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bjh review

An update on the assessment and management of the transmission of variant Creutzfeldt-Jakob disease by blood and plasma products

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Summary

There have been four highly probable instances of variant Creutzfeldt-Jakob disease (vCJD) transmission by non-leucocyte depleted red cell concentrates and it is now clear that the infectious agent is transmissible by blood components. To date there in no reported evidence that the infectious agent has been transmitted by fractionated plasma products, e.g. factor VIII concentrate. This review outlines current and potential risk management strategies including donor deferral criteria, the potential for donor screening, blood component processing and prion reduction filters, plasma product manufacture and the difficulties in identification and notification of those considered 'at risk of vCJD for public health purposes'.

Keywords: Creutzfeldt-Jakob disease, blood, plasma products.

This review offers an update on our recent assessment and management of the risk of transmission of variant Creutzfeldt-Jakob disease (vCJD) by blood components and plasma products (Ludlam & Turner, 2005). As that review surveyed perceptions on the nature of the prion agent, the spectrum of prion diseases in animals and man, and the range of animal studies relating to pathogenicity and infectivity (much of which still represents the current level of knowledge), these topics are not reviewed again here, other than where significant new relevant studies have been published. This current review focuses on the state of the art in relation to the safety of blood components and plasma products, which has also been reviewed elsewhere (Farrugia *et al*, 2005; Dolan, 2006; Ironside, 2006 and Clarke *et al*, 2007).

To date, a total of 203 probable, or definite, cases of vCJD have been reported worldwide, of which 166 have arisen in the UK, 23 in France, four in Eire and Spain, three in the USA, and one in each of Holland, Portugal, Italy, Saudi Arabia, Japan and Canada (http://www.cjd.ed.ac.uk/vcjdworld.htm).

Of these, two of the [illegible] and Japan are thought to have [illegible] third US case is thought to have [illegible] The other cases are thought to [illegible] countries of origin either during [illegible] or exported animals or animal [illegible] vCJD appears to have reached a [illegible] has waned such that in 2007 in [illegible] though the frequency of new c[illegible] France and Spain. All clinically [illegible] have been methionine homozygo[illegible] protein gene (*PRNP*). Mathematic[illegible] current incidence of vCJD might [illegible] estimate of 70 further cases [illegible] (Clarke & Ghani, 2005). The [illegible] estimate, however, if individuals [illegible] are also capable of being infectio[illegible] sions occur from asymptomatic [illegible]

Two observations give pause for [illegible] median age of onset of clinica[illegible] altered over the past 10 years as [illegible] individuals were exposed to infec[illegible] of time. The best estimates [illegible] related exposures [illegible] second in the early [illegible] appendices (Hilton [illegible] showed evidence [illegible] maximum likelihood estimat[illegible] discrepancy between the estim[illegible] clinical incidence [illegible] around 85% of [illegible] pre- or sub-clinical infection. Cl[illegible] consistent with [illegible] studies in patients who [illegible] suggest that individuals [illegible] homozygous at codon 129 [illegible] and a lower incidence of dev[illegible] those who are codon 129 [illegible] observations give rise to concern [illegible] cohort of individuals, maybe a n[illegible] population in the UK may have [illegible]

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First published online 20 October 2008
doi:10.1111/j.1365-2141.2008.07376.x

and so at risk of transmitting the disease through blood and tissue products or surgical and medical instrumentation, despite being asymptomatic themselves.

As there is no currently accepted blood test that reliably identifies vCJD infected individuals (see below), further studies have been carried out to try to refine the estimate of the prevalence of sub-clinical disease. The National Anonymised Tonsil Archive aims to test 100 000 tonsil samples. Currently, there have been no confirmed positive samples out of 43 000 tested (http://www.hpa.org.uk/infections/topics_az/cjd/tonsil_archive.htm). However there are reservations around the interpretation of these data, given that the sensitivity of the assays in detecting subclinical vCJD is uncertain, the frequency of involvement of the tonsil as a site of peripheral infection is unknown, and a large proportion of the study population are too young to have been exposed to dietary bovine spongiform encephalopathy (BSE). The Spongiform Encephalopathy Advisory Committee (SEAC) has therefore not felt it appropriate to amend the current prevalence estimates within the UK at present (http://www.seac.gov.uk).

Infectivity in the peripheral blood

Infectivity remains undetectable in the peripheral blood of patients with vCJD despite the fact that clinical transmission has clearly occurred. This apparent contradiction is probably explained by the presence of a species barrier between man and mice and the limited volumes of blood that can be inoculated in these models.

Studies in hamsters infected with the 263K strain of scrapie have yielded similar results to those in the Fukuoka-1 GSS strain in mice (Brown *et al*, 1998; Ludlam & Turner, 2005), with a point estimate of 1·6 infectious doses (ID)/ml of whole blood of which the plasma (50%) was associated with the leucocytes and most of the remainder in the plasma (Gregori *et al*, 2004). Further studies in this model suggest that the majority of cell-associated infectivity is only loosely bound and can be washed off and therefore that the plasma form of infectivity probably predominates. Further studies in mice suggest that the level of infectivity is similar in vCJD-infected animals (Cervenakova *et al*, 2003). Studies in sheep naturally infected with scrapie, or experimentally infected with BSE, suggest a transmission frequency of up to 50% from blood taken during the preclinical or clinical phase of disease and transfused into recipients from a scrapie-free flock (Hunter *et al*, 2002). BSE has also been transmitted through buffy coat to the primate *Macaca fascicularis* (Houston *et al*, 2002).

Variant CJD transmission by blood transfusion

Within the UK, the Transfusion Medicine Epidemiology Review (TMER) has proved an effective system for collating evidence of possible transmission of vCJD by blood components (Llewelyn *et al*, 2004). The UK CJD Surveillance Unit in Edinburgh shares information about new cases of vCJD with the Blood Transfusion Services, which search their databases to ascertain whether these patients have been blood donors in the past. In this event attempts are made to identify the fate of the blood components (http://www.cjd.ed.ac.uk/TMER) and trace, notify and monitor living recipients. The 'reverse' arm of the TMER study attempts to identify which individuals who develop vCJD have received blood transfusions and to identify the donors.

Eighteen patients with vCJD have, or had previously, been blood donors, from whom a total of 66 recipients have been identified, 26 of whom are still alive. Of those who have died, four cases of transmission of vCJD prions have been identified (see below). Many of these patients however will have died of their underlying conditions within 5 years of the implicated transfusion and will not have had time to show clinical evidence of vCJD if infected.

The first symptomatic case of vCJD disease associated with blood transfusion was identified in December 2003. This individual developed vCJD 6·5 years after transfusion of red cells donated by an individual who developed symptoms of vCJD 3·5 years after donation (Llewelyn *et al*, 2004).

A second case of transmission was identified a few months later in a recipient of red cells from a donor who developed symptoms of vCJD 18 months after donation. This patient died from causes unrelated to vCJD 5 years after transfusion. Postmortem investigations found abnormal prion protein accumulation in the spleen and a cervical lymph node, but not in the brain, and no pathological features of vCJD were found (Peden *et al*, 2004).

