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Creutzfeldt-Jakob disease and blood transfusion safety

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Abstract

Transmissible spongiform encephalopathies (TSEs) are untreatable, fatal neurologic diseases affecting mammals. Human disease forms include sporadic, familial, and acquired Creutzfeldt-Jakob disease (CJD). While sporadic CJD (sCJD) has been recognised for near on 100 years, variant CJD (vCJD) was first reported in 1996 and is the result of food-borne transmission of the prion of bovine spongiform encephalopathy (BSE, "mad cow disease"). Currently, 230 vCJD cases have been reported in 12 countries, the majority in the UK (178) and France (27).

Animal studies demonstrated highly efficient transmission of natural scrapie and experimental BSE by blood transfusion and fuelled concern that sCJD was potentially transfusion-transmissible. No such case has been recorded and case-control evaluations and lookback studies indicate that, if transfusion-transmission occurs at all, it is very rare. In contrast, four cases of apparent transfusion-transmission of vCJD infectivity have been identified in the UK.

Risk minimisation strategies in response to the threat of vCJD include leucodepletion, geographically-based donor deferrals and deferral of transfusion recipients. A sensitive and specific, high-throughput screening test would provide a potential path to mitigation but despite substantial effort no such test has yet appeared~~been approved~~.

The initial outbreak of vCJD appears to be over, but concern remains about subsequent waves of disease among those already infected. There is considerable uncertainty about the size of the infected population, and there will be at least a perception of some continuing risk to blood safety. Accordingly, at least some precautionary measures will remain in place and continued surveillance is necessary~~advisable~~.

Introduction

Transmissible spongiform encephalopathies (TSEs) are a group of unusual neurologic diseases affecting mammals. They are uniformly fatal and no treatment is available. As the name suggests, the agent of the disease can be transmitted; the agent is unusual inasmuch as it is a configurational variant of a common cellular prion protein (PrP^{C}) known as a prion (PrP^{TSE}), and infection seems to occur in the absence of pathogen specific nucleic acid[1]. Human forms of the disease include sporadic, familial and acquired Creutzfeldt-Jakob disease (CJD), familial Gerstmann–Sträussler–Scheinker syndrome (GSS) and sporadic and familial fatal insomnia (FFI). More recently, variant CJD (vCJD) has been recognised: a result of food-borne transmission ~~of the prion~~ of bovine spongiform encephalopathy (BSE, "mad cow disease")[2].

Sporadic CJD (sCJD) is diagnosed at a frequency of approximately one case per million people, per year, globally. Based on aetiological definition, sCJD represents the majority of cases (85%) while familial and iatrogenic ~~acquired~~ cases represent only 15% and 1%, respectively. Of all forms of CJD, vCJD is unique in its aetiology because it has been transmitted through the food chain and is transmissible by blood transfusion[3, 4]. Even before this became apparent, there was concern about the possible transmission of other TSEs via transfusion but, although transmission via blood has been demonstrated in animal models, there have been no reported cases of human transmission by transfusion, other than in vCJD. Nevertheless, a number of precautionary measures to reduce this ~~potential~~ theoretical risk have been implemented.

In this review, we discuss the nature of human CJD and allied diseases and review data on the risk of transfusion-transmission of these agents. We describe current and potential approaches to minimise the risk of such transmission and we consider possible future directions.

Epidemiology

CJD

CJD other than vCJD has been recognised for almost 100 years. sCJD occurs world-wide with an incidence of approximately 1 to 1.5 per million of the population per year. A very small number of cases occur in those less than 50 years old. The annual mortality rate in the UK ~~has been approximately 1.4/ million since 2008 and~~ is comparable to the rate in other European countries and other areas where effective surveillance is in place. Rates for England, Wales, Scotland, and Northern Ireland in the years 1990-2015 varied from 0.82 to 1.35/ million/ year[5] and were not statistically different. Surveillance data strongly support the conclusion that case ascertainment has improved[5] in the UK and elsewhere[6].There do~~es~~ not appear to be any geographic differences in sCJD across the UK, or in other countries, either looking at country, or at region of residence.

Familial CJD (fCJD), GSS or FFI are due to ~~genetic~~ mutations in prion protein gene (PRNP) which cause abnormal forms of prion protein to be formed in the body. Over 30 different mutations have been identified; they are inherited as autosomal-dominant disorders. Different mutations may produce different symptoms, age at onset, or length of disease, even within the same family. In fCJD, symptoms usually arise between the ages of 30 and 60, and disease duration generally ranges from a few months to 5 years. Concern about the potential transmissibility of these familial cases has resulted in the USA in deferral policies for family members of patients.

vCJD

UK cases of vCJD[5] show a slight male preponderance (58%). Median age at onset is 26 years, and at

death 28 years. The youngest case was age 12 at onset and the oldest was 74 years and all were born before 1989. Median duration of illness is 14 months, compared with sCJD where it is 4 months. All patients~~cases~~ who have been genetically analysed were methionine homozygous at codon 129 (129MM) of the *PRNP* gene, with the exception of the latest (2016) case, who was a methionine-valine heterozygote (129MV)[7].

