bjh review

# Dealing with the uncertain risk of variant Creutzfeldt-Jakob disease transmission by coagulation replacement products

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## Summary

The identification of variant Creutzfeldt-Jakob disease (vCJD) in the UK in 1996 led to significant concerns about the possibility of secondary transmission, however the prevalence of subclinical vCJD and risks of vCJD transmission by plasma are not known. In the UK, public health precautions have been implemented in all recipients of coagulation factor concentrates manufactured from UK plasma pools between 1980 and 2001. The recent demonstration of abnormal prion protein in a spleen sample at autopsy of a UK haemophilic patient who received coagulation factor concentrates to which a donor incubating vCJD had contributed most likely represents the first case of vCJD transmission by coagulation factor concentrates. We review the uncertainties that surround risk of vCJD transmission by coagulation factor concentrates, the challenges in dealing with undefined risks, the rationale behind current policies and the implementation of vCJD surveillance and risk management measures in bleeding disorder patients in the UK.

**Keywords:** haemophilia, risk, variant Creutzfeldt-Jakob disease, coagulation factor, plasma.

The prion disease variant Creutzfeldt-Jakob disease (vCJD) was first identified in the UK in 1996 (Will *et al*, 1996), and subsequently shown to be caused by the same prion strain as bovine spongiform encephalopathy (BSE) and is therefore likely to represent the consequences of BSE infection in humans (Bruce *et al*, 1997; Scott *et al*, 1999). As of March 2012, 225 clinical cases of vCJD have been reported worldwide, of which 182 have occurred in the UK (The National Creutzfeldt-Jakob Disease Surveillance Unit [NCJDSU] 2012); the majority of cases have been confirmed by neuropathological examination. Prior to 2004, all cases of vCJD had been attributable to dietary exposure to BSE, however four cases of vCJD infection transmitted by transfusion of non-leucodepleted red cells from asymptomatic donors infected with vCJD have since been recognised (Health Protection Agency [HPA] 2006; Llewelyn *et al*, 2004; Peden *et al*, 2004; Wroe *et al*, 2006). Plasma from these and other donors who subsequently developed vCJD contributed to plasma pools that were used to manufacture factors VIII and IX (FVIII and FIX), which are used in the treatment of patients with haemophilia A, haemophilia B and the severe forms of von Willebrand disease (Zaman *et al*, 2011), as well as factor XI, antithrombin and prothrombin complex concentrates. Over 20 000 patients with inherited bleeding disorders are currently registered in the UK, of whom around one-fifth have received coagulation replacement products manufactured from pooled plasma concentrates. The risk of vCJD transmission by plasma products is not known; however, abnormal prion protein has recently been demonstrated at autopsy in an asymptomatic patient with haemophilia A previously treated with FVIII concentrates to which a donor incubating vCJD had contributed (Peden *et al*, 2010). While this most likely represents the first case of transmission of vCJD by coagulation replacement therapy (Peden *et al*, 2010), its significance remains unclear.

Although the annual incidence of clinical vCJD in the UK has been steadily declining since 2000 and the extent of the primary vCJD outbreak has been several magnitudes less than previously predicted (Cousens *et al*, 1997; Ghani *et al*, 1998), only limited information is available to provide accurate estimation of the number of future cases. It is predicted that only a small number of future vCJD cases will arise as a result of primary transmission (Garske & Ghani, 2010), however it is not known how many asymptomatic individuals are harbouring vCJD as preclinical or subclinical vCJD and this group of individuals pose a risk of onward secondary transmission of vCJD. A characteristic feature of vCJD prions is the lymphoreticular propagation that precedes neuroinvasion (Wadsworth *et al*, 2001) and the subclinical carrier states of prion infectivity has been demonstrated in animal models (Hill *et al*, 2000). It is this prominent lymphoreticular phase that gives rise to the possibility of vCJD transmission via blood and blood products, surgical instruments, dental procedures and transplanted tissue (Head *et al*, 2004). There is

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concern that human-to-human transmission from asymptomatic individuals could lead to a second wave of vCJD infections. In view of this risk, public health precautions were introduced in 2004 in recipients of UK-sourced coagulation replacement products; in 2010 these were extended to include recipients of transfusions of blood or blood components from 80 or more donors who were undergoing surgical procedures involving neurological tissues (ACDP, 2011a). One major caveat in implementing precautions to minimise possible risk of secondary transmission of vCJD is the lack of means to assess the efficacy of these measures: no reliable biological screening test for vCJD is currently available. In this paper we discuss the fundamentals of managing the undefined risk of vCJD transmission by coagulation factor concentrates in a recipient population of patients with bleeding disorders.