A third patient developed symptoms of vCJD 6 years and died 8·7 years after receiving a transfusion of red blood cells from a donor who developed vCJD about 20 months after this blood was donated (Health Protection Agency 2006).

The fourth case of transmission developed symptoms of vCJD 8·5 years after receiving a transfusion of red blood cells from a donor who developed vCJD about 17 months after this blood was donated. The donor to this patient also donated the vCJD-implicated blood transfused to the third patient (Editorial Team, 2007).

All four patients received transfusions of non-leucodepleted red blood cells between 1996 and 1999. Since October 1999, leucocytes have been removed from all blood used for transfusion in the UK.

These data therefore demonstrate clearly that non-leucodepleted red cells from asymptomatic individuals incubating vCJD can transmit the infection by blood transfusion to other individuals and that the risk of them doing so is relatively high.

Donor deferral criteria

There has been little substantive change in blood donor criteria since our previous review (Ludlam & Turner, 2005). Whilst other countries continue to defer those who have spent more than a specified cumulative period of time in the UK, within the UK only those considered by the CJD Incidents Panel to be 'at risk of vCJD for public health purposes' on account of exposure to implicated surgical instruments, blood components or plasma products, and those who themselves have received blood components, are deferred (http://www.hpa.org.uk/infections/topics_az/cjd). There is considerable complexity relating to the introduction of similar donor deferral criteria in the context of cell, tissue and organ donation. Broadly, whilst all forms of donation are excluded for patients with CJD or those considered potentially infected, donation of haematopoietic stem cells and solid organs is permitted from those considered 'at risk for public health purposes' and those previously transfused, subject to a risk assessment that weighs the risk of vCJD transmission against the potentially life-saving nature of an otherwise suitable transplant. Donation of other tissues is based on the same donor deferral criteria as blood. Donor deferral criteria remain, however, blunt risk management tools with potential deleterious effects on blood, tissue and organ supply.

Importation of blood components

Since our last report (Ludlam & Turner, 2005), the use of imported methylene-blue treated fresh frozen plasma (FFP) has been extended to all patients under the age of 16 years and to high users. Solvent detergent-treated FFP is recommended for patients undergoing plasma exchange for thrombotic thrombocytopenic purpura on the grounds that there is some evidence to suggest that methylene-blue treated FFP has a deleterious impact on outcome in this patient group (Alvarez-Larran *et al*, 2004). Consideration continues to be given around the possibility of importing FFP and cryoprecipitate for additional groups of patients. Importation of platelets is likely to be impractical given the short shelf-life of these products. However, it may be possible to import red cell concentrates for some groups of patients, for example for children up to 16 years of age. Consideration also has to be given to cost, quality and regulatory requirements and countervailing risks of transmission of other infectious diseases or of component shortages.

Advances in the development of a screening test

As previously noted (Ludlam & Turner, 2005), neither nucleic acid transmission nor immunological responses have been clearly identified in association with transmission of prion diseases, rendering standard molecular and serological screening assays unfeasible. Surrogate markers, such as 14-3-3, S100 and erythroid differentiation-related factor, have thus far proved insufficiently sensitive and specific to be of clinical value. Considerable progress has however been made in the development of assays for the abnormal conformer of prion protein, PrP^{TSE}.

Normal prion protein (PrP^{C}) is a widely expressed 35 kDa 230 amino acid glycosyl-phosphatidylinositol anchored mem-

Immunocapillary electrophoresis was amongst the first methods that claimed to be able to detect PrP^{TSE} in the peripheral blood. The test material is treated with proteinase and subject to a competitive antibody inhibition assay using a labelled peptide (as the competitor) and a monoclonal antibody that recognises both PrP^{TSE} and the peptide (Schmerr *et al*, 1999; Yang *et al*, 2005). The technique has however proved difficult to reproduce in other laboratories and failed to discriminate between infected and uninfected blood samples in a blinded study (Cervenakova *et al*, 2003b).

Epitope unmasking/masking. More success has been achieved with the conformation-dependent immunoassay (CDI), which is predicated on the observation that some PrP epitopes are masked within the PrP^{TSE} aggregate. An increase in signal intensity produced by a labelled monoclonal antibody by a sample denatured using guanidine hydrochloride when compared with the native (un-denatured) sample denotes the presence of PrP^{TSE} (PrP^{C} gives the same signal intensity under both conditions). The sensitivity of the technique is increased through the use of highly sensitive dissociation-enhanced lanthanide fluorescence immunoassay for antibody detection and, in some versions of the assay, the use of PK to reduce background signal (Safar *et al*, 1998, 2002). CDI appears to achieve greater sensitivity than immunoblot (Bellon *et al*, 2003) and, in the format including PK, may approximate the sensitivity of infectivity assays (Bruce *et al*, 2001). In the absence of PK it appears able to detect PK-sensitive forms of PrP^{TSE}, though it remains unclear as to whether these are infectious or not (Bellon *et al*, 2003).

The epitope-protection assay developed by Amorfix uses a chemical modification process which alters epitopes on normal PrP but not those buried within PrP^{TSE} aggregates. The latter are then disaggregated and the conserved epitopes detected using immunodetection methods (http://www.amorfix.com).

PeopleBio have developed an approach where a single antibody is used for both capture and detection steps leading to the blocking of available epitopes by the capture of PrP^{C} but not PrP^{TSE}.

PrP^{TSE}-specific monoclonal antibodies. Several antibodies have now been developed that appear to be specific for conformation-dependent epitopes present in PrP^{TSE} but not PrP^{C} (Korth *et al*, 1997; Paramithiotis *et al*, 2003; Curin Serbec *et al*, 2004; Zou *et al*, 2004). On these, the antibody 15B3, described by Korth *et al* (1997) and manufactured by Prionics, is the best characterised and has proved capable of detecting infectivity in the peripheral blood of scrapie-infected sheep and BSE-infected cattle in the absence of PK digestion (http://www.fda.gov/ohrms/dockets/AC/06/slides/2006-4240S1_9.ppt). Three other antibodies (Paramithiotis *et al*, 2003; Curin Serbec *et al*, 2004; Zou *et al*, 2004) also appear specific to PrP^{TSE} but have not yet been translated to routine assay format.

PrP^{TSE} – specific ligands. A variety of other ligands have been shown to bind selectively to the abnormally conformed molecule. Plasminogen has been proposed as a means of selective binding PrP^{TSE}, but as it can also bind to a variety of other proteins it is therefore unlikely to be sufficiently specific for assay development (Fischer *et al*, 2000).

Polyanionic compounds are known to selectively bind PrP^{TSE} and this property has been employed in the Seprion assay (Lane *et al*, 2003), which uses coated magnetic beads to capture the molecule. The assay is not dependent on PK treatment and is not species-specific provided a suitable detection antibody is used. It is licensed for postmortem diagnosis of BSE and Chronic Wasting Disease and is reported to be able to distinguish between infected and uninfected blood in scrapie-infected sheep and a small number of human samples.