Cases of vCJD have been spread across the UK, but individuals living in the northern half (Scotland and northern England) have a roughly one and a half times greater chance of developing vCJD. Detailed investigation has not provided any convincing evidence of demographic factors which may have augmented local risks for vCJD.

Non-UK case reports

Although first described in the UK in 1996, cases of vCJD have since been described in small numbers from other countries (Figure 1). Some of these individuals had a period of residence in the UK, and were thus subjected to a UK diet; others may have been exposed to UK beef in their country of residence.

Threat to the blood supply

CJD

As noted, there were concerns about the possibility of transfusion-transmission of CJD even prior to the recognition of vCJD. These concerns were driven by the historical evidence of high rate of transmission of scrapie among sheep, experimental transmission of disease to non-human primates, and by the occurrence of sCJD transmissions in humans via injections with growth hormone and gonadotropin derived from human pituitary glands, through dura mater transplants and by a few other rare treatments[8]: transmissions are attributable to the collection of materials from donor individuals with unrecognized CJD. Animal model studies (described below) showed that infectivity could be present, albeit at low levels, in the blood. Significant efforts were ~~expended~~ undertaken to prevent the possibility of transmission by transfusion. In the United States, the Food and Drug Administration has classified CJD as a "relevant transfusion-transmitted infection", thus requiring specific actions, possibly including the use of a licensed test for donors, should one become available[9]. A particular area of concern was the possibility of contamination of medicinal products manufactured from pooled plasma, because many patients would be exposed if a single infectious donation was included in a fractionation pool~~. In practical terms these concerns are no longer relevant for sCJD~~[10].~~.~~

The actual risk of transfusion-transmitted sCJD has not been quantified and, to date, there has been no definitive report of such transmission in humans. Specific studies have included case-control evaluations of more than 600 cases, several lookback studies involving recipients of blood from donors who subsequently developed sCJD, and autopsy studies on haemophiliacs exposed to pooled plasma products[11-17](summarised in[10]). One additional case-control study did indicate that blood transfusion 10 or more years before occurrence of CJD was more frequent in sCJD than in other neurologic diseases.[18] However, the observation could have been an artefact. Lookback studies reflect several thousand person-years of observation among recipients of blood from persons who subsequently developed sCJD and found no cases of disease[19, 20]. These studies can be interpreted to show that there is no such transmission, or if it occurs, it offers a much lower risk than that from vCJD.

vCJD

Soon after the publication in 1996[21] of the first ~~identified~~ ten cases of vCJD, there were strong suggestions that vCJD would behave differently from sCJD with respect to transfusion transmission. Importantly, this was the first occasion in which a TSE had crossed the species barrier outside the laboratory settings. Secondly, it was suggested that vCJD must have been acquired through the food chain, and that abnormal prions had thus crossed the gut wall and gained access to neural tissue, presumably via gut lymphatics. There was no reason to believe that prions might not also gain access to the blood stream, particularly as the prion was identified in lymphoid tissues. These concerns led to a meeting in April 1996, convened by workers at the UK National CJD Research and Surveillance Unit and involving all four UK Blood Services, and the setting up of the Transfusion Medicine Epidemiology Review (TMER) (see later) to examine whether there was any link between blood transfusion and vCJD.

The first concerns about vCJD and its potential as a threat to the blood supply were followed by animal studies carried out in sheep, which clearly demonstrated that BSE could be transmitted by blood transfusion, using experimentally infected sheep as blood donors before the onset of clinical disease[22]. In December 2003[3] the first link between a human blood donor who had later developed vCJD and a recipient who also later developed vCJD was identified.

Animal studies

Early ~~sporadically entertained~~ experiments to investigate infectivity in the blood of animals or humans with naturally acquired TSEs, usually by intracerebral (i.c.) injection of blood components into rodents or primates, produced negative or inconclusive results [23, 24]. ~~On the other hand~~ However, later systematic studies ~~, infectivity of blood was~~ clearly demonstrated infectivity in blood ~~in small animal studies~~ of experimental small rodents, using mouse- or hamster-adapted TSEs[25-28]. ~~[36-38; consider to use as well: Brown P, Rohwer RG, Dunstan BC, MacAuley C, Gajdusek DC, Drohan WN. The distribution of infectivity in blood components and plasma derivatives in experimental models of transmissible spongiform encephalopathy. Transfusion. 1998 Sep;38(9):810-6].~~

