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The Evidence for Sporadic Creutzfeldt-Jakob Disease being an Acquired Disease

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This thesis is submitted for the degree of Doctor of Medicine

Declarations

I declare that the thesis has been composed by myself and that the work has not be submitted for any other degree or professional qualification. I confirm that the work submitted is my own, except where work which has formed part of jointly-authored publications has been included. My contribution and those of the other authors to this work have been explicitly indicated below. I confirm that appropriate credit has been given within this thesis where reference has been made to the work of others.

The work presented in Chapter 1: The Neuroepidemiology of Human Prion Disease is in the process of being submitted for publication as a chapter in The Oxford Textbook of Neuroepidemiology, titled, "The Neuroepidemiology of Human Prion Disease" by Patrick Urwin (the student and author of this declaration) and Anna Molesworth (one of my supervisors). I carried out the literature review and wrote the first draft. The review process is ongoing, and at each stage both authors have contributed to drafting the work and will approve the final version.

The work presented in Chapter 2: The Evidence for Blood and Blood Product Transfusion Transmission of sCJD consists mainly of two papers. The first was published in Vox Sanguinis, 2016 May, as "Creutzfeldt-Jakob disease and blood transfusion: updated results of the UK Transfusion Medicine Epidemiology Review Study" (1). The work was co-authored by Patrick Urwin (the student and author of this declaration), Jan Mackenzie, Charlotte Llewelyn, Robert Will and Patricia Hewitt. The design of the study followed the earlier Transfusion Medicine Epidemiology Review publication (2); my co-authors all contributed to the research design, acquisition and analysis of data. I contributed to data acquisition and data analysis and wrote the first draft of the paper. All authors contributed to drafting the paper and all approved the final version.

The second paper in Chapter 2 was published in Emerging Infectious Diseases, 2017 June, as "Sporadic Creutzfeldt-Jakob Disease in 2 Plasma Product Recipients, United Kingdom" (3), by Patrick Urwin (the student and author of this declaration), Kumar Thanigaikumar, James W. Ironside, Anna Molesworth (one of my supervisors), Richard S. Knight (my principal supervisor), Patricia E. Hewitt, Charlotte Llewelyn, Jan Mackenzie, and Robert G. Will. The patients were referred to me in my role as the NCJDRSU Research Registrar; I reviewed both patients, interviewed the families, identified their importance and relevance to this subject, and obtained permission from one of the two patient families for publication as well as writing the first draft of the paper. Kumar Thanigaikumar obtained permission from the second family. Robert Will guided and advised me about this work. All authors contributed to drafting the paper and all approved the final version.

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Contents

Abstract

Sporadic Creutzfeldt-Jakob Disease (sCJD) is the most common form of human prion disease. It is a rapidly progressive dementia with other associated neurological abnormalities which is invariably fatal, usually within 4-6 months of first symptoms, and affects around 80-100 patients in the UK each year. It is caused by propagation around the central nervous system of PrP^{Sc}, an abnormal conformational form of the host PrP^{C} protein, which causes the transformation of PrP^{C} to PrP^{Sc}, essentially self-replicating. This process causes neuronal loss and other pathological abnormalities, explaining the clinical presentation. sCJD is believed to occur as a *de novo* convolutional change which seems to occur as an unfortunate chance event.

The existence of other forms of CJD, including variant CJD (vCJD) and iatrogenic CJD (iCJD) highlight the fact that the abnormally folded forms of the prion protein are infective agents, which can be transmitted by diet (from bovine spongiform encephalopathy – BSE – in cattle to humans in vCJD), or by medical intervention (predominantly from contaminated dura mater grafts, and cadaveric pituitary derived growth hormone in iCJD). These two routes of transmission can be associated with long intervals (up to around 40 years has been described) between exposure to the infective agent and subsequent symptom onset.