The approach developed by BioMerieux involves PK digestion, precipitation and denaturation followed by reticulation by streptomycin, chemical capture by calyx-6-arene and detection of the macromolecular aggregates by labelled monoclonal antibody (http://www.fda.gov/ohrms/dockets/AC/06/slides/2006-4240S1_9.ppt). Detection of PrP^{TSE} in a small number of plasma samples from scrapie-infected sheep, BSE-infected cattle and CJD-infected humans has been reported.

Adlyfe have developed a third approach utilising a synthetic peptide based on the region of the PrP molecule involved in the PrP^{C}–PrP^{TSE} conformational transition. The peptide sequence is coupled to its mirror image as a palindromic molecule fluorescently labelled at each end. When incorporated into PrP^{TSE} the peptide folds into a hairpin with a beta-sheet conformation and the flurophores stack and change their fluorescence wavelength. Further, the folded ligand induces further molecules to adopt the folded conformation and thus amplifies the signal (Grosset *et al*, 2005). The assay is reported to have discriminated infected from uninfected plasma in natural and experimental scrapie, BSE and CJD.

Chiron have utilised (http://www.fda.gov/ohrms/dockets/AC/06/slides/2006-4240S1_9.ppt) a synthetic PrP polypeptide to capture PrP^{TSE} on magnetic beads with detection by monoclonal antibody in an ELISA format.

Amplification. Two methods have been used to amplify the detection signal. Screening for intensively fluorescent targets utilises double labelled antibodies, more of which bind to PrP^{TSE} aggregates than to PrP^{C} and giving rise to a stronger fluorescence signal (Bieschke *et al*, 2000). Immuno-polymerase chain reaction (PCR) also provides a method of amplifying the signal from an antibody or ligand conjugated to a nucleotide sequence utilising the PCR (Barletta *et al*, 2005).

Two further approaches have been developed that result in the amplification of PrP^{TSE} itself. The first of these, protein misfolding cyclic amplification (PMCA) has given rise to considerable excitement. PrP^{TSE} seeded into an excess of PrP^{C} leads to formation of new PrP^{TSE}. That PrP^{TSE} is then

fragmented through sonication or shaking and leads to a new round of PrP^{TSE} formation (Kocisko *et al*, 1994; Saborio *et al*, 2001). Recurrent cycles therefore of incubation and fragmentation lead to amplification of the original PrP^{TSE} (Castilla *et al*, 2005). Immunoblot and CDI have been used for detection of PrP^{TSE} and infectivity. Studies show that 140 sonication cycles produced an increase in signal intensity of around 6000-fold, whilst a second 'nested' set of 118 cycles with a fresh source of normal PrP led to an approximate 10^7-fold amplification. The technique has proved capable of discriminating infected from uninfected blood from hamsters experimentally infected with scrapie, however there are recent reports of detection of PrP^{TSE} in uninfected animal brain implying the possibility of low levels of abnormally conformed PrP in 'normal' individuals.

A number of cell-based amplification techniques have been described in which the rodent cell lines N2a (Nishida *et al*, 2000), PK-1 (Klohn *et al*, 2003), Rov9 (Birkett *et al*, 2001) and CAD-5 are infectable by natural or experimental strains of scrapie and demonstrate amplification of PrP^{TSE} detected by immunoblot. No cell-based amplification has yet been successfully reported for CJD.

Both these kinds of amplification take several days (PMCA) to weeks (cell-based assays) and would therefore be better positioned as confirmatory rather than screening assays.

Considerations with regard to assay assessment. Whilst the above is not a comprehensive list of all the assays under development, it does provide a flavour of the range and variety of approaches and their relative strengths and weaknesses. Some of these are now approaching the point at which they may be Council of Europe (CE) marked and marketed as potential clinical assays. There are, therefore, a series of further considerations relating to the potential assessment and utility of prion assays prior to clinical implementation.

The required sensitivity is difficult to gauge because the level, spatial distribution and temporal variation of infectivity in the blood of patients with vCJD or healthy individuals with subclinical infection is unknown. The generalizability of experimental data from mouse and hamster experiments to the human condition cannot be assumed (Castilla *et al*, 2006). Moreover, the relationship between infectivity and PrP^{TSE} is complex. Although many authorities believe PrP^{TSE} to be

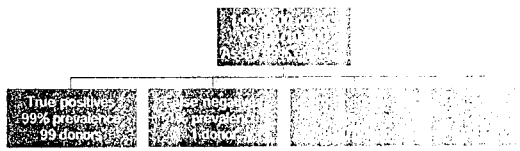

Fig 1. Outcome of screening of a 'normal' population of one million [illegible] variant Creutzfeldt-Jakob disease using an assay with a sensitivity and specificity [illegible] number of false positives greatly exceeds the number of true positives.

[illegible] should not be allowed to continue to donate if they [illegible] it is illegal for example to take the donation [illegible] even if the donor consents to such a [illegible]. Test positive individuals will therefore have to be told of this outcome and (presumably) managed as 'at risk for public health purposes'. Clearly this will cause significant distress and may give rise to psychological and social problems for some people, as well as a disincentive to blood donation and therefore a negative impact on the blood supply. Moreover, it is likely that previous recipients of blood components from donors [illegible] will also have to be traced and contacted (hopefully), giving rise to a much larger group of individuals in the population considered 'at risk of public health purposes' and requiring public precautionary measures to be taken in the event of subsequent medical investigation (see below). A comprehensive health and economic evaluation will therefore have to weigh the positive impact of reducing potential secondary transmission of vCJD against these potential negative consequences.

Blood component processing

Universal leucodepletion was introduced in the UK in 1999 as a measure to reduce the risk of secondary transmission of vCJD. The experimental data from mice infected with the Fukuoka-1 strain of Gerstmann-Sträussler-Scheinker disease (Asante *et al*, 2002) suggests that leucodepletion filters have little impact on plasma-borne infectivity. Studies in the 263K hamster model (Gregori *et al*, 2004) similarly suggest a 4–5 log reduction in whole blood infectivity, consistent with the removal of leucocyte-associated infectivity, but not that present in the plasma. Table I illustrates the likely distribution of residual infectivity in a unit of leucodepleted red cell concentrate prepared by bottom and top processing method (with a residual plasma volume of around 10–15 ml). Assuming 10 ID/ml infectivity in whole blood, just over 130 ID would be left in the unit and that up to a 3 log further reduction is required to impact upon the risk of transmission (i.e. achieve <1 ID/unit). Red cell concentrates prepared by the more common buffy-coat methodology contain greater amounts of residual plasma (around 20 ml) and would consequently require a 4 log reduction. The absence of data on the level of infectivity in human blood means an uncertainty of at least 1 log at any of these point estimates. It can be said in summary, however, that it is unlikely that current blood component processing will suffice to reduce the risk of transmission in more plausible infectivity scenarios.