~~. However, s~~Since 2000, studies in sheep have demonstrated highly efficient transmission of natural scrapie and experimental BSE by blood transfusion [22, 29, 30], and recently, infectivity was detected in blood samples from both vCJD and sCJD patients following inoculation into highly sensitive transgenic mice over-expressing either bovine or human prion protein ~~(*PRNP*) (usually human gene is PRNP and animal is Prnp, therefore I suggest to use "prion protein gene")~~ gene, respectively[31]. Titres of infectivity in blood and the probability of transmission by transfusion appear to correlate with the extent of replication of TSE agents in lymphoid tissues – transmission having been readily demonstrated in species/diseases with widespread lymphoid involvement (e.g. scrapie/BSE in sheep, chronic wasting disease in deer [32]), but not in those where lymphoid replication is limited (e.g. BSE in cattle [33-35]). ~~In humans, the majority of~~ Almost all vCJD patients examined to date show accumulation of PrP^{TSE}, a pathognomic marker of infectivity, to varying degrees in lymphoid tissues including spleen, tonsil, appendix and lymph nodes [36, 37], ~~while~~ although PrP^{TSE} ~~is rarely~~may also be detected in the lymphoid tissues of sCJD cases [38, 39].

Systematic studies in rodents infected with scrapie and human TSE isolates revealed that blood contains more than one thousand-fold lower levels of infectivity (10-100 infectious doses (ID)/ml) than brain (10^7-10^9 lethal doses (LD_{50})/g), and that titres increase as the infection progresses, reaching the highest values during the clinical phase of the disease [25, 27, 40, 41]. Using sheep infected with BSE or scrapie as an experimental model has the advantage that blood and its components can be collected and transfused in similar volumes to those used in human medicine. Thus, sheep experiments demonstrated that TSE infection can be transmitted by transfusion of 180ml – 450ml whole blood from pre-clinical donors during the first third of their incubation period. Transmission rates progressively increased with the time post-infection, reaching 100% for donor sheep in the late preclinical and clinical stages of disease [29, 42].

Cumulative evidence from different animal models supports the conclusion that the highest levels of TSE infectivity in blood are associated with leucocytes[43],[27],[44]. In sheep transfused with blood components from BSE-infected donor sheep, the highest transmission rates were found in those inoculated with buffy coat fractions [45],[30]. In the sheep scrapie model, the minimum number of white blood cells capable of transmitting scrapie following intravenous administration was 10^5[46]. The distribution of infectivity among specific subsets of WBC (e.g. lymphocytes, monocytes, granulocytes) has not been clearly established, but all these cell types may be capable of transmitting infection to varying extents [42, 46-49].

In sheep, prion infectivity associated with other cellular blood components (platelets, red blood cells) can be at least partly explained by the presence of residual leucocytes in those fractions, as leucodepletion appears to substantially reduce infectivity and transmission of infection [45, 50]. However, infectivity has been demonstrated in purified platelets from scrapie-infected sheep[25] and in experimentally infected deer with chronic wasting disease (CWD) by i.c. injection in highly sensitive transgenic mouse models expressing sheep and cervid PrP^C, respectively [25],[47]. Thus platelets may play a role in blood-borne transmission of scrapie or CWD, but the relevance of these findings to humans is not clear.

Plasma contains infectivity sufficient to transmit TSE infection by transfusion in sheep, but with much lower efficiency than whole blood or leucocytes [30, 45, 50]. This is partly due to the presence of leucocytes, since transmission rates were much lower following intravenous administration of leucodepleted or cell-free plasma [45, 50].

vCJD epidemic

Primary epidemic curve and modelling to predict size in UK

In March 1996, the probable link between vCJD and BSE in cattle was first suggested and soon confirmed experimentally ~~(LC: preferred statement because in March there was no experimental data available yet and ref 50 is from 1997) established~~ [21, 51, 52]. In the following 21 years, ~~Currently~~, a worldwide total of 230 vCJD cases have been reported in 12 countries (Figure 1). The peak number of UK cases (28) occurred in 2000, with a declining trend since then suggesting the primary epidemic is essentially over. The last case in the UK was diagnosed post-mortem in 2016. ~~None of the 178 UK cases remain alive; the last case was diagnosed post-mortem in 2016.~~ In France, the peak occurred slightly later, in 2005[53], which likely reflects the peak volume of UK origin beef imports during 1985-1995[54]. None of the French cases remain alive.

