000). Leucodepletion of whole blood from infected hamsters showed only a ~2-fold reduction of blood borne infectivity (Gregori et al 2004). There is no experimental proof that the infectivity from vCJD can be reduced by universal leucocyte reduction (Sachs and Greinacher 2003). Consequently, strategies have been pursued to develop devices for the removal or reduction of prions from blood products by filtration. A prion reduction filtration technology for blood products destined for transfusion has recently become available and is currently evaluated by the Irish Blood Transfusion Service and the English National Blood Service. The values and limitations of prion removal devices for blood products as currently evident have recently been reviewed by Ortolano et al (2005). Blood banks may choose or may not choose to adopt prion removal technologies in the future, depending on the assessment of the risk of vCJD transmission by blood products in the different geographical regions worldwide. However, leucodepletion has been introduced widely as an initial treatment process. For risk assessment purposes the lower value of only 25% removal (i.e. removal of only buffy coat associated infectivity- the more conservative approach) is used.

Conclusion Leucodepletion will result in the following infectivity levels in one therapeutic unit:

- Whole blood (3375iv ID50)
- Plasma (2250iv ID50)
- Red blood cells (1125iv ID50)
- Platelets (175iv ID50) assuming 35 ml of plasma is included in each therapeutic unit

b) Risk from transfusion with red blood cells.

Red blood cells tend to be used in the same geographical area in which they are donated. The risk of a recipient receiving an infective dose from transfusion of packed red cells is directly determined by the likelihood that the donor carries an infectious dose of vCJD in their red cells. Buffy coat removal and leucodepletion is unlikely to result in sufficient infectivity reduction to prevent such infection. It is noted that two of the UK transmission occurrences were in elderly individuals receiving transfusion with red blood cells or non-leucodepleted red blood cells

**Recipient factors**

The likelihood of infection of an individual depends primarily on the exposure factors as presented above being the level of infectivity in the blood or blood components and the total dose administered. In addition recipient factors may be an influence.

- The frequency of administration of vCJD blood or blood components to the individuals (single versus repeated).
- Genetic background of recipients (*PRNP* gene codon 129 alleles being MM, MV, or VV, respectively)

So far, all clinical cases of vCJD have been expressed MM homozygosity at *PRNP* codon 129. Whether being codon 129 MV or VV really absolutely protects against developing vCJD or just results in a longer prolonged (very long) incubation period is as yet unknown. However, the latter is generally thought to be most likely. Recent experiments in mice carrying the human transgene encoding PrP suggest that human MV heterozygotes and VV homozygotes are susceptible to vCJD infection, albeit with a potentially prolonged incubation period compared to MM homozygotes (Bishop et al 2006). The detection of $PrP^{res}$ in one individual (Peden et al 2004) does show that people heterozygous (MV) at codon 129 can at least become infected. In addition, the evaluation of the positive samples of the appendix-tonsils study showed that also VV homozygotes can become infected with the abnormal PrP protein (Ironside et al 2006).

In conclusion, using a series of conservative assumptions made for the purposes of this risk assessment, there is a considerable risk that a vCJD donor could cause infective material to be passed on to one or more recipients of blood and blood components. These worst case assumptions can be summarized as:

- Each donor with vCJD infectivity confers an infectivity of 10 iv ID50/ml whole blood resulting in an average of 4500 iv ID50 in each therapeutic unit of whole blood
- Leucodepletion produces no more than 25% reduction in infectivity.

If a donor is incubating vCJD it is likely that a potentially infecting dose will be transmitted at some stage during the incubation period. The actual risk will be affected by the number of infected donors and the susceptibility of the recipients.

**Risk at the population level**

The above estimate of the risk is dependent on the assumption made of the likelihood of a vCJD infected blood donor being a member of the blood donor population. The

frequency of this is directly proportional to the incidence of vCJD infected donors in the country. This is certain to vary substantially between Member States.

For the estimation of the number of possible infected individuals in the UK the data of the appendix-tonsils study (3.4.3) will be used. These data show that 3 appendices out of 12 ,674 lymphoreticular tissue samples (mostly appendices, but also included tonsils) examined were positive for disease related PrP. This equates to 237 (95 % confidence interval of 49-692) individuals with abnormal PrP in the appendix/tonsils per million of the UK population.

In 1997, 35 million of the UK population were eligible to donate, of these only 4.3 % donated and on average they gave 1.2 times per year (DNV 2003). Using the highest number of the 95% confidence interval (n=692) the following calculation could be made to estimate the number of infected donations per year in the UK:

$692 * 10^{-6} *(1.2 * 35\ 000\ 000 *0.043) = 1250$

This results in 1250 infected donations per year in the UK.

For the number of blood recipients in the UK population who could develop vCJD as a consequence of receiving contaminated blood, the following additional points need to be taken into account

- the proportion of susceptible individuals in the recipient population who would develop vCJD following the administration of contaminated blood. It is not possible to give a numerical value for this, although it is unlikely to be 100%.

- the proportion of individuals in the UK receiving blood transfusions who will survive longer than 3-5 years. This is conservatively estimated as no more than 50% (section 3.6.1.1).

In addition, elderly patients and those who have poorer prognosis are the most likely to receive multiple transfusions.

In order to assess the potential number of recipients affected it is assumed that the blood or blood components from one donor is received by three recipients, and that the number of infective units transferred is sufficient to initiate the disease. This would result in a worst case estimate of the number of 3750 new infections per year as a consequence of blood transfusions. Eventually this would result in 1875 new individuals who will eventually develop vCJD (assuming that 50% of those receiving blood transfusion will live long enough to develop vCJD (Wallis et al 2004).

This figure is implausible given the current declining trend of vCJD cases in UK. There are number of possible explanations for this:

- this is a worst case assumption
- not all infected people may develop disease
- infectivity in blood may not be uniform during the course of infection
- the presence of abnormal prions in the appendix may not be always associated with blood infectivity
- certain genotypes might not be associated with infectivity in blood

- transmissibility may vary according to the identity of the *PRNP* genotypes of donor and recipient
- extrapolation from animal models concerning blood infectivity of vCJD in terms of units of infectivity present in blood may not be correct

These numbers change considerably when the calculations are performed using the lower end of the confidence interval of the data of the appendix-tonsils study. In addition, assuming a lower rate of infection has a considerable influence (Hunter et al 2002, Bird 2004) (Table 4).

**Table 4. Infected donations in the UK**

| Infected donors per million population | Donations p.a (million) | Infection rate % | Infected donations p.a |
|---|---|---|---|
| 49 | 1 806 | 100 | 88 |
| 237 | 1 806 | 100 | 428 |
| 692 | 1 806 | 100 | **1250** (current worst case) |
| | | | |
| 49 | 1 806 | 10 | 9 |
| 237 | 1 806 | 10 | 43 |
| 692 | 1 806 | 10 | 125 |

For other countries there are no data available on prevalence of abnormal PrP in lymphoid organs so similar calculations can not be performed. However, based on the current observed number of cases of vCJD within the Member States which stands at 21 non-UK cases as of September 2005), an estimate of the risk for France (14 cases) would be 10% of that calculated for the UK while for other Member States it will be considerably lower.