Three companies are working on the development of prion reduction filters. One has a CE-marked dead-on filter which is used in series with a leucodepletion filter. Published studies using this filter material show >3 log reduction in infectivity on brain homogenate spikes and to the limit of detection (3–4 log) in exogenous infectivity studies (Gregori *et al*, 2006). Two other companies are working on the development of combined leucodepletion/prion reduction filters. All prion reduction filters will have to undergo independent assessment of clinical safety and efficacy within a series of studies managed by the UK and Irish Blood Services and agreed with SEAC and the Advisory Committee on the Safety of Blood, Tissues and Organs (http://www.advisorybodies.doh.gov.uk/acsbto/index.htm). Part of the problem for both manufacturers and Blood Services is the absence of assays capable of detecting either PrP^{TSE} or infectivity in the peripheral blood of patients with vCJD. Assessment of the efficacy of the technology is therefore based on brain homogenate spikes (where baseline infectivity is sufficient to detect a 3–4 log reduction but the physico-chemical form of the spike is unlikely to be similar to that of plasma based infectivity), and endogenous infectivity studies (where the form of infectivity is likely to be more relevant, but the baseline infectivity is sufficiently low that little more than a 1-log reduction is detectable). There remain, therefore, fundamental questions relating to the clinical relevance of different forms of spike material and general applicability of these kinds of studies to the human situation. The potential for deleterious effects on the red cell concentrate itself are also a matter for concern, both in terms of the possibility of alterations to the rheological or antigenic profile of the red cells and the loss in the volume of the additional filter. The latter would have a particular impact if used in conjunction with bottom and top processing, the combined effect of which may reduce the red cell mass in a concentrate below current standards, necessitating additional transfusions for some individuals.

With regard to platelet concentrates, re-suspension in optimal additive solution rather than plasma would reduce the amount of residual plasma by around 65% to 80–90 ml. This would still contain more than enough infectivity to transmit infection to the recipient under even the most optimistic of the current infectivity assumptions and is likely to be ineffectual. Prion reduction filters are not currently applicable to either platelet concentrates or FFP.

Table I. Residual infectivity distribution in a unit of leucodepleted red cell concentrate.

Log reduction in infectivity	Residual leucocytes	Residual plasma	Residual infectivity
Leucodepletion alone	0·2	130	130·2
1 Log	0·2	13	13·2
2 Log	0·2	1·3	1·5
3 Log	0·2	0·13	0·33
4 Log	0·2	0·013	0·213

The data represents the likely distribution of residual infectivity in a unit of leucodepleted red cell concentrate prepared by a bottom and top processing method (with a residual plasma volume of around 10 ml).

Assuming 10 ID/ml infectivity in whole blood with 40% (i.e.4 ID/ml) being removed by leucodepletion and the remainder residing in the plasma (i.e. for a haematocrit of 0·45 a plasma concentration of approximately 13 D/ml), around 130 ID remains in the unit's plasma. Hence up to approximately a 3 log further reduction is required to reduce the risk of transmission to <1 ID/unit.

Plasma product manufacturing

It is reassuring that to date no recipient of a pooled plasma product has developed vCJD. However in 1997, shortly after the first description of vCJD as a new condition, there was concern that the UK plasma supply might have the potential to transmit the infectious agent and that plasma collected from countries where there were few or no cases of vCJD might pose a lower risk (Ludlam, 1997). Although this view gave rise to controversy, the regulatory authorities moved to a position of allowing, and subsequently mandating that pooled plasma products manufactured in the UK should only be made from plasma imported from parts of the world at low risk of vCJD.

In an attempt to help define the risk of PrP^{TSE} transmission by plasma-derived products, detailed studies have been undertaken to assess how prions are partitioned during the plasma fractionation process, mainly by spiking the starting plasma with 'exogenous' prion derived from brain homogenates of experimentally infected animals. The strengths and weaknesses of this approach are similar to those described above in the discussion around the assessment of prion filters. In general there was least clearance of prion in the manufacture of factor VIII, IX and antithrombin concentrates, greater clearance in the preparation of intravenous immunoglobulin, and greatest clearance in the manufacture of albumin (Foster *et al*, 1999).

The way in which different countries responded to the risk that plasma products might transmit the infectious agent varied and depended partly on the perceived relative number of donors who might be infectious as well as details of the plasma fractionation techniques used in each country.

In the UK, using data on partitioning of prion infectivity during manufacture of plasma products, along with the animal data on the likely range of infectivity in individuals with subclinical infection, a risk assessment was undertaken to quantify the risk of recipients of such products being infected. The CJD Incidents Panel have taken the view that an individual with a >1% additional risk of exposure to an infectious dose of vCJD should be notified and managed as 'at risk for public health purposes'.

To date a total of 174 'implicated' batches of plasma products have been identified as having been manufactured from a pool of plasma to which an individual contributed who subsequently developed vCJD (Hewitt *et al*, 2006). For each of these batches a detailed risk assessment was carried out that included the total number of donations included in the pool, the details of the plasma fractionation process used during manufacture and (conservative) estimates of the likely cumulative reduction in infectivity over the manufacturing process. The outcome was expressed as the likely mass of product to which an individual would have had to be exposed to increase

difficulties in the performance of biopsies with gastrointestinal endoscopes because the samples obtained would probably contain lymphoid tissue. The financial implications are significant because the endoscopes cannot be decontaminated and must effectively be discarded. Both upper and lower gastrointestinal endoscopies without biopsy do not result in the instrument being considered as potentially 'contaminated' and it can therefore be reused on other patients after standard cleaning procedure. The concern about possible contamination of instruments has also led to an increased use of capsule endoscopies, which give good images but cannot be used to biopsy or treat gut lesions.

Although no individuals with haemophilia have thus far developed vCJD and a retrospective study of autopsy samples from individuals with haemophilia in 1998 showed no evidence of sub-clinical infection, it has been important to try and gather more data (Lee *et al.* 1998). This has not been easy and depends upon procuring appropriate tissue samples prospectively from individuals undergoing clinically necessary surgery in addition to consent for autopsy. In addition it has been important to try and develop a record of the extent of exposure of individuals to 'implicated' batches of concentrate, as well as all recipients of UK clotting factor concentrates over the 22-year period of exposure. This is being co-ordinated by UK Haemophilia Centre Doctors' Organisation by accumulating the data for subsequent anonymised studies.

Communication with patients and the general public

Keeping recipients of blood and blood products informed about the current state of knowledge and in particular informing individuals about their individual risks has proved challenging because of the complexity and uncertainty inherent in our understanding of the field. It has been important for there to be close collaboration between those able to assess the risk of vCJD infection, physicians responsible for clinical services and patient organisations representing those potentially affected. For those who have received blood components from donors who subsequently developed vCJD, the risk of exposure to vCJD is judged to be high and these individuals have been contacted on an individual basis and offered counselling and specialist follow-up. Similarly, blood donors who have donated blood administered to a patient who later developed vCJD have been contacted and are managed as 'at risk for public health purposes'. In 2004, all patients with haemophilia were sent a letter stating whether or not they had or had not received UK plasma-derived clotting concentrates between 1980 and 2001, irrespective of whether or not they had received UK plasma products, because in an earlier mailing about this topic only those in the 'at risk' group were contacted and this left non-recipients of letters not knowing whether they had not been potentially exposed or whether their letter had got lost in the post. All were offered the opportunity for individual counselling. It is this attention to the detail of how patients are informed that is critical in trying to ensure that individuals feel confident in the arrangements.