Initial modelling of the epidemic, based on 23 reported cases in the UK by January 1998, predicted from 29 to about 10 million cases[55]. The large upper bound reflected a number of key unknowns, principally the incubation period and number of people exposed per single infected bovine, which was speculated to be as high as 500,000[56]. In 2000, revised predictions estimated between 63 and 136,000 cases within the genetically susceptible population (i.e. 129 methionine homozygotes (MM) of the *PRNP* gene)[57]. At the time, only preliminary data from the first tonsil /appendix study (Appendix-I[58] -see below) was available, with zero infections detected in 3,170 tissues examined. Incorporating this as a UK vCJD 'prevalence' rate within their modelling, and by assuming testing could detect infection in the last 75% of the incubation period (with 100% sensitivity and specificity), Ghani and colleagues[57] noted that the upper bound on total epidemic size in the susceptible genotype population would be reduced to from 136,000 to 80,000.

Current modelling on UK epidemic size

Early modelling to estimate the size of the UK epidemic was restricted to primary transmission cases *via* consumption of BSE contaminated beef and failed to consider either cases among non-129MM *PRNP* genotypes, or secondary transmission. Subsequent to the confirmation that transfusion-transmission was a probable route of infection[3] and the identification of a possible case involving an ~~129MV~~ 129 methionine/valine (MV) genotype[59], new modelling was undertaken which expanded predictions of future cases to include 129MV and valine homozygous 129VV *PRNP* genotypes, as well as transmission via red cell transfusions[60]. Recognising that there remained significant uncertainty on the epidemic 'tail', in 2010 Garske and Ghani[60] predicted a peak annual incidence of around 11 cases, but with the 95% credibility interval between one and 65 cases. Notably, UK surveillance data subsequent to the modelling (from 2010 to 2016) record zero or one clinical case of vCJD per annum (Figure 1).

~~*Blood Safety Response*~~

~~In the late 1990s, before the link between blood transfusion and vCJD had been established, a number of blood safety measures were introduced in the UK[59], based on the worst-case scenario that vCJD could be transmitted by blood transfusion. The precautionary principle was applied, heavily influenced by the Phillips report[60] into the BSE epidemic.~~

~~The first UK blood safety response, started in 1998 and implemented fully by October 1999, was to introduce universal leucodepletion of blood components. A definite scientific basis for this initiative was lacking, although preliminary results suggested that B-lymphocytes had some role in disseminating the infectious prion[61].~~

~~Importation of non-UK plasma for fractionation was implemented over the same time period in the late 1990s. The Department of Health, advised by the Committee on Safety of Medicines, announced in 1998 that the fractionation of UK plasma would cease, and plasma supplies would be obtained from areas with a low prevalence of BSE. This decision pre-dated any decision by the regulators and was in part precipitated by the complexity of the requirement to withdraw batches of product containing plasma from individuals who were subsequently diagnosed with probable or definite vCJD.~~

~~Further risk reduction measures followed. It was assumed that children born after adoption of food safety measures in early 1996 had not been exposed to BSE in the diet and should therefore also be protected, as far as possible, from non-dietary risks of infection, including blood transfusion. Safe~~

~~and sufficient supplies of non-UK red cells and platelets were not available, but fresh frozen plasma (FFP) could be sourced from outside the UK. In 2003, imported FFP was introduced for the "post-1996" cohort and subject to methylene blue treatment to ensure that the reduction of vCJD risk was not replaced by an increase in the risk of transmission of blood-borne viruses. In 2004, the decision was taken to exclude from blood donation anyone who had been transfused since 1980. The following year, donors whose blood had been transfused to individuals who subsequently developed vCJD were also excluded.~~

Geographically-based deferral for residence in affected areas

In the absence of a blood screening test, regulatory authorities[61] and blood services in countries unaffected by primary cases[62, 63] sought to minimise the potential risk from vCJD. Geographically-based deferral was based on 'risk areas' and 'risk periods' as well as defining the duration of 'exposure' resulting in a 'significant' risk of vCJD infection. Risk areas were defined based on the presence of BSE and notified cases of vCJD. Most unaffected countries deferred donors with six months or more cumulative residence in the UK between 1980 and 1996. While the selection of six months exposure period was supported by modelling[61], for some blood services this period represented a compromise based on the associated level of donor deferral (loss), as this directly impacts blood product sufficiency.

Initially, vCJD cases were restricted to the UK and deferral questions were therefore based on residence in the UK and territories. The risk years were based on the timing of the peak BSE epidemic in the UK and the assumed full implementation, by 1996, of measures to preclude the BSE agent from the human food chain. As cases were reported in France, some countries (e.g. USA, Canada) added residence in France -and other affected countries to their vCJD deferral policy. Such policies continue to be adjusted[64].

Deferral for history of blood transfusion

The fact that vCJD may be transmitted by blood transfusion implies that transfusion recipients themselves might offer secondary risk of transfusion-transmission if they had received blood from a~~n exposed~~ donor with unrecognised infection. As a result, a number of countries (e.g USA[10] and France) followed the UK policy of indefinite deferral for presenting donors with a history of transfusion, ~~judged~~ constituting a~~t~~ risk of exposure to vCJD.