## Establishing the prevalence of subclinical vCJD infection

Unlike clinical vCJD, the incidence of which has been shown to decline annually over the last decade, the prevalence of subclinical vCJD remains unclear. While the mean incubation period of primary vCJD has been shown to be around 13 years (Ghani *et al*, 2003; Boelle *et al*, 2004), which is comparable to the orally transmitted prion disease kuru (Wadsworth *et al*, 2008), the incubation period for secondary vCJD transmission by plasma or plasma products is not known. The characteristically long preclinical phase in vCJD gives rise to the potential for many asymptomatic carriers; indeed it has been suggested that the number of clinical vCJD cases represents only a small minority of the total number of infected individuals (Clarke & Ghani, 2005). Furthermore, animal models have shown that infection by subclinical carriers may result in clinical disease (Bishop *et al*, 2006). A polymorphism at codon 129 (encoding methionine or valine) of the human prion protein gene (*PRNP*) appears to confer a powerful susceptibility factor to vCJD; it is not established whether this effect is mediated by variation in incubation period or altered resistance to disease. All confirmed clinical vCJD cases genotyped to date have been shown to be methionine homozygous (NCJDSU, 2012), the genotype found in around a third of the UK population. However, the vCJD haemophilic patient and one of the red cell transfusion transmitted cases, neither of whom displayed any clinical manifestations of disease, were both codon 129 heterozygous (Peden *et al*, 2004, 2010) and a suspected clinical case of vCJD in a codon 129 heterozygous individual has also been reported (Kaski *et al*, 2009). Incubation periods of other acquired prion diseases are known to vary according to *PRNP* genotype; longer incubation periods are generally demonstrated in codon 129 heterozygotes (Collinge, 2001), with some cases of kuru exceeding 50 years (Collinge *et al*, 2006).

Not only is establishing the prevalence of subclinical vCJD infection critical in the assessment of risk of vCJD transmission, it also enables assessment of the efficacy of current precautionary measures described below, thus informing the need for further measures where necessary. Estimates of the prevalence of subclinical vCJD infection in the UK have thus far been derived from the anonymous screening of lymphoreticular tissue removed during routine surgical procedures. A retrospective study of around 12 000 archived appendix samples showed abnormal prion protein accumulation in three, equating to $292/10^6$ [95% confidence interval (CI) 60–853] (Hilton *et al*, 2004). This led to an estimated UK prevalence of vCJD infection of approximately 1 in 4000 (Clarke & Ghani, 2005). However, a second, larger cross sectional opportunistic study of over 60 000 tonsillar tissue samples has recently shown none to be positive for the presence of vCJD prions (95% CI $0–113/10^6$) (Clewley *et al*, 2009). Combining these studies, the assumed prevalence of subclinical vCJD infection in the general population is estimated at approximately 1 in 10 000 (Hilton *et al*, 2004; Clewley *et al*, 2009). Several factors should be taken into consideration when interpreting the findings from these studies; firstly, confirmatory immunoblot testing of positive samples in the appendix study was hampered by the formalin-fixation of the samples, secondly, these results assume the tests to be 100% sensitive and specific throughout the incubation period, and finally, a significant proportion of the subjects in the later study were born after the time of peak BSE exposure. Interim data from a further appendix study have reported four positive samples out of approximately 14 000: this includes patients from earlier birth cohorts (HPA, 2011). Attempts have been made to create a post-mortem tissue archive following the 2006 recommendation by the Spongiform Encephalopathy Advisory Committee (SEAC, 2006a), but this did not receive the backing of the Coroners' Society of England and Wales. A number of concerns were raised, including the effect on the independence of the coronial service should families be asked to consent to the removal of tissue not directly related to the cause of death (McGowan & Viens, 2011).

## Initial risk assessments of plasma coagulation factor vCJD infectivity

In addition to the prevalence of subclinical infection within the donor population and genetic inter-individual variation in susceptibility, several other factors may determine the risk of vCJD transmission by transfusion of coagulation factor replacement therapy (Table I). These include the number of donations contributing to a plasma pool, the effects of the manufacturing process (Foster, 1999; Foster *et al*, 2000, 2004; Reichl *et al*, 2002; Silveira *et al*, 2005; Truchot *et al*, 2006), the infectivity of the donation within the incubation period (SEAC, 2006b), the quantity of infused products and the recipient's age (Swerdlow *et al*, 2003;

Table I. Possible determinants of risk of vCJD transmission by transfusion of coagulation factor concentrates.

| |
|---|
| Levels of infectivity in donor population* |
| Prevalence of sub-clinical infection – geographical variation |
| Exposure of recipient to infected donors |
| Infectivity of donation within incubation period* |
| Number of donors contributing towards plasma pool |
| Quantity of plasma/leucocytes within component |
| Manufacturing process: e.g. leucodepletion, plasma fractionation, viral inactivation procedures* |
| Number of donor exposures |
| Quantity of product received |
| Susceptibility of recipient |
| Genotype e.g. codon 129 *PRNP* |
| Age* |
| Immune function* |
| Other* |