For patients potentially exposed to other implicated plasma products, the issue of traceability and notification have proved more problematic. Whilst patients with primary immunodeficiency share a similar close long-term relationship with their physicians, those receiving immunoglobulin for other clinical indications or high doses of albumin (for example during plasma exchange), are often discharged following their acute care. The absence of a general system of traceability for plasma products and of searchable clinical notes has made the follow-up of the latter groups of potentially exposed patients highly problematic.

Concluding remarks

Three years after our last review (Ludlam & Turner, 2005), the management of the risk of transmission of vCJD by blood and plasma products remains highly challenging. Whilst the diminishing number of clinical cases is reassuring, there are continuing uncertainties surrounding the prevalence of sub-clinical disease, the level of infectivity in peripheral blood of such individuals, and the overall risk of transmission and development of clinical disease. Much progress has been made in the development of new technologies, such as prion filters and prion assays, but assessment of these is problematic and cost and countervailing risks need to be considered. Accurate and timely communication with the general public and with those who are considered to be at increased risk of exposure remains essential given the continuing complexity and uncertainty of the field.

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bjh review

Managing the risk of transmission [illegible] disease by blood products

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Summary

Whereas plasma-derived clotting factor concentrates now have a very good safety record for not being infectious for [illegible] enveloped viruses, concern has arisen about the possibility that prion diseases might be transmitted by blood products. There is epidemiological evidence that classical sporadic Creutzfeldt-Jakob disease (CJD) is not transmitted by blood transfusion. There is now good evidence that the abnormal prion associated with variant CJD can be transmitted by transfusion of fresh blood components and infect recipients. To reduce the risk of the pathological prion in the UK infecting recipients of clotting factor concentrates, these are now only manufactured from imported plasma collected from countries where there has not been bovine spongiform encephalopathy (BSE) in cattle and the risk of variant CJD in the population is, therefore, considered negligible. The safety of these concentrates is [illegible] enhanced because prion protein is, to an appreciable extent, excluded by the manufacturing process from the final product. To help reduce the chance of prion transmission by fresh blood products, donations are leucodepleted, there is increasing use of imported fresh frozen plasma (especially for treating children) and potential donors, who have been recipients of blood since 1980 (the beginning of the BSE epidemic in cattle), are deferred.

Keywords: variant Creutzfeld Jakob disease, transfusion, epidemiology, safety, haemophilia.

Emerging pathogens will always challenge the safety of blood transfusion. Whilst the risk of hepatitis B virus (HBV), hepatitis C virus (HCV) or human immunodeficiency virus (HIV) transmission by blood components and plasma products is now small (http://www.eurosurveillance.org), new potentially transfusion-transmissible pathogens continue to emerge.

Many challenges were posed by the emergence of variant Creutzfeldt Jakob disease (CJD) in 1996 (Will *et al*, [illegible]

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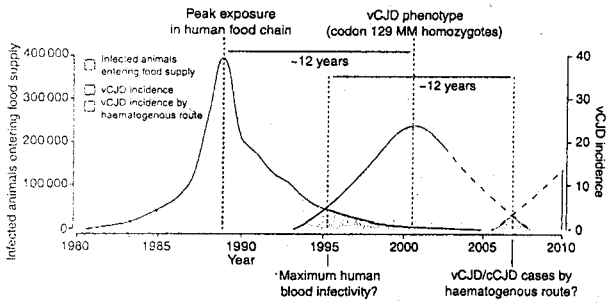

Fig 1. Incidence of bovine spongiform encephalopathy and variant Creutzfeldt Jakob disease in the UK (- - - -, predicted cases). The right hand peak illustrates the potential for secondary spread by haematogenous spread. Reprinted from Collins *et al* (2004) with permission from Elsevier.

addition, a critical polymorphism at codon 129 coding for methionine or valine leads to significant variation in the susceptibility to, and incubation period of, human prion diseases. In the UK, 37% of the general population are homozygous for methionine at this locus, 11% are homozygous for valine and 52% heterozygous. Methionine homozygosity is much more common than expected amongst patients with CJD (*vide infra*). PrP is inserted into the cell membrane predominantly via a glycosylphosphatidyl inositol (GPI) anchor, although transmembrane and soluble forms have also been described. The glycoprotein is predominantly located in calveolar zones in the cell membrane and is estimated to have a half-life of around 6 h, being internalised into endosomes with a proportion recycling to the cell surface (Shyng *et al*, 1993). The function of the protein remains unclear, it has been shown to bind to a laminin receptor precursor protein (Martins *et al*, 1997; Rieger *et al*, 1997) and act as a copper metalloproteinase (Brown *et al*, 1997a). PrP null mice appear to develop normally although some strains show subtle neurological abnormalities (Tobler *et al*, 1996). Prion formation involves changes in the secondary and tertiary conformations of the PrP molecule: up to 40–50% of the molecule can be in the form of beta-pleated sheet, mainly at the expense of the membrane-distal unstructured region. This changes the physicochemical properties of the molecule and engenders relative resistance to proteinase digestion. Prion protein aggregates (PrP^{Sc}) are deposited in cells and tissues leading to the formation of amyloid-like plaques and in the nervous system to neuronal death, astrogliosis and spongiform change.

The mechanism by which PrP^{c} is converted to PrP^{Sc} remains unclear, as does its precise role in the aetiology of the disease. The prion hypothesis (Prusiner, 1998) proposes that the PrP^{Sc} molecule itself converts PrP^{c} to the abnormal conformation, either through a process of heterodimerisation or through nuclear polymerisation (Aguzzi & Weissmann, 1997). PrP^{Sc} is relatively resistant to proteinase-K digestion and different molecular strains of disease can be identified by the balance of di-glycosylated, mono-glycosylated and non-glycosylated species. Several molecular strains of PrP^{Sc} occur in sporadic CJD; however, only a single strain of PrP^{Sc} is found in variant CJD, which is similar to that seen in naturally occurring bovine spongiform encephalopathy (BSE) in cattle, and BSE transmitted naturally and experimentally to other animals (Collinge *et al*, 1996; Hill *et al*, 1997a). Evidence that variant CJD and BSE represent the same strain of prion disease also stems from infectivity studies in a prion disease strain typing panel of inbred experimental mice, where the patterns of incubation period and neuropathological targeting were similar and differed from those seen in sporadic CJD, scrapie and other prion diseases (Bruce *et al*, 1997).

Prion diseases in other species

A range of prion disorders have been described including those involving the SuP35p and Ure2p proteins in yeast, which appear to be non-pathogenic and convey a survival advantage under certain circumstances (Burwinkel *et al*, 2004).

Scrapie was first described as a disease of sheep and goats over 250 years ago and demonstrated to be experimentally transmissible 50 years ago (Aguzzi & Polymenidou, 2004). There is no evidence that scrapie has ever transmitted to man. The only other known self-sustaining animal prion disease is chronic wasting disease in mule deer and elk in several states of the USA. Again there is no current evidence that this disease has transmitted to man.