*3.6.2 Risk assessment on transmission by surgical instruments or invasive procedures*

There have been no confirmed cases of transmission of TSE by virtue of occupation. There have been a small number of reports of sporadic CJD in healthcare workers (including a neurosurgeon, retired laboratory workers and a pathologist) but any link with their occupation is speculative. There are around 300 cases of reported iatrogenic CJD, most linked to contaminated growth hormone injections or the surgical use of cadaveric human dura mater; some other cases are described to be have been linked to other surgical procedures, most during neurosurgery.

There is no evidence at present that occupational exposure to BSE is a risk factor for vCJD, and currently there is no described case of a patient with vCJD linked to previous exposure to contaminated surgical instruments.

Thus the risk of transmission of vCJD due to surgical instruments or procedures may not be great, especially if appropriate precautions are taken with clinically evident cases, but there remains the possibility of contamination via pre or subclinical cases of BSE/vCJD infection. The precautionary principle is applied in many countries for reducing the risk of secondary transmission and thus to avoid an outbreak of nosocomial vCJD infection.

This is based on the risk assessment procedure that has been performed both in UK and France. Event this risk assessment is of limited value because of the scarcity of adequate information for entering into the classical models.

3.6.2.1 Approach used

A risk assessment for the transmission of vCJD via surgical instruments was performed with a modelling approach and numerical scenarios, that was published in 2001 by the UK Department of Health (2001). It confirms that the risk of surgical transmission cannot be ruled out and that the most risky procedures are those involving the central nervous system and the back of the eye. Procedures involving lymphatic tissues, such as tonsils, and front of the eye tissue are of a lesser order of risk.

The risk of transmission of vCJD due to surgical instruments or procedures may not be great, especially if appropriate precautions are taken with clinically evident cases, but there remains the possibility of contamination via pre or subclinical cases of BSE/vCJD infection. The precautionary principle is applied in many countries for avoiding this risk of secondary transmission and to avoid an outbreak of vCJD disease. This is due to the risk assessment which has been performed both in UK and France. This risk assessment is of limited value because of the scarcity of adequate data to enter in the classical models.

3.6.2.2 Consideration of the individual risk factors

**Exposure**

According to the CJD section of the last report of the British Department of Emerging Infections and Zoonosis at the Health Protection Agency's Centre for Infections (CFI), there were 183 reported surgical incidents where instruments, that were considered potentially contaminated with a the CJD agent during use on an index patient, were subsequently re-used on other patients during 4 years (Aug 2000 – Aug 2004). Of these 183 reports, 61 are linked with vCJD (possible, probable or definite) and 86 linked with sporadic CJD (HPA 2005).

Patients can be categorised as follows, in descending order of risk, according to Table 5. It is, at present, very difficult to clearly differentiate between CJD or vCJD and other forms of human TSEs CJD in these categorisations.

**Table 5. Categorisation of patients by risk**

| | |
|---|---|
| 1. Symptomatic patients | 1.1. Patients who fulfil diagnostic criteria for definite, probable or possible CJD, including vCJD<br>1.2. Patients with neurological disease of unknown aetiology, not fulfilling current CJD diagnostic criteria, but where a diagnosis of CJD, including vCJD, is being actively considered<br>1.3 Patients with non-focal cerebral disease undergoing diagnostic brain biopsy. |
| 2. Asymptomatic patients | 2.1. Individuals who have been shown by genetic testing to be at considerable risk of developing CJD |

| | |
|---|---|
| | or who had two or more relatives affected by CJD or other prion disease or having a genetic mutation indicative of familial CJD<br>2.2. Recipients of hormone derived from human pituitary glands (e.g. growth hormone.)<br>2.3. Individuals who have received a graft of *dura mater*, or neurosurgical procedures or operations before 1992<br>2.4. Patients with previous exposure to instruments used on, blood, plasma derivatives, organs or tissues donated by a patient who went to develop any form of CJD 'Patients with exposure to instruments previously used on patients with CJD, or who went on to develop CJD. Patients exposed to blood or blood derivatives donated by patients with CJD, or who went on to develop CJD. Patients who have received organs or tissues donated by patients with CJD, or who went on to develop CJD.' |

In these patients it could be assumed, as presented in Table 1, section 3.2.3 that tissue infectivity may be classified, according to WHO (1999) or ACDP/SEAC (2003), ranging from low (dental pulp, peripheral nerve) to medium (anterior eye and cornea) and high (brain, spinal cord and ganglia, cranial nerves, posterior). Although such studies are limited with vCJD, the preliminary data are a valuable support as it is believed that their distribution is more or less similar for other forms of CJD although with considerably greater lymphoid involvement. This is especially important in view that lymphoid involvement in vCJD can be preclinical,

There is a lack of direct evidence for how prion material attached to an instrument is likely to behave on re-use. However there are indications that the $PrP^{sc}$ protein remains bound to instrument surfaces. The expectations would then be that only a fraction of it would be detached on re-use. It is expected than 10 % or less may be detached, but scenarios must consider a wide range from less than 10 % to 100 %. Direct evidence is also lacking about the initial mass of infected tissue potentially adhering to the instrument. For simplification purpose it could be assumed that 10 mg could adhere to each instrument, and across all operations around 20 instruments are used (and 12 during tonsillectomies (section 3.5.1).

Given some infective burden after the first decontamination and sterilization cycle, the eventual number of infections will depend on how much of this is subsequently transferred to other patients rather than being removed or deactivated. Both the transfer percentage and second and subsequent decontamination efficacy are unknown. Thus the worst case scenario must always be employed. Although certain instruments are obviously dedicated to specific procedures, some others may circulate more widely and crossover of instruments between specialities may occur, thus increasing possibilities for transmission, even if such crossover appears to be the exception rather than the rule.

**Hazard factors**

A titre of $10^8$ intracranial ID50/g. could be encountered in brain and other CNS tissue in the pre-clinical stage of the disease. Once the disease reaches its clinical stage, even higher titres may be encountered, but instruments used on such patients should already be quarantined as a matter of routine. Posterior eye tissue should be regarded as having a similar potential infectivity to CNS. However the anterior chamber can be regarded as having a potential titre 1-2 logs less.