*Not known in humans

Boelle *et al*, 2004) and immune function (Brown *et al*, 2009). The partitioning of prion infectivity during the manufacture of plasma products has been extensively investigated and is detailed elsewhere (Foster, 1999; Foster *et al*, 2000, 2004; Reichl *et al*, 2002; Silveira *et al*, 2005; Truchot *et al*, 2006). While these studies largely demonstrate removal of prion agents in the manufacture of plasma products, this should be interpreted with caution in view of significant limitations in the methods used to model and estimate levels of infectivity. An independent assessment of the risk to patients of exposure to vCJD infectivity in blood products was carried out on behalf of the UK Department of Health (DH) by Det Norske Veritas Consulting (DNV) and reported in 1999 (Comer & Spouge, 1999). In order to estimate the numbers of new infections and possible resultant vCJD cases, the authors attempted to estimate the proportion of UK blood donations that may be infected with vCJD, the possible level and distribution of vCJD infectivity in blood components and plasma products derived from those donations, and the likely level of exposure to infectivity of defined sets of patient groups. Substantive data surrounding several of the variables used in these calculations were lacking, necessitating that various assumptions be made and that data be extrapolated from spiked animal models (Brown *et al*, 1998, 1999). Assuming blood to be equally infective throughout the incubation period of the disease, the likely proportion of infected donations was estimated as being between 1/200 and $1/10^6$, depending on the median incubation period of the disease (Comer & Spouge, 1999). Over the same range of infected donations the recipient's risk of infection was predicted to range between unity and $1/10^6$, depending on the patient group. Each infected donation was estimated to result in 2·6 infected recipients (assuming roughly equal contributions from red cell and plasma product transfusions), approximately 80% of whom may live long enough to develop vCJD (Comer & Spouge, 1999).

## Measures implemented to reduce the risk vCJD transmission pre-2004: use of recombinant coagulation factor concentrates, product recalls and patient surveillance

The widespread transmission of hepatitis C (HCV) and human immunodeficiency viruses (HIV) by coagulation factor concentrates in the 1970s and 1980s in patients with bleeding disorders heightened awareness and concern amongst haemophilia physicians about the emergence of future blood-borne pathogens. Of the (approximately) 20 000 patients registered with inherited bleeding disorders in the UK, over one fifth have been treated with coagulation factor concentrates derived from UK-sourced plasma donations (Millar *et al*, 2010). At the time of the first reports of vCJD, recombinant FVIII and FIX concentrates were becoming available for the treatment of patients with haemophilia A and B. By 1997 the United Kingdom Haemophilia Centre Doctors' Organisation (UKHCDO) had recommended the use of recombinant and non-UK plasma sourced concentrates (UKHCDO, 1997). Funding for treatment with recombinant factor concentrates became available in 1998 for children with haemophilia and was extended to include all adult patients between 2003 and 2006. Where plasma coagulation factors are used, since 1999 these have been fractionated from plasma imported from the United States; a chronological summary is shown in Figure 1.

Following the 1996 reports of vCJD, the Transfusion Medicine Epidemiology Review (TMER) was established as a collaborative study between the NCJDSU and the four UK blood services with the aim of identifying an association between CJD (including variant) and blood transfusion (www.cjd.ed.ac.uk/TMER/TMER.htm). At that time, 17 patients were recorded as having donated blood prior to being diagnosed with vCJD and there was concern that there may be many more subclinically infected individuals in the donor population. The uncertainty of vCJD transmissibility by plasma products led to the recommendation by the Committee for Proprietary Medicinal Products (CPMP) in 1997 that a product be recalled where a donor subsequently diagnosed with vCJD had contributed to the plasma pool (termed an 'implicated' batch) (CPMP, 1998). This resulted in two recalls that year of intermediate and high purity FVIII concentrates issued between 1995 and 1997 by Bio Products Laboratory (BPL), the plasma fractionator for the UK National Blood Service at that time, with a further BPL recall in 2000 of FVIII and FIX concentrates issued between 1996 and 1998 (Millar *et al*, 2010). In Scotland, two donations from an individual later diagnosed with vCJD had contributed to the Scottish National Blood Transfusion Service fractionation pools, resulting in the 2001 notification of FVIII and FIX products issued between 1987 and 1991 (Millar *et al*, 2010). No public

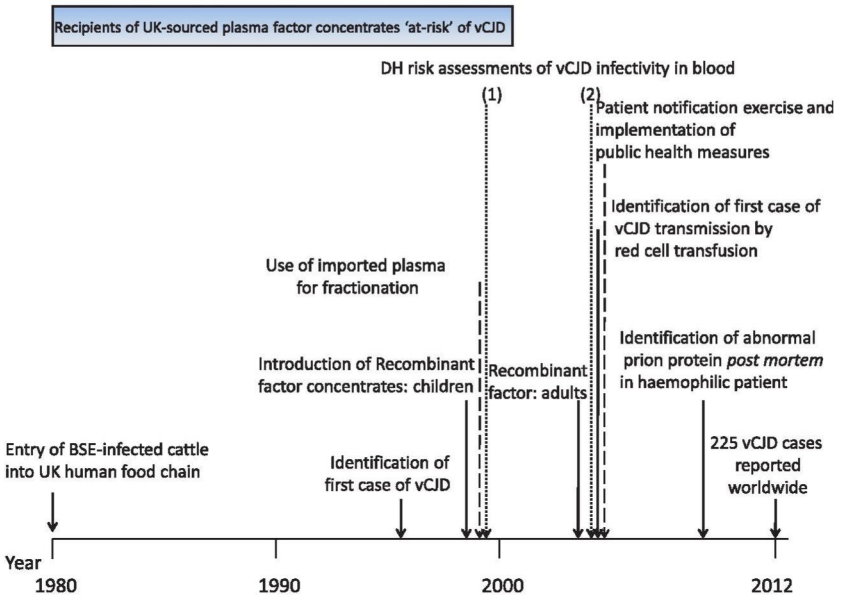


**Fig 1.** Timeline summarising key events and vCJD risk management measures adopted in bleeding disorder patients in the UK. BSE, Bovine Spongiform Encephalopathy; DH, department of health; vCJD, variant Creutzfeldt-Jakob disease.