BSE was first described in UK cattle in 1985 (Wells *et al*, 1987) and is thought to have spread through oral consumption of ruminant-derived meat and bone meal (Wilesmith *et al*, 1988; Brown, 1998). The disease spread widely, peaking in 1992 with over 180 000 clinical cases in the UK, although mathematical estimates suggest that 1–2 million cattle could have been infected but slaughtered and entered the human food chain before they were old enough to demonstrate evidence of clinical disease (Fig 1) (Anderson *et al*, 1996). BSE has crossed into up to 20 other species, including domestic and exotic cats (Wyatt *et al*, 1991; Kirkwood & Cunningham, 1994) and exotic ungulates in British zoos. In July 1988, the spread of BSE led the UK Government to restrict the use of ruminant-derived meat and bone meal as an animal feed and in November 1989 specified that bovine offals were banned for human consumption.

Sporadic Creutzfeldt Jakob diseases

Sporadic CJD was the first described human prion disease, is of uncertain aetiology, has a worldwide distribution and an incidence of around one per million population per year (Will *et al*, 1998). The median age at onset is around 68 years and the disease is characterised by a rapidly progressive dementia leading to death in around 4–6 months. The incidence of the disease varies with the codon 129 genotype of the *PRNP* gene, with 83% of patients homozygous for the expression of methionine at this locus (Deslys *et al*, 1998). Molecular strain typing suggests that six forms of disease are dependent on codon 129 phenotype and strain of prion disease. One of the pathological hallmarks of sporadic CJD is the restriction of accumulation of plaques of prion protein to the central nervous system (CNS). However, with recently developed, more sensitive techniques, prion accumulation has also now been reported to be present in peripheral nerve (Favereaux *et al*, 2004) as well as in muscle, lymphoid tissue and olfactory epithelium (Glatzel *et al*, 2003) at an advanced stage of clinical disease.

Although there are a small number of reports claiming transmission of sporadic CJD by inoculation of blood from patients with clinical disease into experimental rodents (Manuelidis *et al*, 1985; Tateishi, 1985), these results have not been supported by further studies in primates (Brown *et al*, 1994). Similarly, although there are a handful of reports of sporadic CJD arising after blood or plasma product transfusion (Klein & Dumble, 1993; Creange *et al*, 1995, 1996; de Silva, 1996; Patry *et al*, 1998), in none of these has a causal link to a donor with CJD been established. Moreover a series of epidemiological case control (Kondo & Kuroiwa, 1982; Davanipour *et al*, 1985; Harries-Jones *et al*, 1988; Will, 1991; Wientjens *et al*, 1996; Van Duijin *et al*, 1998; Collins *et al*, 1999), look-back (Esmonde *et al*, 1993; Heye *et al*, 1994; Operskalski & Morley, 1995) and surveillance (Evatt, 1998; Evatt *et al*, 1998; Lee *et al*, 1998) studies carried out over almost 25 years have failed to demonstrate evidence of transmission of sporadic CJD by blood components or plasma products. It seems likely therefore that the preclinical incubation period in sporadic CJD is sufficiently short, or peripheral blood infectivity is sufficiently low, as to make transmission of the disease by blood components and/or plasma products at worst a very rare event (de Silva & Mathews, 1993; Brown, 1995; Ricketts *et al*, 1997; Will & Kimberlin, 1998).

Thus, although individuals suspected of having sporadic CJD are permanently deferred from blood donation, no other precautions, such as withdrawal of plasma products if the donor has contributed to the plasma pool, are undertaken. This is because although sporadic [illegible] large number of donors is exposed [illegible] to frequent with drawal and [illegible]

Familial human prion diseases

Familial human prion diseases [illegible] Gerstmann–Sträussler–Scheinker [illegible] insomnia and familial CJD, [illegible] in the prion gene (*PRNP*). All [illegible] familial human prion disease transmit [illegible] individuals with two or more [illegible] disease, or who have been advised [illegible] disease as a result of *PRNP* gene [illegible] blood donation as a precautionary [illegible]

Acquired human prion diseases

The transmission of human prion [illegible] reported in the fore parade of Papua [illegible] 1950s (Gajdusek & Zigas, 1957) [illegible] transmitted during ritual cannibalistic [illegible] rites. The clinical features [illegible] with more prominent cerebellar [illegible] one time Kuru was a leading cause [illegible] people and into the late [illegible] around 1960, [illegible] clinical disease [illegible] period in prion disease [illegible] Iatrogenic transmission of [illegible] direct inoculation of the [illegible] surgical instruments [illegible] mater and corneal [illegible] occurred via neurosurgical procedures [illegible] gonadotrophins [illegible] (Buchanan *et al*, 1981; Brown *et al*, [illegible] presentation varies depending [illegible] centrally transmitted cases [illegible] period of around 2 years [illegible] dementia [illegible] transmitted cases [illegible] have a [illegible] incubation period of around 15–[illegible] ataxia and sensory disturbance [illegible]

Variant CJD

Variant CJD was first described [illegible] 1996) as a result of systematic [illegible] clinical phenotype of CJD in the [illegible] Surveillance Unit in Edinburgh. [illegible] unusual in that it presents with [illegible] such as anxiety or depression [illegible] develop progressive dementia, [illegible] with an average [illegible] (Will, 2003; Will & [illegible]

Table I. Iatrogenic transmission of Creutzfeldt Jakob disease.

	Number	Incubation period (months)
Neurosurgical instruments	5	12–28
Intracerebral electrodes	2	16–20
Dura mater graft	120	18–216
Corneal graft	4	16–320
Human growth hormone	142	550–456
Human gonadotrophin	5	144–192

The incubation period for infections transmitted by peripheral inoculation is shorter than that when infection is directly in the brain (from Ironside and Head, 2003, with permission from Blackwell Publishing).

pathogram changes are observed, but magnetic resonance imaging (MRI) is more informative, with changes in the pulvinar (posterior thalamus) in the majority of cases.

Neuropathologically, the disease is characterised by neural cell loss, astrogliosis and spongiform change with particularly florid amyloid plaques as a pathognomic feature (Fig 2) (Ironside & Head, 2003; Peden & Ironside, 2004). To date all clinical cases of variant CJD have occurred in methionine 129 homozygous individuals; it seems likely that valine homozygous and methionine/valine heterozygous individuals are more resistant to infection or, if infected, to the development of clinical variant CJD. In this context it may be relevant that methionine 129 human prion protein oligomises more rapidly with beta-sheet formation whereas 129 valine tends to form alpha-helix rich monomers (Tahiri-Alaoui *et al*, 2004). Furthermore it is of interest that following inoculation with prions, mice homozygous for human methionine developed 'typical' variant CJD, whilst those that were homozygous for valine appeared more resistant to infection and when this occurred, the clinical and pathological features were more similar to sporadic CJD (Wadsworth *et al*, 2004). It is noteworthy, in this context that the second case of probable variant CJD prion transmission by blood transfusion was recorded in a methionine/valine heterozygous patient who did not develop clinical features of the disease despite surviving 5 years after transfusion (Peden *et al*, 2004). This patient had been identified as part of the variant CJD lookback process and postmortem examination was requested following death from unrelated causes (*vide infra*).