It is possible that some cases classified as sCJD may in fact be acquired through other means of transmission. As part of the UK National CJD Surveillance process, the NCJDRSU collect data on potential routes of CJD transmission. In this thesis, I describe and analyse the data concerning three potential such routes for the definite or probable cases of sCJD reviewed by the NCJDRSU between 2010 and 2015 inclusive:

1) Packed red cell blood transfusion has been associated with iatrogenic transmission of variant CJD. We found no evidence of such transmission in sporadic CJD. I also describe the first two reported cases of sCJD occurring in patients with clotting disorders who had received numerous transfusions and other blood products; there have been no further published cases since this report, and these two cases are considered likely both to represent the chance development of sCJD.

2) Tissue and organ transplantation is another recognised route, with iatrogenic transmission of CJD described in corneal transplantation. A small number of sCJD patients have undergone surgery involving transplantation of tissues or organs. Although a few cases of interest were identified as recipients of potentially infective materials, including three patients who are suspected to have received dura mater grafts and one patient who underwent definite corneal transplantation, it was not possible to confirm the exact nature and source of these materials, meaning it is not certain that these cases definitely represent iatrogenic transmission. Therefore, I found no evidence that tissue or organ transplantation is responsible for the development of cases of iCJD among this patient cohort.

3) Other surgical procedures may convey the risk of transmission of PrP^{Sc} through incomplete sterilisation of instruments. I assessed the potential for UK sCJD patients to have come into contact with instruments used on another CJD patient, looking at associations between surgical procedures occurring within the same year, at the same hospital, and within the same surgical domain. While some such associations were identified, interpreting these associations is extremely difficult, in large part due to the absence of a suitable control group, as well as incomplete data availability.

Overall, this work has identified no definite transmission of iCJD masquerading among the sCJD 2010-2015 patient cohort. There are significant limitations to each

aspect of this work, in large part pertaining to incomplete medical records; these limitations are addressed in the relevant chapters. Some of these limitations may be difficult to overcome if future these studies are repeated in the future with a new cohort.

Lay Abstract

Sporadic Creutzfeldt-Jakob Disease (sCJD) is the most common form of human prion disease. It is a rapidly progressive dementia (usually memory and language loss) with other associated neurological abnormalities (such as coordination problems and involuntary jerking movements), and is invariably fatal, usually within 4-6 months of first symptoms. It affects around 80-100 patients in the UK each year. It is caused by the spread around the brain and spinal cord of PrP^{Sc}, an abnormally shaped version of a protein found in all humans (and many other animals) which is called PrP^{C} when found in its normal, healthy form. This PrP^{Sc} protein replicates itself by changing the normal protein into the abnormal, diseased form. This process causes damage to, and loss of brain cells, as well as other abnormalities which are detectable both pathologically (looking at tissue under the microscope) and biochemically (using molecular techniques to study the proteins themselves, which are too small to see directly with a microscope). The damage to, and loss of, brain cells explains symptoms experienced by patients. sCJD is believed to occur as a spontaneous protein shape change which occurs as an unfortunate chance event.

Other forms of CJD have been transmitted by diet – from bovine spongiform encephalopathy (BSE) in cattle to humans as variant CJD (vCJD) – or by medical procedures –called iatrogenic CJD (iCJD). This medical transmission predominantly occurred through injection of hormones extracted from glands located next to the brain of deceased humans, and through implantation dura mater grafts. The dura mater is a tough membrane which surrounds and protects the brain. Until 1992, dura mater obtained post mortem from human cadavers was commonly used as a patching material in neurosurgery, for example to repair cerebrospinal fluid leaks, and also in some other (non-neurosurgical) procedures. The fact CJD can be transmitted in these forms highlights that the abnormal protein, PrP^{Sc} is an infectious particle which can transmit certain forms of the disease.

It is possible that some cases which are believed to be the sporadic (random, chance) form of CJD may in fact have been acquired through contact with other patients who have suffered from CJD, perhaps through transplantation of body tissues or whole organs, or through contaminated blood transfusion or the use of surgical instruments. In this thesis, I describe and analyse the data collected by me and others at the National CJD Research and Surveillance Unit to look for evidence of transmission of CJD by these means for patients with sCJD referred to the NCJDRSU between 2010 and 2015 inclusive.