Throughout the incubation period, infectivity of $10^6$-$10^7$ ic ID50/g may be dispersed through the body in the lymphoreticular system and some other peripheral material. In a non-human primate model large amounts of $PrP^{Sc}$ (vCJD/BSE, sCJD, or iCJD) were detected in lymphoreticular organs, while low amounts were observed in organs associated with nervous structures including muscles, adrenal glands and enteric nervous system after intracerebral inoculation (Herzog et al 2005).

As previously described $PrP^{sc}$ protein is very resistant to different decontaminating and "sterilizing" processes, thus it must be considered that only a limited number of cleaning/decontamination processes are effective (see 3.5.2).

3.6.2.3 Use of the above risk factors for risk assessment

Assuming that some individuals in the population may currently be incubating vCJD and be potentially infective, a series of models addressed the following key questions:

- how many secondary infections might be transmitted from one operation on an infective patient, as instruments are re-used?
- what would be the resulting rate of secondary infections within the population as a whole ?
- how could the transmission rate develop over time, and how might these infections eventually be translated into additional clinical uses of vCJD?

Uncertainties accumulate in moving from each question to the next, because at each calculation additional key variables need to be estimated each adding to the overall uncertainty. Specific numerical scenarios must therefore be regarded as illustrative only. Given the scarcity of direct data on vCJD in humans, many key inputs are based using scrapie as a model of the disease (implying that infectivity may be widely-distributed through the body, in contrast to classical CJD).

Despite the uncertainties, some interim conclusions may be reached on the basis of current knowledge:

- surgical transmission of vCJD cannot be ruled out as a risk to public health; according to scenarios, with a starting titre log ID50/g of respectively 8 and 5 logs, expected numbers of infections are ranging from 1 to 0.01
- the total number of vCJD cases caused by surgical transmission could be around 5-10 % of the number infected in the primary outbreak, but the absolute scale of such a risk cannot be determined until more is known
- the most important way of reducing the risk is to ensure that decontamination of instruments is as effective as possible
- operations on the Central Nervous System and those ophthalmic procedures involving the posterior eye carry the highest risk of transmitting vCJD (CNS/PO

surgery). Results of illustrative scenarios for infections per operation are the following.

- Inter-CNS/PO[1], initial infectivity $10^8$ ID50/g, 1 to $10^{-3}$ secondary infections per operation according to effect of cleaning and sterilization (3 logs to 6 logs).
- Inter-LRS[2] or anterior eye ($10^6$ ID 50/g), $10^{-5}$ to $10^{-2}$ secondary infections per operation (efficiency of reduction from 3 to 6 logs).

3.6.2.4 Conclusions

Because of the actual limitations for conducting a full risk assessment process (lack of knowledge both on the level of contamination of the instruments before reprocessing and on the minimal infectious dose linked to new variants of prion according to the route of transmission) the edited guidances are based on the precautionary principle:

- specific precautions for symptomatic patients (definite, probable and possible) and asymptomatic patients potentially at risk of CJD
- general precautionary measures for surgical procedures and endoscopy.

A patient 'at high risk' is defined as a patient exposed to materials from individuals who are thought to have been infected with vCJD. For all symptomatic patients, use of single-use protective clothing and single-use disposable surgical instruments and equipment are necessary. All single use items must be destroyed by incineration. In some Member States general precautions have been taken in respect to high risk procedures like tonsillectomy.

For asymptomatic patients but who are known to be at risk the same precautions apply, but where single-use instruments are not available, the handling of re-usable items depends on the kind of activity and the tissue in contact with the instrument (tissue considered in the WHO classification as possibly contaminated or not). Thus according to the French SEAC it is possible to consider the following situations and best practice (Table 6).

**Table 6. Schedule for cleaning and disinfection treatment of surgical instruments**

| | **Activity with high risk (nervous system, eye)** | **Activity with low risk (coeliosurgery, delivery)** |
|---|---|---|
| Patient with clinical vCJD | Destruction | Destruction |
| Patient with high risk | Destruction or Procedure I | Procedure II |
| Patient without risk | Procedure II | Procedure III |

*Procedure I: Cleaning + quarantine + chemical inactivation + physical inactivation*
*Procedure II: Cleaning + chemical inactivation or physical inactivation*
*Procedure III: Cleaning + classical disinfection or sterilization*
*High risk patient: see Table 5.*

In certain instances, the quarantine of equipment is advisable until definitive diagnosis can be made. In conclusion, the following Figure 6 could be proposed for precautions for surgical procedures on known, suspect or patients at risk for vCJD.