health precautions were advised at the time of any of these recalls. The consensus given by the DH at the time was that patients would 'not benefit from this knowledge, and that uncertainty created by informing patients could cause unjustified worry and create a permanent blight on their lives' (Millar *et al*, 2010). In spite of this, and given the experience of the public health responses to HIV and HCV, and the resultant impact on the lives of affected patients and relatives, haemophilia physicians advocated for the right of recipients of implicated batches to be informed, even in the absence of known risk. Many haemophilia physicians therefore either directly informed patients who had received an implicated batch, or provided all their patients with information about vCJD, giving them the option to be informed whether or not they had received an implicated batch (Millar *et al*, 2010).

The establishment of the CJD Incidents Panel (CJDIP) in 2000 provided an independent expert committee that advised on issues involving possible vCJD transmission in healthcare settings. The following year vCJD surveillance of UK patients with haemophilia was commissioned and funded by the DH and co-ordinated by the UKHCDO. The tripartite aims of surveillance were to determine and record the extent of exposure of individual patients with inherited bleeding disorders to implicated batches of replacement coagulation products, analyse relevant tissue biopsy and autopsy material for evidence of vCJD infection and notify possible and confirmed clinical cases of vCJD in this patient population (Millar *et al*, 2010). It was anticipated that, in addition to facilitating the appropriate monitoring and long-term follow-up of patients, the findings from this study would inform future assessments of the risk of vCJD transmission posed by plasma products.

## Second risk assessment, CJDIP recommendations and the identification of vCJD transmission by blood transfusion

Meanwhile, concern was growing about the possibility of vCJD transmission by blood and blood products following the demonstration of blood transmission of BSE in a sheep model (Houston *et al*, 2000). Unlike previous experimental models in which prions were inoculated via the intracerebral route, the sheep in this study had been orally infected with BSE and were therefore more representative of the human scenario. Of particular importance was the demonstration of vCJD transmission by blood taken during both the preclinical and clinical stages of infection (Hunter *et al*, 2002). These data prompted a second DNV risk assessment undertaken on behalf of DH to provide estimates of the potential infectivity of the various blood and plasma fractions to inform the management of individuals who had received implicated batches of blood and plasma products (DNV, 2003). As neither the nature of the blood-associated vCJD infective agent nor the effects of blood processing and manufacture of plasma products on vCJD infectivity are known, this DNV assessment was also based on data from published animal studies (Brown, 2000; Holada *et al*, 2002; Hunter *et al*, 2002). Infectivity was quantified using $ID_{50}$, with one $ID_{50}$ being the dose resulting in infection in 50% of recipients. Together with batch-specific manufacturing data, the DNV infectivity estimates were used by the HPA in 2004 to estimate the vCJD infectivity of each implicated batch (Zaman *et al*, 2011). The likely risk to treated patients was compared to the 'at-risk' threshold developed by CJDIP to guide the management of other 'at-risk' patient groups (CJDIP,

2004a). If patients had been exposed to a 'threshold' of 0·02 $ID_{50}$ (1%) or greater potential risk of infection over and above the general risk to the UK population believed to have resulted from dietary exposure to the BSE agent, CJDIP advised that they should be notified and requested to take public health precautions to minimise the risk of secondary vCJD transmission. This 1% additional risk equates to an exposure of 0·02 $ID_{50}$, the level of risk at which public health measures are implemented in patients exposed to vCJD via surgical instruments (DH, 2001). For each of the major assumptions underlying the risk assessment, the most precautionary option was adopted. Firstly, the separation of whole blood into blood components and plasma fractions was assumed to be the only step that reduces infectivity in the production of plasma products (DNV, 2003). A similarly cautious approach was adopted in the assumption of equal infectivity in plasma and red cell components, despite the known diluting effect of the plasma pool (DNV, 2003). Finally, the dose-response relationship for vCJD infectivity was assumed to be linear with no threshold or ceiling effect (DNV, 2003). Such a combination of assumptions would be expected to overestimate infectivity risks: indeed, other risk assessments that take the clearance factors achieved at different stages to be at least partly additive, have shown much lower infective loads.