1) Packed red cell transfusion has been associated with accidental medical transmission of variant CJD, but we found no evidence of such transmission in sporadic CJD. I also describe the first two reported cases of sCJD occurring in patients with clotting disorders who had received numerous transfusions and other blood products; these two patients are thought most likely to have developed sCJD by chance, and there have been no further published cases of sCJD among clotting disorder patients.

2) A small number of sCJD patients have undergone surgery involving transplantation of organs or suspected implantation of relevant tissues. There are significant limitations to the available data, meaning it is not possible to be completely certain regarding the details and origins of these organs and tissues. There was no evidence of tissue and transplant transmission of CJD among the patient group studied.

3) Other surgical procedures convey the risk of transmission of PrP^{Sc} through incomplete sterilisation of instruments. I assessed the potential for UK sCJD patients to have come into contact with one another through associations between surgical

procedures occurring within the same year, at the same hospital, and within the same surgical domain. While such connections were identified between pairs of patients, without a non-CJD group to compare for the same connections, it is not clear what these connections represent, as such connections could occur by chance alone. There are also significant limitations caused by incomplete data availability which further hampers any analysis.

Overall, while this work has identified no definite transmission of CJD by blood or blood product transfusion, or tissue and organ transplantation. Although it is possible that sCJD patients may have contact with one another by surgical instruments, these episodes of potential interconnection may represent chance occurrences, and a non-CJD control group would help interpretation of these results, if further data was collected in the future. There are significant limitations of the analysis possible from NCJDRSU data, which are detailed in the relevant chapters.

Acknowledgements

I owe a great deal of thanks to my supervisors, Anna Molesworth and Prof. Knight. Both have been full of patience and encouragement, despite my stuttering work output, and distractions with my efforts to obtain further employment at the end of my time in the NCJDRSU. Anna has kindly shown me, as developing neurologist, the ropes in terms of starting out in epidemiology, and has been a therapeutic counsellor, sharing many whinges about the bumpier aspects of politics, medicine, and the world of CJD, as well as plenty of happier chats about gin, Scottish island hopping and coffee. Prof. Knight had a huge part to play in extracting me from London and dragging me up to Edinburgh, and I am forever grateful that he employed me, as my life has improved immeasurably since moving to Edinburgh. He also spent many years gently helping me as a developing neurologist, including teaching me the importance of admitting, "I don't know" to both patients and colleagues, and educating me in the finer points of century eggs and permafrost electricity pylons.

Anna Williams, my pastoral supervisor, ensured we kept on topic, that I was never unsupported, and fought throughout my time in the NCJDRSU for more time for me to get on with writing. She also helped me to appreciate the importance of bringing dark chocolate ginger biscuits to each and every important meeting.

While Prof. Will did not have a formal role as supervisor for my work, his patient assistance was of huge benefit, particularly his help with the two papers published in Chapter 2, and in allowing me to discuss the more complex patients with him.

The NCJDRSU could not run without Jan Mackenzie, whose knowledge of all CJD patients and all the finer workings of the surveillance database was critical in my thesis work. I am also extremely grateful to all the other administrative and clinical

staff who keep the Unit running, and have all contributed to my work, including through data entry, IT support, discussion of pathological findings, and nursing assistance on patient visits; it has been a great pleasure working with each them.

Finally, I must thank my wife, Caroline, for gentle encouragement, pertinent reminders, and firm pokes to keep things ticking along with my original submission and subsequent corrections. Cups of tea and tolerance for my stresses and grumps have also been warmly received. Her proof reading and admin help has also been generous and most welcome, although I should have given her more time to help with the former! I'm really looking forward to getting out walking in the mountains with her soon.

Chapter 1

The Neuroepidemiology of

Human Prion Disease

Introduction

Prion diseases affect both humans and other animals, and comprise a number of rare and ultimately fatal neurodegenerative conditions that result from the accumulation in the central nervous system (CNS) of an abnormal form of a naturally occurring protein, called the prion protein (4). This protein, coded for by the prion protein gene (*PRNP*), is widely expressed in many different tissues although it is predominantly found in the CNS (5). In its normal, protease sensitive, form, designated PrP^{C}, it is thought to be neuroprotective even though its exact function remains unclear. In disease states, the PrP^{C} is converted to an abnormal form, designated PrP^{Sc}, following a "seeding" event. The PrP^{Sc} is protease resistant and able to propagate through further contact with PrP^{C}, building up over extended incubation periods and causing pathology in the CNS tissues affected.