[1] CNS/PO Central Nervous System/ Posterior eye
[2] LRS Lympho Reticular System

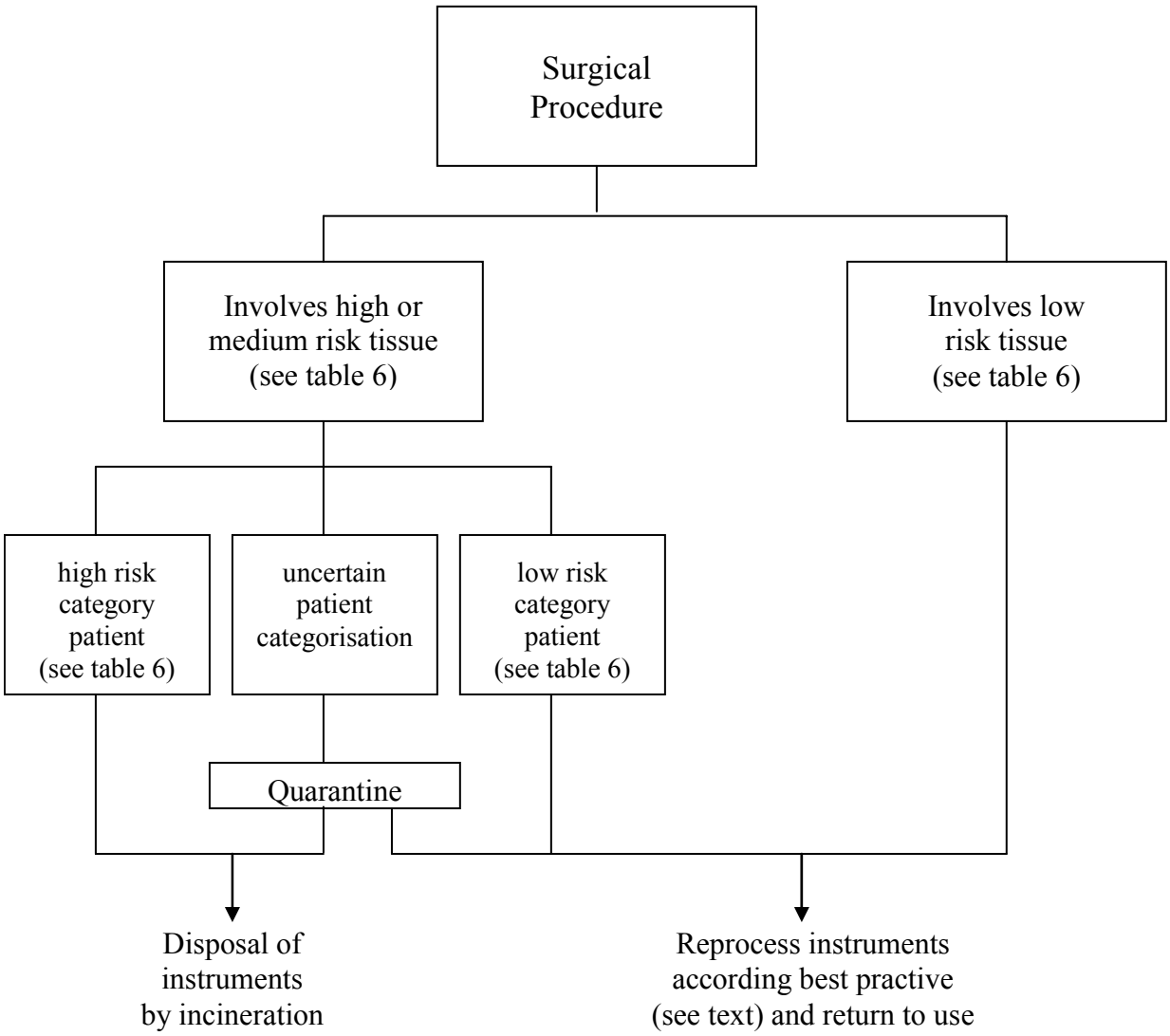


**Figure 6. Algorithm for precautions for surgical procedures**

*3.6.3 Risk assessment on vertical transmission*

The increase in exposure of humans to transmissible prion agents and the recent possibility of blood borne infection raises the possibility of vertical transmission. This could occur in the uterus, during birth or via breast milk. One important aspect of this question is whether there is any evidence of increased infectivity of the vCJD agent as a result of it being transmitted from human to human, (i.e. the removal of the species barrier).

3.6.3.1 Placental structure

There are marked differences in placental structure among species affected by TSEs – including domestic farm animals, laboratory animals and humans. These differences

mean that when attempting to extrapolate between species considerable caution must be exercised.

The placenta of sheep and cattle are of the cotyledonary epitheliochorial type in which the maternal endometrial epithelial surface and the foetal epithelial trophoblast become closely apposed. In cattle there is some fusion of trophoblast and endometrial cells (hence the term "synepitheliochorial" which is sometimes used by Wooding and Flint 1994). In sheep the trophoblast binucleate cells occasionally fuse with maternal epithelial cells to form trinucleate cells. In both species there are zones of maternal haemorrhage where the red cells are lysed and phagocytised by trophoblasts. This facilitates the transfer of iron to the developing foetus.

In commonly used laboratory rodents and man the placentas are discoid and of the haemochorial type. Maternal blood is in intimate contact with the foetal trophoblast. Transfer of gas, nutrient and waste products occurs directly between maternal blood and the trophoblast. In rats and mice this takes place in the labyrinth region of the placenta across a three distinct layers of trophoblast. In humans the structure is of the villous type and there are only 2 layers of trophoblast.

### 3.6.3.2 Biodistribution of TSE agents

There is considerable variation in the tissues containing infectivity or immunoreactive protein depending on the animal species and the TSE agent used. For example in scrapie infected sheep $PrP^{Sc}$ was widely distributed in nervous and lymphoid tissues but is also found in the placenta (Andreoletti et al 2002) However in BSE infected sheep no immunoreactivity was detected in the placenta, albeit in a very small number of animals tested (Foster et al 2001). In BSE in cattle peripheral deposition was very low (Terry et al 2003). For vCJD, there is no report of any testing of the placenta or mammary tissue.

### 3.6.3.3 Evidence for vertical transmission in animals

Vertical transmission has been difficult to demonstrate with certainty since it is hard to conclusively exclude transmission from other sources, (contaminated feed or environment etc). To control for these factors studies were undertaken in which challenged ewes and unchallenged animals were housed together. These studies suggest that transmission by contact is very low (Foster et al 2004) and this is broadly comparable with studies in cattle with BSE (Wilesmith et al 1997). One study has directly addressed the question of *in utero* or perinatal transmission of BSE in susceptible sheep and did not observe any vertical transmission in 29 offspring. The statistical evaluation of the result allowed for an estimation of the occurrence of transmission being at most 1 in 4 (95% CI) (Foster et al 2004). Recently, preliminary results were reported indicating the vertical transmission of BSE in sheep either *in utero* or perinatally (Bellworthy et al, 2005). In this experiment susceptible sheep were dosed orally with 5 gram of BSE cattle brain inoculum. Two lambs developing BSE were born of ewes which developed BSE after the oral administration of BSE cattle brain.

### 3.6.3.4 Evidence for vertical transmission in man

There are no data available relating to the bio-distribution of $PrP^{Res}$ in human placental tissue. Human and placental tissues vary during gestation and lactation so potential of transmission of infectivity may similarly vary. In addition there is no evidence of vertical transmission of Kuru (Ridley and Baker 1995).

3.6.3.5 Conclusion

Currently there are no proven instances of vertical transmission of any human prion disease. The available animal and human data are inadequate to allow firm conclusions concerning vertical transmission to be drawn. There are no data indicating that breast milk transmits human prion disease.

*3.6.4 Risk assessment on transmission by transplantation of Umbilical Cord Stem Cells*

The collection and storage of cells from umbilical cords is becoming increasingly common. These cells can be used as a source of haematopoietic stem cells for transplantation following myeloablation. They can be used in both allogeneic and autologous transplantation in children and adults (Laughlin et al 2001).

The cells that are transplanted are of foetal origin although the possibility of low levels of contamination with maternal blood can not be definitively excluded.

3.6.4.1 The Risk

Umbilical cord blood stem cells are of foetal origin and the likelihood of infection is extremely low, since vertical transmission in humans has not been observed in any prion disease. In addition, it should be recognised that the recipients of the cells may be seriously ill with life-threatening conditions. There remains the possibility that small amounts of maternal blood may be present as a contaminant in cord blood.

3.6.4.2 Conclusion

Since vertical transmission in humans has not been observed in any prion disease, the risk posed by the use of cord blood is considered to be negligible.

*3.6.5 Conclusions*

Using a series of conservative assumptions made for the purposes of this risk assessment, there is a considerable risk that a vCJD donor could cause infective material to be passed on to one or more recipients of blood and blood components. In the worst case scenario each therapeutic unit of blood donated could contain as much as 4500iv ID50.

In view of the distribution of vCJD infectivity over the various blood compartments (plasma, platelets and leucocytes) leucodepletion may produce no more than a 25% reduction in infectivity. Taken into account the population eligible for blood donation, the number of donations and the percentage of the population actually donating blood, up to 1250 infected donations may occur, per year, in the UK.