Tissue studies (tonsil/ appendix)

The first UK tissue study looked at removed appendices for evidence of deposition of abnormal prion protein[65]. One out of 8318 appendices examined had positive findings, giving an estimated prevalence of 120 per million of the population. A further study was carried out between 2007 and 2011, analysing tonsils by two independent ~~EIAs~~immunoassays, immunohistochemistry & western blot[66]. In total, approximately 150,000 tonsils were tested, and none was unequivocally positive. The appendix study was repeated (Appendix II) in a retrospective study on appendix samples collected between 2000 and 2012[67]. The samples were screened by immunohistochemistry, and 16 of 32,441 were positive (age range born between 1941 and 1985), giving an estimated prevalence of 493 per million (95% CI: 282-801/million).

Various caveats were expressed, perhaps most importantly that only appendix samples were confirmed reactive. It was hypothesised that the tonsil is only affected late in the disease process, and might therefore not be the tissue of choice for examination. Although vCJD occurs more

commonly in the younger age groups, reactive appendix samples were found across all age groups. It was proposed that many normal healthy persons could have peripheral PrP^{TSE} accumulation in the appendix, and it was therefore important to carry out a similar study in a BSE/vCJD free population. The Appendix III study looked at samples outside of the presumed BSE exposure period: those removed before 1980, and from young people born after 1996.

The results of the Appendix III study have not yet been reported in detail, but a preliminary report[68] revealed that positive samples were found in both groups examined, but not in any appendix removed before 1976 or in any individual born after 2000. It could be that there is a low background prevalence of abnormal prion protein in appendices, unrelated to the intensity of exposure to BSE, or that it is related to BSE exposure and that human exposure began in the late 1970s and continued until the mid-1990s, although at a lower rate than in the central years in the mid-1980s.

Second wave probability

Although the peak of vCJD cases occurred in the UK in 2000, there remains uncertainty about the possibility or probability of a second wave of infection. There are two possible sources of a second wave. First, the development of clinical disease in those infected through diet in the past, perhaps due to an extended incubation period in individuals of a non-129MM *PRNP* genotype. The first case of vCJD in such an individual was reported from the UK in 2016, and was in a 129MV *PRNP* heterozygote[7, 69]. Does this represent the start of a second wave, or a random event? Epidemiological studies of kuru, a disease in aboriginal tribes of Papua New Guinea practicing cannibalistic rituals, and acquired CJD have indicated that persons with any 129MM, 129VV, 129 MV *PRNP* genotype have been infected [70, 71], although the incubation periods were more prolonged in 129MV individuals[72, 73].

A second wave could also occur due to person-to-person transmission, for example though blood transfusion or surgical instruments. The four cases of transfusion transmission occurred in 1999 or earlier. If there are significant numbers of infected (and potentially infectious) carriers of vCJD in the UK population, as suggested by the appendix studies, it is difficult to explain why further cases of transfusion-associated vCJD have not appeared. There is a detailed assessment of every new case of vCJD, and the possibility of blood donation and/or blood receipt is examined every time a new case is diagnosed, so it is unlikely that there has been under-recognition of such cases. Furthermore, there has been no case of vCJD in a recipient exposed to multiple transfusions of blood components. It is estimated that there are several thousand such recipients, for example those regularly transfused in management of haemoglobinopathies and aplastic anaemia, and many more who are intensively transfused.

It thus appears, at present, that a second wave cannot be discounted, but is most likely to be due to past infection through diet becoming manifest after a prolonged incubation period in non-129MM *PRNP* genotype individuals, rather than person-to-person transmission. Only time and surveillance will answer this question.

Transfusion transmission

Risk assessments

The US FDA has developed models to estimate the residual risk of vCJD transmission from transfusion in the USA. The primary approach was to estimate the residual risk, based upon the assumption that some donors would have been exposed to BSE as a result of travel or residence in areas of significant BSE prevalence. The risk estimate was based upon data developed in the UK, based on the frequency of clinical vCJD (the low estimate) or a study of appendices (the high estimate). The overall risk estimates for the US were one transmission in 134 million(low) to 1 in 148,000(high) transfusions. Overall, the low value was thought to be most likely[74]. A subsequent model looked at the relative risk attributable to donors with exposure in different countries, concluding that deferral focused on the UK and most European countries, along with leucodepletion, was only marginally more effective than deferrals based upon the UK, France and Ireland (90.4% vs 89.9%), with 35% fewer deferrals[75].