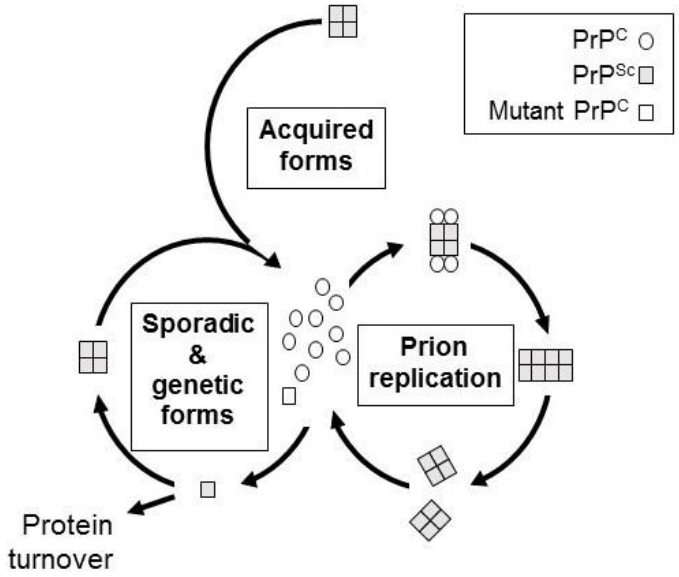
Prion diseases in humans are characterised clinically by prolonged incubation periods (in the forms where the timepoint of exposure is known) followed by a relatively rapid progressive loss of cognitive and motor functions. Case identification relies principally on the investigation of suspected cases by neurologists and neuropathologists, although other specialities, for example general medicine, geriatric medicine, and old age psychiatry may also be involved. Clinical diagnostic criteria include abnormal neurological features and investigation results, the latter principally involving magnetic resonance imaging brain scans and cerebrospinal fluid tests. Definitive diagnosis, however, requires neuropathological evidence – usually obtained at post mortem – of aggregated PrP^{Sc}, gliosis, neuronal loss and brain tissue vacuolation or spongiform change (6) (see also http://www.cjd.ed.ac.uk/sites/default/files/criteria_0.pdf, accessed 31.07.17). Currently there are no diagnostic blood tests for pre-clinical disease, although this is

an area of ongoing research in vCJD (further details concerning blood testing in vCJD can be found on page 26).

Human prion diseases can be classified as genetic, sporadic or acquired, according to the initial seeding event thought to have occurred (see Figure 1.1). Genetic (or familial) prion disease in humans is associated with the presence of (one or more of) over 30 established *PRNP* mutations that destabilise PrP^{C} and cause or predispose to conversion (7). In sporadic disease, the initial seeding event is thought to be a spontaneous protein misfolding event, likely associated with a somatic mutation or a chance conversion of PrP^{C} to PrP^{Sc} of unknown cause. Acquired disease results from exposure to exogenous PrP^{Sc}, from an animal or another human. A methionine-valine polymorphism at codon 129 of the *PRNP* gene affects disease phenotype as well as susceptibility, with methionine homozygosity at this locus predisposing to prion disease and the presence of valine generally associated with longer incubation periods and slower disease progression (8, 9).

Figure 1.1: Simplified schematic of the prion hypothesis.

Source: Mark Head, NCJDRSU, Edinburgh, UK.



In recent years there has been increased interest in the field of human prions, with evidence that the proteins associated with other neurodegenerative diseases, for example Aβ associated with Alzheimer's disease, may also propagate in a prion-like manner (10). However, I have not included these other conditions in this thesis, instead focusing on the classical definition of prion disease. The focus is on all forms of Creutzfeldt-Jakob Disease (CJD), although we will mention other forms of human prion disease, as well as animal prion diseases relevant to humans.