Following the DNV risk assessment, all implicated plasma products were stratified into three groups based on the assessed vCJD infectivity risk (CJDIP, 2004b). Amongst those considered to pose a high risk were FVIII, FIX, and antithrombin concentrates, of which as little as one vial of treatment could lead to an exposure in excess of the defined risk threshold. Products in the medium-risk group included those in which exposure to substantial quantities was required to reach the risk threshold, such as immunoglobulins, and the low-risk group comprised products with such low levels of potential infectivity as could effectively be ignored as causing any additional vCJD risk. The low-risk group also included some high purity FVIII products that had been manufactured using implicated albumin as an excipient. To minimise the possible risk of onward transmission of vCJD, in 2004 the CJDIP recommended that public health precautions be taken in recipients of high- and medium-risk implicated plasma products who had exceeded the 1% additional risk threshold. Around the time of these recommendations the first clinical case of vCJD transmission by blood transfusion was reported (Llewelyn *et al*, 2004). TMER surveillance has since established two further clinical secondary cases and one asymptomatic case of vCJD within the cohort of 24 surviving recipients identified from the 66 patients who had received red cell transfusions linked to individuals subsequently diagnosed with vCJD (Peden *et al*, 2004; HPA, 2006; Wroe *et al*, 2006). All affected red cell donations are known to have been sourced relatively close to the onset of clinical symptoms in the donor, consistent with the increasing level of prion infectivity demonstrated throughout the incubation period in some animal models (SEAC, 2006b). The inoculation times in the confirmed red cell transmitted vCJD cases varied between 5 and 8·5 years, around half the length of that estimated for primary oral infections from BSE (Peden *et al*, 2004; HPA, 2006; Wroe *et al*, 2006). Furthermore, as a significant proportion of patients in the TMER recipient cohort did not survive long enough to develop clinical disease should they have been infected by vCJD, it is possible that the observed number of infected recipients underestimates the transmissibility of vCJD by blood transfusion.

## 2004 public health notification of recipients of UK plasma-derived coagulation factors

By the time of the 2004 CJDIP recommendations the fate of products manufactured from 23 plasma donations derived from nine UK plasma donors who later developed vCJD had been established. These donations had undergone fractionation to produce albumin, immunoglobulin and coagulation factor concentrates, which included 17 batches of FVIII and eight batches of FIX that had been distributed and used throughout the UK between 1987 and 1999 (Millar *et al*, 2010). Some of these batches had been notified in the previous BPL recalls, although three of the previously notified high purity FVIII batches were now reassigned as having a low infectivity risk (CJDIP, 2004b) and therefore no longer considered to be 'implicated'. TMER surveillance identified that these donations included plasma from at least one donor who probably already transmitted vCJD via red cell concentrates (Hewitt *et al*, 2006). Given that small volumes of implicated FVIII or FIX treatment may cross the 1% additional risk threshold and the perceived high likelihood of further vCJD implicated batches of coagulation factor concentrate being identified as future vCJD cases arose, a policy decision was taken to adopt a population approach to minimise the risk of secondary spread. In this, all patients with bleeding disorders who had been treated with UK-sourced pooled factor concentrates between 1980 (when BSE was believed to have entered the human food chain) and 2001 (latest expiry date of UK-sourced concentrates) were considered to have potentially been exposed to vCJD and therefore be at risk of vCJD for public health purposes.

Since 2004, infection control policies in patients considered to be at-risk of vCJD have been informed by guidance from the ACDP Transmission Spongiform Encephalopathy Working Group (ACDP, 2011b), which hospitals are required to implement. These include detailed precautions required at times of surgery, dental work and endoscopy according to the classified infectivity of the relevant tissues. For example, procedures involving tissues considered to be of high or medium infectivity (brain, spinal cord, posterior eye, tonsil, spleen, appendix, thymus, lymph nodes, gut lymphoid tissue, adrenal gland, olfactory epithelium), require the tissues to be destroyed by incineration and, where single-use instruments are not available, instruments should be

destroyed or quarantined for exclusive re-use in the same patient only (ACDP, 2011b). Separate guidance is available for endoscopy (ACDP, 2011c).

The UK notification process was conducted in September 2004 on behalf of the DH by the HPA and is described in detail elsewhere (Millar *et al*, 2010; Zaman *et al*, 2011). In brief, haemophilia clinicians were requested to inform all patients with inherited bleeding disorders of the reasons for the notification, identify all recipients of UK-sourced plasma products between 1980 and 2001, inform their general practitioners of their 'at-risk' status, offer counselling to all patients and the opportunity to discuss the public health measures as well as the option of knowing whether they were 'at-risk' and whether they had received any of the FVIII or FIX batches known to be implicated. Unless requested otherwise, 'at-risk' patients were recorded on the National Haemophilia Database including details of any implicated batches received (Millar *et al*, 2010; Zaman *et al*, 2011). Medical directors were contacted to trace recipients of other products, such as antithrombin and prothrombin complex concentrates, used in general medical patients. The public health exercise was not limited to patients in the UK. Implicated plasma donations had also contributed towards pooled plasma products distributed outside the UK and it is estimated that there are patients who have been exposed to a level of infectivity exceeding the 'at-risk' threshold residing in at least four countries worldwide (Millar *et al*, 2010; Zaman *et al*, 2011).