A quantitative risk assessment based on the data of the UK appendix-tonsils study and the worst case scenario, shows that there may occur 3750 new infections each year in the UK. Of these 3750 new infections 1875 new individuals will eventually develop vCJD being the subgroup living long enough after the transfusion to develop vCJD.

There is no evidence that individuals working in hospital settings have developed vCJD by virtue of their profession. Transmission via surgery remains a concern, but to date there is no evidence that it has actually occurred. For prevention of transmission of vCJD by surgical instruments a system is proposed based on the probability of the patient under investigation/treatment being infected with vCJD (or any other TSE), and the precautionary principle.

For use of instruments on clinical vCJD patients single use materials should be used, For patients with high risk, single use materials or alternatively materials followed by specific cleaning plus chemical inactivation plus physical inactivation may be used. For low risk surgical procedures, cleaning plus chemical inactivation or physical inactivation may be used.

For patients without risk the procedure cleaning plus chemical inactivation or physical inactivation, or cleaning plus classical disinfection or sterilization may be used, depending on whether the treatment is an activity with high or low risk, respectively.

There are no proven instances of vertical transmission of any human prion disease. The available animal data are inadequate to allow firm conclusions concerning vertical transmission to be drawn. There are no data indicating that breast milk transmits human prion disease. In view of the lack of vertical transmission in humans the risk posed by the use of cord blood is considered to be negligible.

At this moment there is a declining incidence of trend for the occurrence of vCJD in the UK. All clinical cases to date have been homozygotes MM at codon 129 in the *PRNP* gene. However, vCJD infection was also demonstrated in one MV heterozygote and two VV homozygotes. It is unknown whether MV heterozygosity or VV homozygosity protects against developing vCJD or just results in a longer incubation period. If the latter is the case, a second wave of vCJD could develop in the (near) future.

### 3.7 Evaluation of previous opinions of the SCMPMD on human product safety for vCJD transmission with regard to current scientific knowledge

Three opinions have been issued by SCMPMD on the subject of human derived products and the risk of transmission of vCJD. These opinions are discussed step by step and carefully evaluated which statements no longer are valid due to new scientific data.

#### *3.7.1 Opinion on Quality and Safety of Blood (Adopted by SCMPMD on 16 February 2000)*

In this opinion the key elements for establishing high standards of quality and safety for the collection, processing, storage and distribution of whole blood, blood components and blood precursors were identified, namely:

- Inclusion of the complete transfusion chain into the consideration

- Establishment of a Quality System
- Need for a haemovigilance system
- Blood donor recruitment and retention policies
- Need for criteria for the suitability of the donor and for his protection
- Testing requirements
- Storage conditions
- Requirements for labelling
- Introduction of new preparations and products
- Importance of a common terminology
- Mechanisms for establishing and maintaining harmonised quality standards

*Comment: The report is very general and the conclusions are still valid. The report stresses the importance of taking into consideration all the steps of the blood transfusion chain. Specifically, in regard to vCJD, it is indicated at page 8, that "although at present testing of vCJD in blood donors is not possible, it is likely that once a sensitive and specific test for vCJD becomes available, such a test will be implemented in all member states". As discussed in section 3.3.6 of the present report, the consequences of a positive test should be evaluated very carefully, taking into account the prevalence of the positive samples, the possible occurrence of false positive tests and the difficulties in determining whether a positive test is a false-positive one.*

*3.7.2 Opinion on update of the opinion given by SCMPMD on the risk quantification for CJD transmission via substances of human origin (Adopted by SCMPMD on 16 February 2000)*

This update concerns an opinion adopted by SCMPMD in October 1998. Three issues are treated in this opinion

1. Leucodepletion. It is stated that the efficiency of leucodepletion is uncertain. TSE infectivity is predominantly associated with the buffy coat but infectivity is also found in plasma. It is speculated that removal of leukocytes might cause shedding of prions and thus increase the infectivity of the plasma. It is recommended to perform further studies and in the meantime introduce leucofiltration which also for other reasons would be of benefit for recipients.
2. Screening assays. SCMPMD encourages efforts to develop easily applicable screening tests for vCJD.
3. Exclusion of donors. The rules applied at that time in USA and Canada are cited: Donors that had stayed in UK more that six months (cumulated) in the period 1980 to 1996 are deferred. It is recommended by SCMPMD that an evaluation is made in Europe on the basis of 1) travel patterns, 2) exposure to UK bovine material in different member states, 3) prevalence of HIV, HBV and HCV in first time donors in member states.

*Comment: According to the EMEA report (June 2004, www.emea.eu.int/pdfs/human/press/pos/287902rev1.pdf), studies in UK on leucoreduction show that these procedures do not provoke fragmentation or cell lysis. A study in hamsters indicates that infectivity of whole blood decreases by 42% after leucoreduction, (Gregori et al 2004). Leucodepletion is now adopted in most member*

*countries for other reasons. The recommendations of this report are limited and there are no new data. See section 3.6.1.2. of the present opinion.*

*3.7.3 Opinion on The Safety Of Human-Derived Products With Regard To TSE's (Adopted by SCMPMD on 18 January 2002)*

The working group consisted of members from SCMPMD, the scientific steering committee (SSC) and its TSE/BSE ad hoc group. The report of the working group is divided into the following sections:

1) Methods and techniques for the detection of vCJD infectivity
   a) Bioassays
   b) In vitro diagnostic assays
   c) Epidemiology
2) vCJD infectivity of blood and other human substances
3) Application of vCJD detection tests
   a) Clinical diagnosis of vCJD ("screening in sufferers of neurodegenerative disease")
   b) Surveillance of vCJD
   c) Donor screening tests for vCJD
4) Transmission of vCJD/ TSEs via blood: Risk assessments in different countries
   a) Europe
   b) Countries outside Europe (USA, Canada, New Zealand)
   c) Appendix: Selected official documents (assessments, guidance)
5) Compatibility of different risk assessments
6) Transmission routes of vCJD: Risk factors in regard to blood donors
7) Processing procedures for blood and blood components and vCJD transmission risks
   a) Leucoreduction of blood components
   b) Leucoreduction or filtration of plasma for fractionation
   c) Nanofiltration of plasma derivatives
8) Acknowledgement
9) Appendix: Selected official documents (assessments, guidance)
10) References; Opinions of SSC and SCMPMD; Abbreviations

*In the following section 1-7 will be discussed.*

1. Methods and techniques for the detection of vCJD infectivity

   a) Bioassays
   b) In vitro diagnostic assays
   c) Epidemiology

*The subsections a) and b) are valid; bioassays are still needed in order to detect vCJD infectivity. Subsection c) is no longer correct; it is for instance indicated that epidemiological studies reveal no evidence of human TSE transmission by blood. This statement is in conflict with the probable cases reported recently.*