Lookback studies

CJD

The American Red Cross (ARC) has been working with the US Centers for Disease Control for more than 20 years, in order to monitor the extent to which donors who are presumed to be incubating CJD may transmit the disease to recipients of their blood. When a confirmed case of CJD is identified and the patient is known to have donated blood, the relevant blood collection site is asked to identify those hospitals that received components from the affected donor. The recipients of those products are identified and their current vital status is determined and/or their identifying information is sought and vital status is determined by searches in the National Death Index on an ongoing basis. Direct and contributing causes of death are obtained for all decedents. As of the most recent report, 65 donors were identified: they had contributed a total of 1,816 components to the blood supply, 826 of which could be traced to recipients of whom 799 could be fully tracked. Of these recipients, 654 were deceased and 154 were still alive. The total follow-up was over 3900 person-years and no cases of CJD were identified. It is of interest to note that 414 recipients were transfused with blood collected just prior to the donor's diagnosis and that 264 recipients survived more than 5 years post-transfusion, 44 of whom survived for more than 20 years[20].

vCJD

As noted, the TMER[19] was set up to establish whether there is any link between blood transfusion and CJD. All types of CJD are included, but most interest has been in the vCJD arm.

All individuals old enough to have been blood donors who have a diagnosis of probable or definite vCJD are notified to the UK blood services, and a search is made of donor databases to establish whether the case was a blood donor. If there is a record of the individual, a lookback is carried out to establish the fate of all blood donations and associated issued blood components. Receiving hospitals are notified of components issued to them, and they establish the ultimate fate of the components from their laboratory records. If the blood was transfused, the recipient is identified to the blood service and the details shared with the National CJD Research and Surveillance unit (NCJDRSU). Health service records are then flagged so that a copy of the death certificate will be forwarded to the NCJDRSU when the individual dies, and cause of death and associated illnesses can be determined. At the start of the study, because there was no known link between vCJD and blood transfusion, the identified recipients were not notified. When the first link was made in December 2003[3] the surviving identified recipients were informed of the situation and told they were at risk of vCJD. In total, 67 donors developed vCJD subsequent to their donations and 68 recipients have been identified.

Three of the 68 recipients developed vCJD and died some years after transfusion from blood from donors who developed vCJD after blood donation. Two of the cases were linked to a common donor. Recipient disease was diagnosed 6.5 to 8.3 years after transfusion, which occurred in 1996 or 1997. The implicated donors developed vCJD 18 to 40 months after the transmitting donation. All of these clinical cases were 129MM homozygous[19, 76].

One further recipient (129 MV heterozygous) who received transfusion from a third donor died five years later without any clinical signs of vCJD, but abnormal prion protein was found at post-mortem in the spleen and one lymph node but not in the brain. [77].

Other deceased recipients have either had no post-mortem, or negative findings. Fourteen of 68 identified recipients remain alive and symptom-free, and all have now passed the tenth anniversary since the transfusion in question.

The TMER has performed exhaustive investigation of the donor and recipient cohorts and have found no further evidence of transfusion-transmitted vCJD. Similar examinations of sporadic and familial CJD have failed to demonstrate any evidence of transmission[19].

In the reverse part of the study, people with vCJD with a history of blood transfusion are notified to the blood services together with the identity of the treating hospital. The blood service establishes the transfusion history and traces the relevant blood donors; their NHS records are also flagged. In this process, ten people who developed vCJD have had a history of blood transfusion confirmed, but only three of them are linked to donors who are known cases of vCJD. These three recipients had already been identified through the "forward arm" of the study, as described in the preceding paragraphs. So, in this reverse process, no additional cases of transfusion-transmission have been uncovered, which were not already known. The identified blood donors relating to the other cases are considered to be possible sources of the vCJD in the recipient, and are therefore at risk of vCJD. They have been notified accordingly and withdrawn from the donor panel, as described in an earlier section, but none is known to have developed vCJD, after ~~many~~ almost 2400 person-years of follow-up[78].

After the link between blood transfusion and vCJD was established, the part of the TMER concerned with vCJD was reassigned from a research study to routine CJD surveillance. The research study continues to operate for sporadic and familial CJD cases, with negative findings to date.

Blood safety response and e~~E~~fficacy of risk mitigation strategies ~~*Blood Safety Response*~~

In the late 1990s, before the link between blood transfusion and vCJD had been established, a number of blood safety measures were introduced in the UK[64], based on the worst-case scenario that, vCJD could be transmitted by blood transfusion. The precautionary principle was applied, heavily influenced by the Phillips report[79] into the BSE epidemic.

The first UK blood safety response, started in 1998 and implemented fully by October 1999, was to introduce universal leucodepletion of blood components. A definite scientific basis for this initiative was lacking, although preliminary results suggested that B lymphocytes had some role in disseminating the infectious prion[80].

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in 1998 that the fractionation of UK plasma would cease, and plasma supplies would be obtained from areas with a low prevalence of BSE. This decision pre-dated any decision by the regulators and was in part precipitated by the complexity of the requirement to withdraw batches of product containing plasma from individuals who were subsequently diagnosed with probable or definite vCJD.