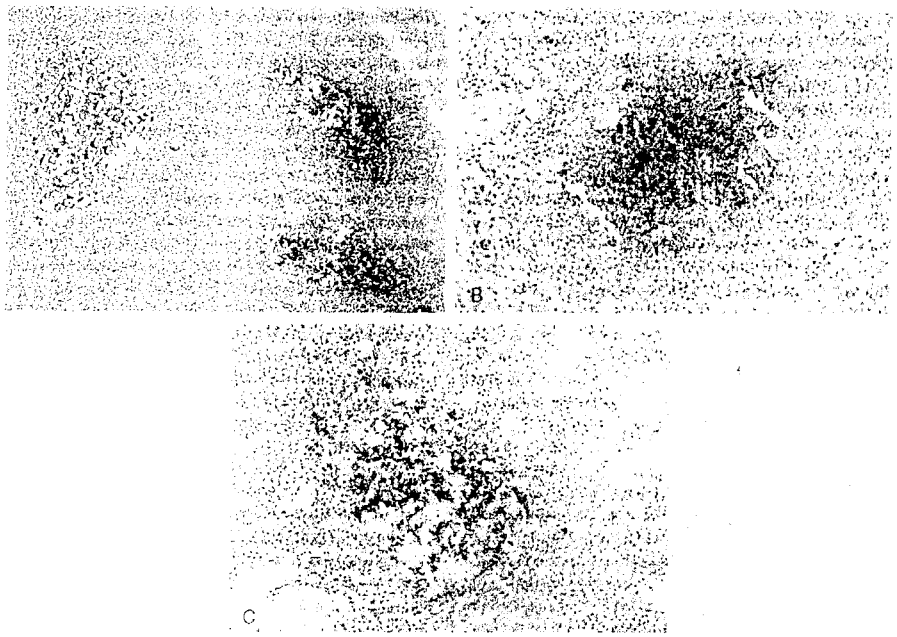

Fig 2. Immunocytochemistry for the prion protein (PrP) in lymphoid tissues in variant Creutzfeldt Jakob disease shows staining of follicular dendritic cells and macrophages in (A) the tonsil, (B) spleen and (C) lymph node. Anti-PrP antibody (KG9) with haematoxylin counterstain [from Ironside and Head (2003) with permission from Blackwell Publishing].

Unlike sporadic and familial forms of CJD, patients with variant CJD show evidence of abnormal prion accumulation in follicular dendritic cells in peripheral lymphoid tissue including tonsils (Hill *et al*, 1997b; Kawashima *et al*, 1997), appendices, spleen (Hilton *et al*, 1998) and lymph nodes (Hill *et al*, 1999). In two patients, appendices removed 8 months and 2 years prior to the onset of clinical disease have also shown evidence of prion accumulation, although a sample removed 10 years prior to onset of clinical disease did not (Glatzel *et al*, 2004).

The median age at death is 29 years (range 14–74 years) and has not altered over the first 10 years of the outbreak, suggesting an age-related susceptibility or exposure (Ghani *et al*, 1998a; Boelle *et al*, 2004). At the time of writing there have been 154 definite and probable cases of variant CJD in the UK, nine in France, two in Ireland and one in each of the USA, Canada, Italy, Saudi Arabia and Japan. In the UK, the incidence of clinical disease appears to have peaked around 2000 and has since fallen significantly (http://www.cjd.ed.ac.uk). However, although the outbreak thus far has been very much less than that which was initially feared (Cousens *et al*, 1997; Ghani *et al*, 1998b), with an upper boundary of around a further 70 new cases now predicted based on the pattern of clinical disease (Will, 2003; Smith *et al*, 2004; Sneath, 2004), a recent retrospective study of tonsil and appendix samples demonstrated three of 12 500 samples positive for abnormal prion accumulation, suggesting that up to 3500 people could be infected with a prevalence of pre- or subclinical disease amongst the 10 to 30-year-old UK population of one of 10 000 (Hilton *et al*, 2004). Ghani *et al* (1998a) have suggested that up to 90% of individuals infected may have prolonged preclinical or true subclinical disease and that this could be related to codon 129 genotypes encoding valine homozygosity or methionine/valine heterozygosity. If transmissible prion infectivity is present in the peripheral blood of such asymptomatic individuals, the concern is that blood-derived products could provide a route to long-term persistence of variant CJD within the population.

Animal studies of peripheral blood infectivity and transmissibility

The route by which the prions disseminate and replicate following peripheral inoculation is of importance in understanding the likely distribution of infectivity and has been recently reviewed (Mabbott & Turner, 2005). Studies in knockout mice with deficiencies in PrP expression, or lacking various cellular compartments of their immune systems, have led to the conclusion that initial accumulation or replication in follicular dendritic cells is essential to peripheral transmission (McBride *et al*, 1992; Bueler *et al*, 1993; Fraser *et al*, 1996; Brown *et al*, 1997b; Klein *et al*, 1997, 1998; Mabbott *et al*, 1998). Indeed, infection and abnormal prion accumulation can be demonstrated in the lymphatic tissues of scrapie-infected rodents and sheep prior

The reverse arm of the surveillance scheme addresses the question as to whether any of the patients who have developed variant CJD could have become infected via a previous blood transfusion. The transfusion history of all patients developing variant CJD is assessed and the donors are traced and also flagged to the UK Office of National Statistics.

To date 17 variant CJD patients are known to have been blood donors (15 in the UK and two in France). Of the 50 recipients of blood components, 17 are still alive. Plasma from 23 donations was fractionated to produce albumin, immunoglobulin and clotting factor concentrates that were used in the UK, France, Belgium, Germany and Italy. In the UK it appears that the incidence of variant CJD peaked in about 2001 and is now declining (Fig 1).

To date there have been two cases of probable transmission of variant CJD prions via non-leucodepleted red cell concentrates. In the first episode, a 24-year-old individual gave a blood donation in 1996 (Llewelyn *et al*, 2004). Three years later he developed variant CJD and died the subsequent year. The recipient of this donation in 1996 was aged 62 years and also received four other units of red cell concentrate to cover a surgical operation. In 2002 he became depressed and developed blurred vision, motor difficulties including a shuffling gait and cognitive impairment. An MRI of his brain was reported as normal. In 2003 he died of dementia. At autopsy, histology of his brain revealed characteristic features of variant CJD, and this was confirmed by proteinase-K resistance and typical features on Western blotting. Analysis of his *PRNP* gene revealed him to be homozygous for methionine at codon 129. A statistical assessment concluded that there was only a 1:15 000–1:30 000 chance of this occurring by coincidence.

A second individual was reported in 2004 as a result of the national surveillance of recipients of transfusions from donors who later developed variant CJD. This patient very likely became infected with variant CJD prions by a unit of red cell concentrate in 1999 from a donor who developed variant CJD 18 months later (Peden *et al*, 2004). Although this patient died 5 years after the transfusion of unrelated causes with no clinical features of variant CJD, analysis of her lymphoid tissue at autopsy revealed that prion accumulation was present in the spleen and one cervical lymph node. There were no histological features or evidence of prion accumulation in her CNS. The other unusual feature as noted above, was that the *PRNP* gene was heterozygous at codon 129 for methionine/valine.