## Evaluation of vCJD risk and surveillance of recipients of vCJD implicated batches

Three thousand and seven hundred and thirty-five UK patients with bleeding disorders have now been identified as having received UK-sourced coagulation replacement products between 1980 and 2001, 787 of whom have been notified as having received at least one of the 25 known implicated batches of FVIII or FIX concentrate between 1987 and 1999 (Table II) (Zaman *et al*, 2011). However, as only around half of the implicated FVIII and FIX batches have been accounted for to date (Millar *et al*, 2010), the true size of the cohort of recipients of implicated batches is likely to be significantly greater. Individual risk assessments performed by the HPA in this patient cohort are based on the $ID_{50}$ estimates for each batch by DNV risk assessment and quantity of treatment. These show wide variation in estimated cumulative lifetime infectivity of vCJD, median $ID_{50}$ 0·443, range 0·010–9·593 (Zaman *et al*, 2011) (Fig 2). Follow up of this cohort over a 13–25 year period has shown no clinical cases of vCJD to date. Of the four patients in this cohort in whom autopsy material has been investigated for evidence of vCJD infection, abnormal prion protein has been identified post-mortem in an isolated single spleen specimen of one patient, which most likely represents subclinical vCJD infection (Peden *et al*, 2010). As well as receiving implicated batches of coagulation factor, this patient had received nonimplicated batches and red cell transfusions and had undergone an endoscopic procedure with biopsy – all potential sources of vCJD infection in addition to the background dietary risk and UK residence. Estimation of the relative levels of risk of vCJD posed by these multiple routes of exposure has identified UK-sourced coagulation replacement therapy as being the most likely route of transmission (Peden *et al*, 2010). This patient died of causes unrelated to vCJD eleven years after treatment with two known implicated FVIII batches (total of 9000 units) with an estimated vCJD infectivity $ID_{50}$ of 0·21, equating to an additional vCJD risk of approximately 10%. The additional vCJD risk from treatment with non-implicated, UK-sourced FVIII concentrate (about 400 000 units), was estimated to be >100% (Peden *et al*, 2010; Zaman *et al*, 2011). Of note, all other autopsy tissues tested for the presence of abnormal prion protein in this patient were negative (Peden *et al*, 2010). These tissues included 23 other samples from the spleen, brain, heart, liver, lymph nodes and appendix and would therefore have been considered negative should this patient have been included in

Table II. Patients with inherited bleeding disorders registered in the National Haemophilia Database on 1st March 2011 by diagnosis and subgroups at risk of vCJD for public health purposes.

| | Number of patients with bleeding disorders by diagnostic subgroups | | | | |
|---|---|---|---|---|---|
| Patient group and subgroups | Haemophilia A | Haemophilia B | Von Willebrand disease | Other | Total |
| a. Total registered in the National Haemophilia Database (NHD) | 5337 | 1140 | 9125 | 6863 | 22 465 |
| b. Registered patients designated 'at risk' of vCJD: (treated with UK-sourced coagulation factor concentrates between 1980 and 2001) | 2246 | 562 | 518 | 409 | 3735 |
| c. Registered patients at risk of vCJD known to have received implicated FVIII and FIX batches | 556 | 168 | 39 | 24 | 787* |

*11 million units (about 50%) of implicated batches remain unaccounted for (Millar *et al*, 2010). Because of this under-notification, it is estimated that the 787 patients represent around one half of all patients who have received implicated batches.

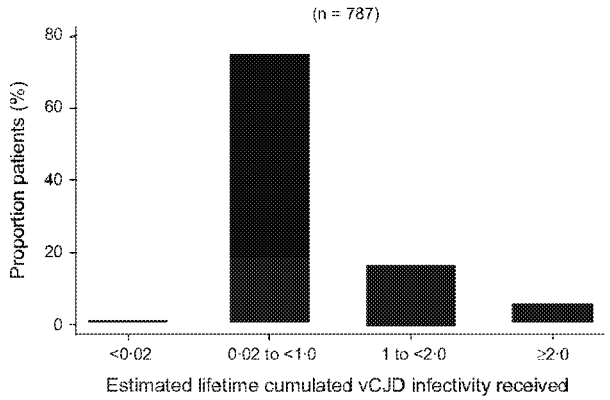


Fig 2. Distribution of patients with bleeding disorders by estimated lifetime cumulated vCJD infectivity received ($n$ = 787).

the vCJD prevalence studies described above. Other than being a marker of subclinical vCJD infection, the significance of the findings in this patient, in particular with regards to infectivity risk, is not clear.

Over 600 patients in this cohort have now been followed up for 15 years, of whom the vCJD risks in addition to the background UK population risk resulting from dietary exposure have been estimated to be $\geq$1% in 595, $\geq$50% in 164 and 100% in 51 patients (Zaman *et al*, 2011). Although this period of follow up is longer than both the incubation period estimated for primary vCJD infection (Ghani *et al*, 2003) and that observed in secondary transmission via non-leucodepleted red cell transfusions (Peden *et al*, 2004; HPA, 2006; Wroe *et al*, 2006), it is feasible that the incubation period for secondary transmission of vCJD by plasma products could be longer.

## Surveillance of recipients of UK plasma coagulation factors not known to be implicated

Of the remaining 2948 patients known to have received non-implicated UK-sourced coagulation replacement products, no clinical vCJD cases have been identified. Within this patient cohort, autopsy material has been investigated in seven patients and biopsy in a further six (Peden *et al*, 2010); no evidence of vCJD has been demonstrated in any of these samples although clearly this represents testing of only a very small proportion of the UK haemophilia population considered at risk of vCJD. As discussed above, treatment with non-implicated FVIII concentrates was identified as being the most probable source of vCJD infection in the haemophilic patient with likely subclinical vCJD. Considering that the plasma pools from which coagulation replacement products are manufactured each contain in the order of 20 000 donations, it is highly likely that many pools contain plasma from unidentified infective donors, based on the prevalence estimates for subclinical vCJD described above (Hilton *et al*, 2004; Clewley *et al*, 2009). Since the 2004 notification no further blood donors have been identified as having donated plasma prior to developing vCJD, thus no further implicated batches have been identified. While this may appear to offer some reassurance, currently available prevalence data do support the existing approach that considers all recipients of UK-sourced coagulation replacement products to be at significantly increased risk of vCJD infectivity to warrant public health measures, rather than only those with known exposure to implicated batches. Furthermore, risk calculations using lower estimated $ID_{50}$ have shown an increase in the relative infectivity of non-implicated batches compared to implicated batches.