2. vCJD infectivity of blood and other human substances

*It is indicated that "currently there is neither a disproof nor a proof for vCJD infectivity of human blood". In sections 3.2.4 and 3.4.4.5 of the present opinion, the three probable cases of transmission of vCJD or vCJD infection by blood are detailed. It is considered unlikely that these cases were due to dietary transmission. In contrast, there has been no evidence that plasma pools that include donations from patients that later developed vCJD, could transmit the disease.*

3. Application of vCJD detection tests

a) Clinical diagnosis of vCJD
b) Surveillance of vCJD
c) Donor screening tests for vCJD

*The methods have evolved and the reader is referred to section 3.3 of the present report.*

4. Transmission of vCJD/ TSEs via blood: Risk assessments in different countries

a) Europe
b) Countries outside Europe (USA, Canada, New Zealand)
c) Appendix: Selected official documents (assessments, guidance)

*This section describes the risk assessment policies applied by European countries (a) and countries outside of Europe. It represents an important work which however needs updating because the situation has evolved since 2002. Table 1 reflects the situation in 2001-2002, the reader is referred to the updated table: risk of vCJD transmission by blood and donor deferrals with data provided by national blood experts as of 6 September 2004 (FMD/D (2004) 360136), see the following link: europa.eu.int/comm/health/ph_threats/human_substance/documents/blood_vcjd_en.pdf*

5. Compatibility of different risk assessments

The risk assessments employed by Member States as well as by other countries consist of two separate parts:

1. Assessment of the risk whether or not TSE infectivity can be transmitted by blood (via blood components and plasma derivatives) of individuals infected with the vCJD agent.
2. Assessment of the risk that a blood donation was donated from a vCJD infected individual in populations of different geographical areas (prevalence of vCJD infected blood donors).

ad 1.

*Recent data demonstrate that TSE infectivity can be transmitted by blood transfusion in animals and probably in man (see sections 3.2.4 and 3.4.4.5 of present report).*

ad 2.

*The number of vCJD cases in UK was 115 in the previous report (Dec 2001) and 157 in September 2005 (NCJD surveillance unit, UK). In France the number was 5 in 2001 and is 14 in September 2005 (Institut de veille sanitaire). In other European countries*

*confirmed vCJD cases are rare (3 in the Republic of Ireland, 1in Italy, 1 in the Netherlands and 1 in Portugal and 1 in Spain). Outside Europe one case has been reported in each of the following countries: USA, Canada, Japan, Saudi Arabia (see section 3.4.2).*
*As stated in the report, the estimation of the number of expected vCJD cases in each country is very uncertain, either based on simple extrapolations of UK data or on sophisticated statistical methods. The conclusions of the section are not clear and the reader is referred to the present report.*

6. Transmission routes of vCJD: Risk factors in regard to blood donors

*As stated in the report, there are in principle two different possible ways to contract vCJD:, exposure to bovine material carrying BSE infectivity (primary transmission) and exposure to human derived material carrying vCJD infectivity (secondary transmission). The secondary transmission also includes surgical and investigative diagnostic instruments that have been in contact with infected tissues and there is a risk that individuals infected secondarily become themselves a source for secondary transmission. In addition, these also pose a risk for other forms of CJD transmission.*
*Measures have been taken to interrupt primary transmissions, notably the removal of specified risk material at slaughtering and the exclusion of animals scoring positive in a BSE rapid test.*

*The first round of secondary vCJD transmissions depends on the prevalence of individuals infected by primary transmissions. If there are differences between different areas in the prevalence of those individuals it might be sensible to prevent, in lower risk areas, the use of human material originating from higher risk areas. This consideration may justify attempts to evaluate or even quantify the extent of the primary vCJD epidemic in different Member States.*

*The rest of the section concerns considerations about the primary exposure in different countries taking into account trade, extent of BSE epizootic and different genetic susceptibility (methionine homozygosity for codon 129). This is out of scope of the present opinion that only concerns secondary transmission.*

7. Processing procedures for blood and blood components and vCJD transmission risks

a. Leucoreduction of blood components
b. Leucoreduction or filtration of plasma for fractionation
c. Nanofiltration of plasma derivatives

*In experimental rodent systems, 90% of the vCJD infectivity was estimated to be found in the white cells, while a more recent work reported that only about 42% of the infectivity was in the buffy coat (Gregori et al 2004). As indicated in the report, leucoreduction has other benefits and is applied in most member states.*
*The subsections on leucoreduction for plasma fractionation and nanofiltration have already been discussed by EMEA and will not be commented upon.*

*In conclusion, most conclusions and recommendations of the previously published Opinions of the Scientific Committee on Medicinal Products and Medical Devices (SCMPMD) and the Scientific Steering committee (SSC) are still valid. Two important issues do need re-evaluation/consideration, the first being the possible transmission of vCJD by blood, and the second being the presence of vCJD in the UK population as indicated by a large scale study of surgical appendix-tonsil specimens .*

# 4 COMMITTEE OPINION

In 2004 two instances were reported indicating the possible transmission of variant Creutzfeldt-Jakob Disease by blood transfusion (Llewelyn et al 2004, Peden et al 2004). A third instance was discovered in the beginning of 2006 (HPA 2006). Llewelyn et al (2004) reported on a survey of recipients who were identified as having received blood from donors who developed vCJD. One of the recipients developed symptoms of vCJD 6.5 years after receiving blood transfusion.

Peden et al (2004) reported on an asymptomatic individual who had received a blood transfusion from a donor who subsequently developed vCJD. Protease resistant protein ($PrP^{res}$) was detected by immunohistochemistry in the spleen and cervical lymph node of the blood recipient who was found to be an MV heterozygote at the prion gene *PRNP* codon 129. In addition, the appendix-tonsil study demonstrated infection with vCJD in VV homozygotes. All clinical cases to date have been *PRNP* codon 129 MM homozygotes. Whether being codon 129 MV or VV protects against developing vCJD or results in a longer incubation period is as yet unknown. If the latter is the case, further waves of vCJD may develop in the future.
The three human instances of vCJD infections cannot be definitively proven to be caused by the preceding blood transfusion. However, it is highly likely that they did originate from infection by blood transfusion from preclinically infected donors. Consequently, it must be assumed that in an asymptomatic vCJD infected individual the prion is present in peripheral blood. This is consistent with animal experimental data.

The present risk assessment of exposure to vCJD infectivity in whole blood and in blood components allows a rationale to define precautionary measures to prevent vCJD transmission within the human species by the intravenous or other routes.