Sporadic Creutzfeldt-Jakob Disease

Clinical-pathological features:

CJD was first described in 1920 by Neuropathologist Hans Creutzfeldt (11) and shortly afterwards by Neurologist Alfons Jakob (12). Although rare, CJD is the most common human prion disease and the majority of cases are idiopathic, occurring sporadically worldwide. Most sporadic CJD patients suffer rapidly progressive cognitive decline with myoclonus, ataxia, cortical visual impairment, pyramidal and/or extrapyramidal signs, progressing to akinetic mutism before death. Disease duration from first symptoms to death is usually short, with a median of around 4 months, however there can be significant variation, with death occurring within one month of onset or after several years. Sporadic CJD is, however, clinically heterogenous, with the patient's *PRNP* codon 129 methionine-valine polymorphism status and the subtype (type 1 or type 2) of the protease-resistant component of PrP^{Sc} found when investigating PrP^{Sc} by Western Blot, designated PrP^{res}, associated with the clinical phenotype; together, codon 129 status and PrP^{Sc} subtype are used to define the strains of sporadic CJD (8, 13). Longer survival has been associated with younger

age at onset, female sex, *PRNP* codon 129 methionine-valine heterozygosity and type 2A PrP^{res} (14).

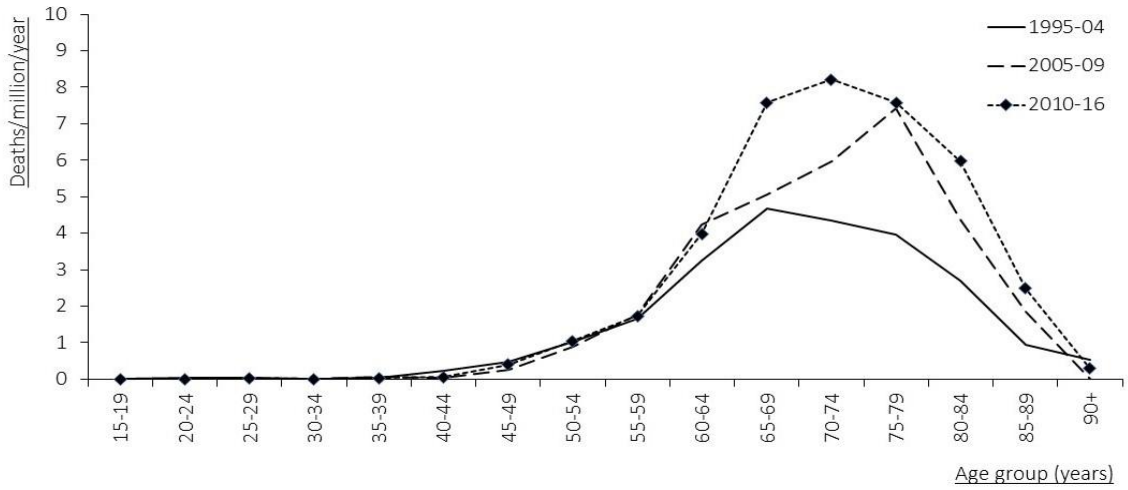
Incidence and basic demographics:

Sporadic CJD occurs at rates of between 1 and 2 cases per million population worldwide (see http://www.eurocjd.ed.ac.uk/surveillance_data_1.html, accessed 31.07.17). It affects males and females approximately equally, and is a disease mainly of the older population. In the United Kingdom (UK), the median age at death is 68 (range 20-95) with a peak in mortality between 65 and 79 years of age and fewer cases in individuals either under 50, or above 80 years of age, although cases have been reported (outside the UK) in adolescents, and in adults over 90.

In the UK and other countries with well-established surveillance systems, annual mortality rates for sporadic CJD have increased over time (15) (see figure 1.2 overleaf). This may reflect better ascertainment of cases across all ages, particularly in older patients who – as a group – have perhaps become better investigated as new treatments and diagnostic tools are developed for certain forms of dementia. However, the low rate of disease seen in the most elderly is noteworthy, in that perhaps more cases might be expected in this age-group from a sporadic protein misfolding event. It is unclear whether this decline in incidence at older ages is real, or perhaps reflects incomplete case ascertainment in the most elderly, with these "missed" diagnoses perhaps being lost amongst more common dementias, resulting in cases of prion disease being misidentified (16, 17).