## Other determinants of vCJD infectivity

The HPA risk assessments assume infectivity to be equal throughout the incubation period of disease and therefore do not consider potentially important donor-related determinants of vCJD infectivity. The interval between the time of donation and diagnosis of clinical vCJD was shown to range from six to 143 months (median 88 months) in the eight donors linked to implicated batches of FVIII and FIX (Zaman *et al*, 2011). Animal models have shown an increase in prion infectivity throughout the incubation period (SEAC, 2006b). The implicated batches used to treat the single haemophilic patient who demonstrated abnormal prion protein at autopsy were linked to two plasma donations made by a donor who developed clinical vCJD less than six months after the second donation (Zaman *et al*, 2011). In total, the plasma from this donor had contributed to four batches of FVIII and FIX concentrates, which had been used to treat 257 patients, 149 of whom had received FVIII concentrates derived from the second donation. It appears that the infectivity dose of the FVIII batch of the second donation was lower than the other batches to which this donor contributed (Zaman *et al*, 2011). Other factors not considered in the individual risk assessments include geographical variation, recipient genotype and age. The majority of clinical primary vCJD cases to date have been aged under 40 years, despite the likely exposure of the whole UK population to BSE (NCJDSU, 2012). This observed age distribution could result from a higher rate of dietary exposure, reduced incubation period or a higher susceptibility in younger individuals (Boelle *et al*, 2004), e.g. due to a greater volume of gut-associated lymphoid tissue (St Rose *et al*, 2006). Human data for secondary vCJD is limited to recipients of growth hormone, in whom age has also been shown to be a risk factor (Swerdlow *et al*, 2003). Recent studies in mice have shown advancing age to impair splenic prion accumulation and neuroinvasion following peripheral prion transmission; aged mice were shown to develop subclinical but not clinical prion disease found in young mice (Brown *et al*, 2009). These age-related differences are not seen following direct intracerebral prion inoculation and imply that early lymphoid prion accumulation and subsequent neuroinvasion may be significantly impaired by the reduction in follicular dendritic cell function associated with advancing age. Extrapolating these findings to

humans suggests that, in addition to age, vCJD neuroinvasion may also be impaired as a result of blood borne virus-mediated immune modulation, leading to a greater likelihood of subclinical rather than clinical vCJD infection in recipients of implicated coagulation factor concentrates.

## Impact of uncertain risk of vCJD transmission on patients with bleeding disorders

While a significant proportion of the 'at-risk' cohort comprises patients with severe bleeding disorders who receive regular coagulation factor replacement therapy and have been exposed to many thousands of units derived from UK-sourced plasma in the affected period, there are many patients with milder bleeding phenotypes who received only occasional treatment during this time. One such example would be a patient with a milder bleeding disorder undergoing a surgical procedure, who would otherwise have had little or even no contact with haemophilia services. This has several important inferences at the time of a notification that include the nature of the doctor-patient relationship, as well as practical issues such as the ability to trace patients. Many of the 'at-risk' patients with bleeding disorders had been affected, both directly and indirectly by HIV and HCV, and it is likely that those previous experiences, at least in part, influenced their response to the vCJD notifications. At the time of the 2004 notification, 'at-risk' patients were informed that their clinical care would not be compromised by the public health measures being implemented. Whether this will been borne out over time remains to be seen. What is known is the level of confusion that surrounded some aspects of the notification exercise (Millar *et al*, 2010). Furthermore, the need to quarantine surgical instruments, including endoscopes where a biopsy has been performed, has had a negative impact in the delivery of haemophilia care in the UK (Millar *et al*, 2010). An important area that has not been studied in detail is the significant psychological impact of dealing with the uncertainties of exposure to UK products and being labelled as "at risk for vCJD for public health purposes".

## vCJD in other countries

Outside the UK, 49 cases of vCJD have been reported worldwide to date (NCJDSU, 2012), 41 of these have been in Europe, the majority in France. While a small minority are understood to have acquired the disease in the UK, most patients are believed to have been infected in their country of origin. Other countries have adopted less precautionary approaches than the UK. Authorities in France concluded that the risk posed by implicated batches, even in the most pessimistic scenario, was very low and consequently continued to manufacture plasma sourced from their domestic blood supply, with the introduction of nano-filtration as an additional step in the process (Agence française de sécurité sanitaire des produits de santé [AFSSAPS] 2003). In their risk assessment, the US Food and Drugs Administration (FDA) concluded the risk of vCJD infection by plasma products to range from 1 in 9·4 million to 1 in 15 000 (FDA, 2006).