These two cases are therefore of great importance because they have demonstrated that variant CJD prions can be transmitted by blood transfusion from donors who are in a preclinical phase of disease at the time of donation and that methionine/valine heterozygous individuals can also be infected, although whether they are as susceptible to infection and/or the development of clinical disease as methionine homozygous individual remains uncertain (Aguzzi & Glatzel, 2004).

Blood donor selection

Many countries have instituted policies of donor deferral for those who have spent time in the UK, France or more broadly Europe, based on the likely comparative level of risk with their indigenous population, the extent or pattern with which their population visit affected areas and the likely impact on their blood donor base.

In the UK, there are few epidemiological criteria that would allow identification of a 'high-risk' donor population. In response to the blood transfusion related transmissions of variant CJD, in 2004, a policy of deferral of donors who themselves have been recipients of blood components since 1980 was instituted to reduce the risk of tertiary or higher-order transmissions leading to a self-sustaining outbreak. This policy also has the advantage of reducing the risk of other blood borne infectious agents being recycled in the community by transfusion. There was concern that this would lead to a significant reduction in the donor base and that a sometimes precarious blood supply would be further compromised. Whilst about 5–10% donors have been lost from the UK blood donor panels, the impact has been mitigated by proactive recruitment campaigns to enlist more new donors.

Importation of blood components

It is not likely to be feasible to import red cell or platelet concentrates due to the large volumes required, the short shelf life and lability of these components and concerns over the risk of other transmissible agents in some overseas donor populations. To reduce the risk of variant CJD transmission to children, in 2002 the decision was made to only use imported non-UK plasma to treat those born after 31 December 1995. This date was chosen because it was considered that BSE-infected foods had been largely eliminated from the diet by this date, and therefore, children born after this time were unlikely to be infected from food. In addition, with relatively small volumes of plasma, the product can be stored, transported frozen and be virus-inactivated.

Donor screening

No immunological response to prion infection has yet been identified nor has DNA been found associated with disease transmission. Therefore, traditional serological and molecular biological approaches to donor screening are not currently feasible.

Several groups have looked at the possibility of using surrogate markers. The proteins 14·3·3 (Zerr *et al*, 1998) and S100 (Otto *et al*, 1998) are non-specific markers of CNS damage and are therefore likely to be elevated only in the clinical stages of disease. It has been shown that transcription of erythroid differentiation associated factor (EDAF) is depressed in the peripheral blood of animals suffering from prion disease (Miele *et al*, 2001). The cause of this observation

is uncertain and it also currently remains unclear whether this could be translated into the setting of human clinical and preclinical disease and whether an appropriate differential exists between patients incubating variant CJD and normal individuals.

Infectivity has not thus far been detected in the peripheral blood of patients with clinical variant CJD by intracerebral inoculation into rodents despite the evidence of clinical transmission, reflecting the limitations of infectivity bioassays due to the species barrier and the small amounts of blood inoculated.

A central difficulty in the development of molecular assays is the differentiation of PrP^{Sc} from PrP^{c} (Minor, 2004). There are currently no monoclonal antibodies or other reagents of sufficient analytical specificity to differentiate between the normal and abnormal isoforms. Most assays therefore depend on differential physicochemical characteristics, such as resistance to proteinase-K digestion or display of additional or novel PrP epitopes following treatment with chaotropic agents, such as guanidine hydrochloride. The level of sensitivity required is challenging. Brown *et al* (1999) has estimated that in the order 1 pg of PrP^{Sc}/ml may be present in the peripheral blood of individuals in the pre- or subclinical phases of disease, in the context of around 100 ng/ml of PrP^{c}, i.e. a ratio of 1 PrP^{Sc} molecule:1 million PrP^{c} molecules. There are also significant challenges in validating such assays. This would normally be undertaken using samples from individuals with the disease in question. However, there are very few patients alive at any one time with variant CJD and large amounts of blood cannot be drawn for ethical reasons. As it is not currently possible to determine who may, or may not, be incubating the disease, the assays will therefore need be validated on brain homogenate-spiked human blood or animal endogenous infectivity samples posing questions around the extrapolation of the data to the human setting. Finally it should be borne in mind that it will not be possible to determine which of the donors with positive assays are actually incubating variant CJD and which of these are likely to go on to develop clinical disease. There is no treatment available at the present time to offer such individuals. There is concern, therefore, over the number of donors who may need to be deferred due to positive assay results and the potential impact of the introduction of such assays on the willingness of donors to donate (Blajchman *et al*, 2004; McCullough *et al*, 2004).

Blood component processing

In October 1997, the UK Spongiform Encephalopathy Advisory Committee advised that universal leucodepletion be considered. The UK Departments of Health commissioned an independent risk assessment by Det Norske Veritas Consulting (DNV) and asked the Blood Services to consider the feasibility (Comer & Spouge, 1999). Implementation was recommended in July 1998 and completed by the autumn of 1999 (Department of Health, 1998a,b). The measure was

predicated on st[illegible] to be involved [illegible] leucocytes were [illegible] peripheral blood [illegible] animal studies [illegible] plasma and is likely to [illegible] by only about 4[illegible] St Romaine *et al*, 2004). [illegible] considered to [illegible] reduction in [illegible] cytomegalovirus and human T-cell lymphotropic [illegible] of alloimmunisation, immunomodulatory effects [illegible] sion-mediated graft *versus* host disease [illegible]

Other approaches under consideration [illegible] infection risk include the greater use of [illegible] a single donor [illegible] donors), exten[illegible] cryoprecipitate [illegible] further reduction [illegible] plasma [illegible] concentrates.

Two compan[illegible] developing [illegible] reduce the prion concentration [illegible] magnitude. [illegible] likelihood of [illegible] sub- or preclinical [illegible] a significant [illegible] naturally infected human [illegible] therefore [illegible] enised infected [illegible] infected rodent [illegible] these models.

Plasma product manufacture

In 1997, the Committee for Proprietary [illegible] (Committee for Proprietary Medicinal Products [illegible] licensing authority, recommended recall of [illegible] date, factor VIII concentrate that had been manu[illegible] plasma pool containing donations from two [illegible] subsequently developed variant CJD. The UK [illegible] Centre Doctors Organisation (UKHCDO) [illegible] recommended that as variant CJD occurs [illegible] the UK, it is likely that any risk of transmission [illegible] by using concentrate prepared from [illegible] collected in other countries [illegible] of variant CJD [illegible] Ludlam, 1997). The UK [illegible] keen to try to evaluate the risk of blood [illegible] CJD so that [illegible] safety measures [illegible] 2004, the original DNV risk assessment was [illegible] further [illegible] animal stud[illegible]

Although [illegible] degradation [illegible] severe [illegible] plasma [illegible] feat[illegible] suggest that they will [illegible]