Figure 1.2: Age -specific mortality from sporadic CJD in the UK, 1995-2016

Source: NCJDRSU, Edinburgh, UK.



Risk factors:

Codon 129 of the prion gene may be regarded as a risk factor for the development of sporadic CJD, with overrepresentation amongst cases of the homozygote states, particularly methionine, relative to the general population (18).

Although some exposures to tissues containing abnormal prion proteins (PrP^{Sc}) from patients with sporadic CJD in medical settings have resulted in transmission from person to person, associated with the accidental transplantation of infective tissue or inoculation of small amounts of residual tissue adhering to reusable surgical instruments (see section on iatrogenic CJD below), no consistent risk factors for the development of sporadic CJD have been identified. Some case-control studies have identified an increased risk of sporadic CJD associated with prior surgical exposure (19-21) or through blood transfusion (22) but this has not been convincingly demonstrated by others (19, 23, 24). In addition, there is no evidence of any increased

occupational risk (25), even though people employed in certain professions, for example neuropathologists or neurosurgeons, might be exposed to infectivity in the course of their work. Very occasionally, sporadic CJD cases are identified in people who have lived close together at some point in their lives, raising the possibility that the cases may be linked, for example, a husband and wife pair, both of whom developed sporadic CJD (26). One analysis has also provided evidence of increasing geographic clustering of sporadic CJD cases with increasing time interval before disease onset, consistent with some sporadic CJD cases perhaps resulting from a past exposure to a common external factor (27). Identifying a common source of transmission many years after the event is difficult, however, and there are other limitations to the above studies, not least bias and confounding factors (28). But while it is plausible that a small number of cases classified as sporadic CJD have resulted from transmission associated with other, as yet unidentified, causative factors, the evidence for this is weak. Overall the findings are consistent with the hypothesis that sporadic CJD is not an acquired disease.

Variably Protease Sensitive Prionopathy

Recently a new disease called variably protease sensitive prionopathy (VPSPr) has been identified with distinct biochemical characteristics: the accumulating PrP^{Sc} shows a markedly different response to the proteases used in biochemical analysis than as is seen in other forms of CJD, with variable sensitivity in different brain regions (29, 30). The disease affects those in mid-to-later life (range 48-81), patients have a non-specific clinical profile and a duration of illness ranging from under 1 to over 5 years; the neuropathology at post mortem would be atypical for sporadic CJD.

Currently, the true incidence and underlying epidemiology of this novel prion disease are uncertain. Diagnosis is neuropathological and consequently case identification has been retrospective, with most cases of VPSPr worldwide having been identified in the UK (13 cases reported to date, see http://www.cjd.ed.ac.uk/sites/default/files/figs_4.pdf, accessed 31.07.17), as well the USA and other parts of Europe. Cases have been identified in all codon 129 *PRNP* polymorphism groups, but the disease has predominantly been identified in valine homozygotes, the rarest codon 129 status. Although research suggests that VPSPr has limited transmission potential (31), its longer disease course and non-specific early clinical findings may mean VPSPr is falsely diagnosed as other forms of dementia and likely to be under-ascertained.

Variant Creutzfeldt-Jakob Disease

Clinical-pathological features:

In 1996 a new disease, variant Creutzfeldt-Jakob Disease (vCJD), was identified in the UK (32). It was differentiated from the more commonly encountered sporadic form of CJD by the young age of patients (the median age at death based on UK cases observed to date is 28, range 14-75) and unusual neuropathological and clinical features. Cases typically presented with a neuropsychiatric prodrome of apathy or withdrawal, followed by early ataxia, early and prominent sensory features, and a notable absence in early stages of memory problems or dementia. Neuropathological examination identified plaques of PrP^{Sc} deposition, described as "florid" and largely distinct from any seen in other forms of CJD, alongside the more typical features of spongiform changes, loss of neurones and astrocytosis, as seen in other prion diseases. Cases had a longer disease duration from symptom onset to death than was

typical of other forms of CJD; the median duration based on UK cases is currently 14 months (range 6-114) (15).