## Strategies to reduce vCJD risk

The introduction of recombinant coagulation factors has essentially eliminated the infection-related risks in users. Currently, the recombinant products available are FVIII, FIX and FVIIa, whilst FXIII and von Willebrand factor are undergoing clinical trials. For situations where plasma coagulation factors have to be used, such as fibrinogen, FXI and prothrombin complex concentrates, these are manufactured from non-UK plasma. As far as non-fractionated plasma products are concerned, avoidance through the use of alternatives such as cell-salvage, perioperative haemodilution, use of restrictive transfusion practices and the selective use of desmopressin and tranexamic acid should considered. It is possible to reduce the transfusion-transmitted vCJD risk of fresh frozen plasma (FFP) and cryoprecipitate by the universal use of solvent detergent FFP prepared from non-UK plasma and fibrinogen concentrate respectively, yet rather surprisingly this has not been adopted in the UK. Prion-reducing filters are being developed but at present none are in routine use in the UK.

## Development of a screening test for vCJD

Improved evaluation of subclinical vCJD infection prevalence rates and the efficacy of the risk-reduction measures described would be greatly facilitated by the availability of a blood test that reliably detects prion infectivity in asymptomatic individuals, notwithstanding the issues that would be encountered surrounding interpretation of the significance of a positive test on likelihood of development of clinical vCJD. The unique pathogenesis of prion diseases poses major challenges to the development of such tests: the misfolded host cellular prion protein ($PrP^{sc}$) does not generate a humoral immune response, nor is there agent-specific nucleic acid. While immunoassays that detect $PrP^{sc}$ in affected tissues use protease to degrade normal prions ($PrP^{c}$), some protease-sensitive $PrP^{sc}$ forms have also been reported (Pastrana *et al*, 2006). Furthermore, to enable successful identification of infectivity in blood, an assay is required that can detect much lower levels of $PrP^{sc}$ than those found in tissues of clinical vCJD. Taken together with the higher $PrP^{c}$:$PrP^{sc}$ ratio found in blood than any other tissue and scarcity of blood samples from vCJD patients, this poses significant challenges to the development of a sufficiently sensitive and specific assay. These have been partly overcome by the demonstration of avid prion binding to some surfaces, including metals (Zobeley *et al*, 1999), which have been used in the development of a quantitative blood-based assay for prion infectivity

(Edgeworth *et al*, 2009). Levels of sensitivity and specificity of 71·4% and 100% respectively, have been reported in a study of clinical vCJD cases (Edgeworth *et al*, 2011). This level of specificity can be interpreted as being sufficient for neurological diagnostic use, however extensive further validation would be required before these assays could be used as screening tests for the detection of subclinical vCJD infection and there is no currently available blood test for vCJD detection outside the research setting.

## Conclusion and future directions

The resources expended on the inquiries scrutinising the policies and procedures following the tragic consequences of widespread HIV and HCV transmission in this patient cohort have dwarfed those that were available to inform those policies. Lessons learned from the public health responses to these previous transfusion-transmitted infections have paved the way for heightened awareness for emergent pathogens and the adoption of more cautious and prudent strategies. Major public health concerns have arisen following the identification of vCJD in the UK in 1996. There are many challenges involved in policy decision-making where risk is concerned, in particular where this is not well defined. We have highlighted the many scientific uncertainties that surround the risk of vCJD transmission by blood and blood products, and outlined the need for multiple assumptions to be made in informing policy. Conveying these uncertainties and acknowledging the lack of knowledge in this field poses a significant challenge, however it is a widely held view that this approach is safer than overstating certainty where it is not known. The demonstration of abnormal prion protein in one spleen sample from the autopsy of a UK patient with haemophilia most likely represents the first case of vCJD transmission by coagulation factor concentrates. What this means, other than being a marker of subclinical vCJD infection, is not clear, particularly in relation to subsequent clinical disease and infective risks. No clinical vCJD cases have been identified in UK haemophilic patients to date.

The implementation of public health measures using the precautionary population approach adopted in the UK demands ongoing employment of substantial human and fiscal resources and in the absence of a validated screening test, the efficacy of such an approach is unknown. For the reasons discussed, it is questionable whether the inclusive UK approach continues to be necessary, particularly in view of the decreasing incidence of new clinical vCJD cases, the lack of clinical vCJD cases in the cohort of exposed haemophilic patients and the lack of identification of any further implicated batches since the 2004 notification. However, these indicators are unlikely to represent the best measure of vCJD infectivity in the population given the current estimates of subclinical prevalence. Furthermore, the subclinical vCJD prevalence estimates are consistent with a significantly higher vCJD infectivity risk associated with non-implicated than implicated concentrate batches, which suggests that the additional risk in an implicated batch is insufficiently high to require more targeted public health measures. Individual risk assessments are currently undertaken in the UK in other patient cohorts, such as primary immunodeficiency patients.

Unlike currently applied risk calculations, which make many assumptions, do not consider several relevant variables and are likely to be overly pessimistic, much lower loads of vCJD infectivity have been estimated by risk assessments that take the clearance factors achieved at different stages of coagulation factor concentrate production to be at least partly additive. Future improved models of risk calculation may result in a more targeted policy and revision of the current inclusive population approach used to inform public health measures. Continued active surveillance of bleeding disorder patients is essential for the future assessment of risk of vCJD transmission by coagulation factor therapy.

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