. The possibility of minimizing risk of vCJD transmission via blood transfusion through exclusion of certain donors, although of limited impact, has been implemented in most EU countries on the basis of time spent in the UK during the period of risk of BSE exposure through food. Concern over possible transmission by blood is increased by the fact that an assay for routine screening with respect to vCJD is not yet available. This has important implications for possible iatrogenic transmission by blood and/or cells and organs from donors in the incubation phase or with subclinical disease, and transmission through surgical instruments used in invasive procedures.

Thefirst two instances of a possible transmission of vCJD infection by blood transfusion initiated several questions which were presented to the Scientific Committee on Emerging and Newly Identified Risks (SCENIHR). These questions (section 2) were as follows:

- to review the previous scientific SCMPMD Opinion on the ‘Safety of Human-Derived Products with regard to TSEs’ adopted on 18 January 2002, and the SCMPMD Opinion on ‘Quality and Safety of Blood’ adopted on 16 February 2000 in order to quantify, if possible, the nature and magnitude of the risks of transmission of

the vCJD agent through blood donated by preclinical cases of vCJD, through surgical instruments or by instruments used in invasive procedures,

- to evaluate the risk of vertical transmission of vCJD in pregnant women and
- to evaluate the risk of vCJD transmission by the tissues stored in umbilical cord cell banks.

The pathogenesis of prion-related diseases is widely considered to be related to defective protein folding resulting in abnormal protein conformation. Normal cellular protein is converted into an insoluble, aggregated, beta-sheet rich form which is deposited in the brain. The mechanism of prion-related diseases is believed to involve disease transmission by replication of this protein conformation. The accumulation of protease resistant prion protein ($PrP^{Res}$) in tissues is a characteristic feature of prion disease. vCJD is a typical prion disease in this respect.

Current evidence on the pathogenesis of vCJD indicates that orally ingested prions enter mainly via the gut lymphoid tissue, and are transported to the other lymphoid organs. Prions are replicated in the periphery in lymphoid tissues such as the spleen, the appendix and tonsils, and are then transported to the brain mainly by peripheral nerves. Direct penetration into the brain across the blood-brain barrier may occur. Thus, peripheral blood may be involved in propagation and transport of vCJD prions. The two clinical cases of vCJD (as of February 2006) and one instance of detection in tissues of abnormal prion protein, in recipients of blood donations from donors incubating vCJD, support the involvement of peripheral blood. However, it has not yet been possible to detect abnormal PrP ($PrP^{Sc}$ or $PrP^{Res}$) in human blood of vCJD patients, which is most likely due to the lack of sensitivity of available tests.

Although the immune system may facilitate vCJD infection, an immune response against vCJD prions is not initiated. However, antibodies against either the normal or disease related forms of PrP can protect against TSE disease in animals. vCJD protein has been found to be present in various tissues including brain, spinal cord, spinal ganglia, cranial nerves and ganglia, posterior eye, tonsil, appendix, spleen and thymus and other lymphoid tissues, with varying degrees of infectivity.

Important advances in test methodologies for prion detection have been made in recent years, and the application of these advances in the diagnosis of vCJD has, in particular, been fruitful. However, no diagnostic system has yet emerged with the high level of sensitivity and specificity required for routine screening of blood or urine. It is essential that confirmatory assays are available, and all ethical implications are considered and carefully taken into account before implementing testing. Independent validation of any new methodology should be mandatory prior to implementation, and it is recommended to adopt the EU procedure used for the BSE testing. For validation carefully controlled vCJD reference materials should be used. However, the availability of blood from individuals at increased risk of vCJD or diagnosed with vCJD is very limited. Therefore, ethical collection of such valuable material should be considered a priority. Special strategies are then needed to evaluate potential blood tests in order to conserve material. Collection of urine should be considered which could be tested when more sensitive tests for abnormal prion protein suitable for testing urine become available. The issue of false positives needs careful consideration, as even minute percentages of these may actually involve a large number of individuals. The ethical issues of informing an individual of test results, without providing any certainty as to the likelihood of progression to clinical disease, should be considered seriously.

The incidence of vCJD in the UK is decreasing but there remain considerable uncertainties and concerns over future numbers of cases. While other countries have not been exposed to BSE to the same extent, France continues to identify new cases of vCJD and additional countries have reported vCJD cases.. the control of BSE in cattle and the precautions taken to prevent BSE infected material entering the human food chain, there remains the issue of controlling secondary human-to-human transmission. The monitoring of vCJD cases, vigilance for any new phenotypes of human TSE infection and the identification of any actual secondary transmission events are essential. Human population studies are entirely dependent on having efficient and appropriate surveillance systems in all relevant coutries. It is therefore essential that the EU wide surveillance for human TSEs is continued and that there is a coordinated collection and publication of information on vCJD as done for BSE by the OIE World Organisation for Animal Health.

For surgical instrument cleaning and disinfection there is a need to validate the effectiveness of the methodology employed. The animal assay with intracerebral inoculation is still the best method for determining TSE infectivity. No procedure for the decontamination of surgical instruments has yet been validated to the extent that its universal introduction can be recommended. Cleaning and protein removal from the instruments is essential. Drying of instruments before cleaning or decontamination is likely to reduce the effectiveness of the decontamination procedure. Disinfectants with fixative properties such as those containing aldehydes must not be used for decontamination of instruments suspected to be contaminated with TSEs as they tend to stabilise rather than inactivate prions.

The specific answers to the questions presented to SCENIHR are presented below:

Question 1:

- to review the previous scientific SCMPMD Opinion on the 'Safety of Human-Derived Products with regard to TSEs' adopted on 18 January 2002, and the SCMPMD Opinion on 'Quality and Safety of Blood' adopted on 16 February 2000 in order to quantify, if possible, the nature and magnitude of the risks of transmission of the vCJD agent through blood donated by preclinical cases of vCJD, through surgical instruments or by instruments used in invasive procedures.

The general conclusions and recommendations of the previous Opinions on the safety of human derived products including blood and blood components are still valid. Research on detection methodology has further evolved and an update is presented. Two aspects need our current attention; the possibility of transmission of vCJD by blood and blood components, and the presence of vCJD infected individuals in the population. The possibility for transmission by blood or blood products seems likely in view of the three instances of vCJD infection in persons who received previously a blood donation from a donor who developed vCJD. In addition, the UK study evaluating anonymised routine appendicectomy samples (Hilton at al, 2004a) indicates that vCJD infectivity may be present in the UK population at higher levels than the present numbers of identified clinical cases suggest.

There is a risk that a vCJD infected donor could pass infective material to one or more recipients of blood and blood components. In the worst case scenario each therapeutic unit of blood donated could contain as much as 4500 infectious units.

In view of the distribution of vCJD infectivity over the various blood compartments, leucodepletion may produce no more than a 25% reduction in infectivity. Based on the data of the UK appendix study and the worst case scenario with an infectivity of 100%, and taking into account the population eligible for blood donation, the number of donations and the percentage of the population actually donating blood, up to 1250 infected donations may occur, per year, in the UK.