Further risk reduction measures followed. It was assumed that children born after adoption of food safety measures in early 1996 had not been exposed to BSE in the diet and should therefore also be protected, as far as possible, from non-dietary risks of infection, including blood transfusion. Safe and sufficient supplies of non-UK red cells and platelets were not available, but fresh frozen plasma (FFP) could be sourced from outside the UK. In 2003, imported FFP was introduced for the "post-1996" cohort and subject to methylene blue treatment to ensure that the reduction of vCJD risk was not replaced by an increase in the risk of transmission of blood-borne viruses. In 2004, the decision was taken to exclude from blood donation anyone who had been transfused since 1980. The following year, donors whose blood had been transfused to individuals who subsequently developed vCJD were also excluded.

It is difficult to assess the efficacy of risk-mitigation strategies for CJD ~~or indeed, for vCJD~~. The absence of any definitive evidence of transmission of sporadic CJD by transfusion is really not informative. Suffice to say that clearly there has been exposure of recipients to blood from donors who have been incubating the disease; a situation not amenable to any rational intervention. Routine deferral of those considered to be at risk has a minimal impact on blood availability, although the policies may be ~~challenging~~ confusing for those potential donors who are ~~impacted~~deferred.

A number of measures have been implemented to attempt to manage the risk of transmission of vCJD by transfusion. Again, however, it is not possible to assess the efficacy of these methods, although with definitive evidence of transmission, it can be argued that the absence of continuing transfusion-associated cases may be meaningful, albeit in the face of a decline in the number of cases of vCJD in the general population. In the UK, an early measure to combat such transmission was the implementation of universal leucodepletion. ~~The importance of this measure is evident when one considers~~In this context, it is of interest to note that all four transmissions reported from the UK were traced to non-leucodepleted red cells. Subsequently, in the UK, the use of locally derived plasma was eliminated from transfusion for young people born after 1996, and further manufacture into fractionated plasma products. Outside the UK, the broad focus has been on deferral from donation of potential donors with a history of travel to, and/or residence in the UK and parts of Europe. Outside the UK, there have been no cases of transfusion-transmitted vCJD reported, and vCJD reported cases have been attributed to probable dietary exposure outside the country, or to exposure to UK-derived beef in the country of residence. Thus, the ~~outcome of the apparent success of the deferral program is not possible to evaluate~~absence of transfusion-transmission of vCJD outside the UK cannot necessarily be attributed to the deferral policies. It appears likely that deferral policies will be modified as the risk of infection from the food chain is eliminated from countries affected by travel deferrals.

Filters intended to remove TSE prions from blood or plasma have been developed, ~~and were shown, in~~but laboratory studies~~,~~ to ~~achieve a modest, but not complete, reduction of infectivity~~assess their usefulness were inconclusive[81]. Such filters were evaluated for potential use in the UK and the Republic of Ireland [82], but were not recommended for adoption. Currently, available methods for pathogen reduction of blood components are not effective against TSE~~R~~ infectivity.

vCJD donor screening test

Developing an appropriately sensitive and specific donor screening test has been very challenging and to date elusive, despite major efforts. Detection of the PrP^{TSE} by classical serological methods is prohibited by the absence of any immune response by the host. As well, PrP^{TSE} levels in blood are extremely low (in the femtomolar range) and indistinguishable by general characteristics from PrP^{C}, which is present in very large excess.

~~While the value of a~~A vCJD donor screening test is anticipated to be beneficial, but ~~undeniable,~~ the performance requirements for such a test must be very stringent given the serious negative consequences of incorrect results in the context of notification for an incurable disease with a long incubation period. The importance of defining appropriate performance standards for candidate donor screening tests led to the establishment of a European Union (EU) regulatory standard (EU Commission Directive 2011/100/EU) for licensing for human use which requires that tests achieve at least 90% sensitivity and 99.5% specificity[83].

Presently there are two promising candidate test methods, Direct Detection Assay (DDA) developed by the UK MRC Prion Unit and protein misfolding cyclic amplification (PMCA), which have demonstrated the capacity to accurately identify vCJD prion infection in whole blood or urine[84-90]. Bougard and colleagues recently reported their PMCA assay was able to detect 18 patients with clinical vCJD among 256 plasma samples from the two most affected countries, with 100% sensitivity (95%CI: 81.5 to 100%) and 100% diagnostic specificity (95%CI: 96.5 to 100%)[88]. Critically, their assay was able to detect PrP^{TSE} in two samples collected from asymptomatic blood donors 1.3 and 2.6 years before they developed symptoms of vCJD, the first time silent carriage has been identified. In a related study, PMCA correctly identified 14 vCJD cases among 153 controls, which included patients with sCJD and other neurological or neurodegenerative disorders[89].