If there are 1250 infected donations per year they will result in 3750 new infections each year in the UK assuming that donations are typically split between 3 recipients. Of these 3750 new infections the subgroup living long enough after the transfusion to develop vCJD is approximately 50 %, so 1875 new individuals are eventually expected to develop vCJD. However, this worst case scenario does not fit the current data on vCJD case trends in the UK and may therefore largely overestimate vCJD transmission. The presence of subclinical infection may account for this discrepancy. It is unknown how vCJD may develop in non-MM genotype individuals. They may be not susceptible for developing clinical vCJD, but they also could have a prolonged incubation period. Regarding persistence of such a vCJD infection in the population by blood transfusion, preliminary data from one mathematical model suggest that it is unlikely that a vCJD infection could establish itself in the population by blood transfusion only.

The risk assessment is directly applicable only to the UK situation. In order to perform similar risk assessments for other Member States, it would be necessary to collect data on the presence of infection ($PrP^{sc}$ or $PrP^{Res}$) in the general population similar to the UK appendix-tonsil study. However, it is recognised by SCENIHR that there are considerable difficulties in the collection of such data due to the rather low vCJD prevalence in other Member States. Alternative approaches for possible estimations of vCJD prevalence may therefore be used such as calculations based on BSE exposure. The scarcity of data on vCJD tissue distribution in preclinical patients and on the prevalence in the population hampers every risk assessment for vCJD. The best approach for the risk assessment is yet unknown.
The current decline in clinical vCJD in the UK and the general low number of cases in the older age groups that comprise the majority of blood recipients, suggest that this worst case scenario as used in the current risk assessmentoverestimates transfusion-related vCJD disease development.

There are several potential explanations, namely:

- infections may have a very long incubation period so that the individual dies before disease develops,
- infections in some groups such as the MV heterozygotes may not be associated with blood infectivity,
- different genotypes may not transmit efficiently to each other even if the blood unit transfused is infectious.

Taking the lower limit of the confidence interval of the prevalence from the UK appendix study and if it were assumed that only ten percent of infectious donations actually transmit the infectious agent, the number of infections resulting would be 9 per year in contrast to the 1250 predicted by the worst case scenario. Even small changes in the assumptions can lead to large changes in the prediction. Independent of the method of calculation transmission by blood transfusion may occur. Based on current data, the frequency cannot be reliably estimated, but even in the UK it is low. The frequency is largely dependent on the number of

asymptomatic infected individuals in the general population which is likely to differ from one Member State to another.

There is no evidence that individuals working in hospitals have developed vCJD by virtue of their occupation. Transmission of TSEs during surgical procedures remains a major concern, but to date there is no evidence that it has actually occurred in relation to vCJD. Surgical iatrogenic transmission of CJD has been observed after dura mater transplantation, neurosurgery and ophthalmic surgery. To minimize the risk of transmission of vCJD by surgical instruments, procedures (see 3.5) are recommended based on the probability of the patient under investigation/treatment being infected with vCJD (or any other TSE).

- For clinical vCJD patients potentially contaminated instruments must be destroyed.
- For patients at high risk single use instruments are recommended, alternatively cleaning plus chemical inactivation plus physical inactivation for an activity with high risk, or cleaning plus chemical inactivation or physical inactivation for an activity with low risk may be used.
- For patients not deemed to be at risk for TSE, cleaning plus chemical inactivation or physical inactivation for an activity with high risk, or cleaning plus classical disinfection or sterilization for an activity with low risk may be used.

In view of contact with blood of potential infectious patients, besides surgery also dental procedures may be considered a potential route of transmission. Some dental procedures involve contact with nerves.

Question 2:

- to evaluate the risk of vertical transmission of vCJD in pregnant women

There are no proven instances of vertical transmission of any human prion disease. However, the available animal and human data are inadequate to allow firm conclusions concerning vertical transmission to be drawn. It is recommended that there is a follow-up for children that are born to mothers who had or developed clinical vCJD. There are no data indicating that breast milk transmits human prion disease but the relevance of limited data on other TSEs to vCJD is not known.

Question 3:

- to evaluate the risk of vCJD transmission by the tissues stored in umbilical cord cell banks.

As cord blood is entirely fetal in origin, and as there are no proven instances of vertical transmission, there is no indication that prion infectivity is transmitted by cord blood cells. However, contamination with maternal blood during collection remains a possibility.

Conclusions:

As long as there is a risk that infectious prion protein ($PrP^{Sc}$ or $PrP^{Res}$) is present in blood and blood components, there will be a risk of transmission of vCJD disease by transfusion. Blood transfusion appears the most likely route for inter-human transmission of vCJD, although other routes of transmission also should be considered like surgery, dentistry, and organ or cell transplants. The Committee does not consider that additional specific measures are needed to reduce the risk from vCJD infectivity in blood. In the UK and some other countries

measures have already been taken including donor exclusion of blood transfusion recipients, leucodepletion, import of fresh frozen plasma, and reduction of amounts of plasma in blood components for transfusion. When there is a concern for spreading of vCJD by blood transfusion, donor exclusion of blood transfusion recipients is the appropriate measure. In addition, there are good practices to reduce any risk for transmission of infectious diseases such as optimal use of the transfusion to reduce the number of patients exposed, and optimal blood donation techniques and blood transfusion practices which minimize the number of blood donors to which an individual patient is exposed.

## 5 MINORITY OPINION

Not applicable.

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[3] Declared Interest (see minutes of the SCENIHR plenary meeting of 28-29 September 2005 : http://europa.eu.int/comm/health/ph_risk/committees/04_scenihr/docs/scenihr_mi_007.pdf).

## GLOSSARY

**CJD** Creutzfeldt-Jakob Disease

**BSE** Bovine spongiform encephalopathy

**TSE** Transmissible spongiform encephalopathy

**sCJD** Sporadic CJD, the commonest form of human prion disease

**vCJD** Variant CJD, the human prion disease thought to be due to transmission of BSE from cattle to man

**Prion** Infectious agent of TSE

**PrP (prion protein)** A protein of uncertain function that exists in a normal form (in health) and also an abnormal form (in prion diseases)

**$PrP^{C}$** The normal prion protein (c=cellular)

**$PrP^{Sc}$** The abnormal form of the prion protein associated with prion diseases (Sc= scrapie)

**$PrP^{Res}$** The protease resistant core of $PrP^{Sc}$ that remains after treatment with proteases such as proteinase K. Immunocytochemical detection methods generally detect $PrP^{Res,}$ rather than $PrP^{Sc,}$ but the presence of $PrP^{Res}$ is obviously a marker of the presence of $PrP^{Sc}$ ,

**NCJDSU** The UK National CJD Surveillance Unit