While there has been significant progress in vCJD test development, most notably the detection of PrP^{TSE} in pre-clinical samples[88], there remain substantial hurdles in respect of a high-throughput screening test. The PMCA assay has demonstrated the capacity to detect the minute amounts of PrP^{TSE} in sub-clinical samples but requires further validation on a larger sample set including non-129MM *PRNP* genotype samples. Also, in its current format it is not practical as a high throughput screening test as it requires several days to complete, although its use as a vCJD diagnostic, or confirmatory method for screening test-reactive samples, looks promising. The DDA assay is more suited to development for high throughput screening than PMCA. However, to date its capacity to detect samples with vCJD is restricted to those with a clinical diagnosis and with a sensitivity of 70%, compared to 100% for PMCA. It remains to be seen if the test has the capacity to interdict samples taken from pre-clinical vCJD cases and the rarity of such samples complicates clarification of this issue.

In the event that a suitably sensitive and specific high-throughput test is commercialised, it appears unlikely that implementation for universal donor testing can simply be assumed. The moral and

ethical issues associated with testing for an incurable disease are complex and given the low risk level outside countries directly impacted by vCJD, universal testing is unlikely to be cost-effective. Indeed, the potential refusal of donors to be tested leading to donor loss might precipitate supply shortages resulting in a net increased risk to recipients. The issues associated with counselling donors and recipients have been discussed in detail [91, 92]. The availability of a suitable confirmatory test is viewed as an essential pre-requisite to implementing universal screening. In the absence of a suitable confirmatory test, opt in/opt out testing (where donors could indicate their preference for notification in the event of a screening test reactive or confirmed positive result) is one suggested option.

Unanswered questions and future directions

Future management of the risk of transfusion-transmitted vCJD and CJD is unclear. Current evidence suggests that the transmission of vCJD from the food-chain has been effectively eliminated, at least in the UK and, ~~at least~~ in the USA, regulators have established that donors are considered at risk only if their exposure in the UK was between 1980 and 1996. It is to be presumed that such cut-off dates will also be implemented as other countries eliminate food-borne risk. Nevertheless, a taxing question is the extent to which those exposed before 1996 may be incubating infection; incubation periods beyond 40 years have been noted for kuru. One concern is that all but one of the clinically apparent vCJD cases have occurred among those with the 129MM *PRNP* genotype and this raises the question that the 129MV or 129VV genotypes may have a much longer incubation period. As noted, the latest UK case of vCJD was in a 129MV individual [7], which may indicate the beginning of a second wave of the epidemic.

In the UK, individuals born after 1996, and in theory not exposed to BSE in the food chain, might form a "lower risk" cohort for vCJD. Their donations could then be preferentially used for recipients who also belong to the "lower risk" cohort having been born after the precautionary measures for food were enacted. It was suggested, for example, that FFP from this donor cohort could be ear-marked "lower risk" and could replace the supplies of FFP being imported from outside the UK. The results of the Appendix III study have naturally led to more uncertainty about when exposure to BSE through diet in the UK can be said to have ceased also leading to a lack of confidence that a date can be defined for any cohort of "lower risk" donors. It also raises a question about the definition of a "lower risk" group of recipients and continued use of imported FFP for this group.

The current outbreak of vCJD appears to be over for PRNP codon 129 ~~methionine~~MM homozygotes, although there is some degree of concern about subsequent waves of disease among those already infected. There is considerable uncertainty about the size of the infected population, and as long as the cohort that was exposed to BSE survives, there will be at least a perception of some (albeit small) risk to blood safety. Accordingly, some precautionary measures will remain in place. Whether feasible testing methods for potential infectivity will be available or, if available, will be used, is an open question. Certainly cost-benefit assessments have not favoured the adoption of prion filters, especially in view of existing evidence that their efficacy appears to be less than optimal. It is possible that the apparent resolution of the BSE and vCJD epidemics will result in a reduction of public, political and financial interest in this field, which will be unfortunate, because there is much yet to be learned about TSE diseases and their management. It is also reasonable to consider that there may be lessons for the future. Is it possible that there could be further outbreaks of novel TSE diseases of zoonotic origin? CWD of cervids is extraordinarily infectious in

nature and there have been some studies indicating the possibility of limited cross-species infection. As is true for other agents that may impact blood safety, continued alertness and surveillance is ~~advisable~~necessary.

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Legend for Figure 1.

Title - vCJD Cases Worldwide

Worldwide cumulative vCJD cases (n=230) by country and year compiled from; http://www.eurocjd.ed.ac.uk/surveillance data 1.html as of May 28, 2015 and http://www.cjd.ed.ac.uk/sites/default/files/figs050117.pdf as of April 24, 2017

vCJD Cases Worldwide