Incidence worldwide:

Following reports of the first vCJD cases in 1996, the vCJD epidemic comprised a single wave of epidemic cases, peaking in the UK in 2000, later elsewhere, and subsequently declining to under 1 new diagnosis currently reported per year worldwide (see Figure 1.3). At the time of writing, a total of 231 cases of vCJD have been identified worldwide, mostly (178, 77%) within the UK (see http://www.cjd.ed.ac.uk/sites/default/files/worldfigs_0.pdf, accessed 31.07.17). The non-UK vCJD cases have occurred mainly in France (27 cases), with no more than 5 cases reported in each of a small number of other countries in Europe and the rest of the world.

Figure 1.3. BSE and vCJD deaths by year in the UK and other European Union (EU) countries.

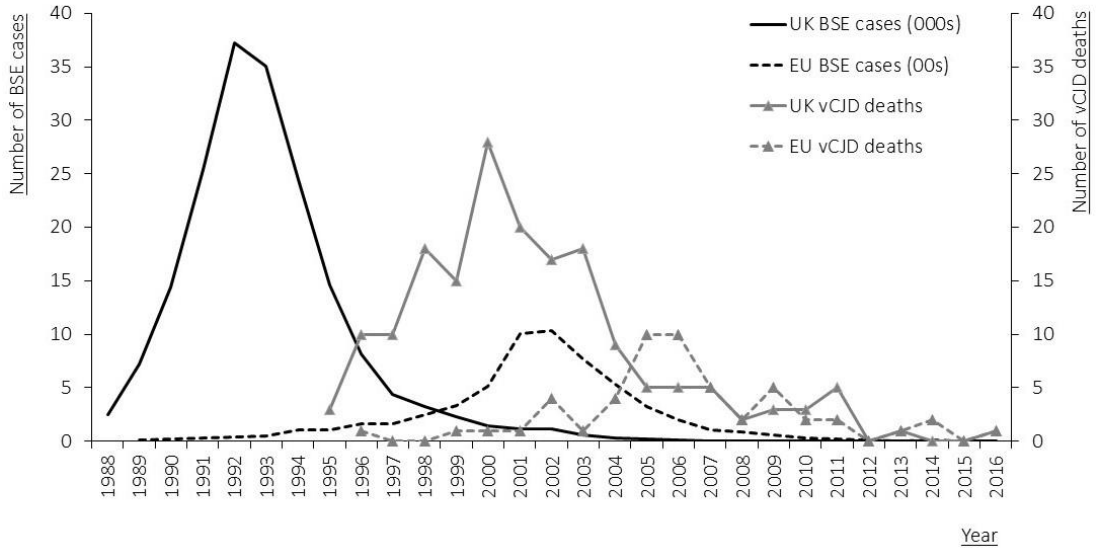


Figure 1.3 source: NCJDRSU, Edinburgh, UK and World Organisation for Animal Health. Please note different units for UK and EU BSE cases.

Biochemical similarities, transmission studies and strong geographic and temporal associations with the animal zoonotic bovine spongiform encephalopathy (BSE) have provided strong laboratory and epidemiological evidence that vCJD and BSE are linked (33-36). BSE is addressed in more detail in the section on animal prion diseases that follows in this chapter (page 38), but those aspects of direct relevance to the vCJD epidemic are covered within this section. The first cases of BSE were reported in the UK in 1986 (37, 38). In total, one million cattle are thought to have been infected during the ensuing BSE epidemic, with over 400,000 of these animals estimated to have entered the human food chain (39). The epidemic peaked in 1992 and 1993, and then subsequently declined to one or two cases per year (see http://www.oie.int/en/animal-health-in-the-world/bse-specific-data/ accessed 31.07.17). Far fewer BSE cases have been diagnosed outside the UK (e.g. Ireland reported 1656 between 1989-2016 inclusive, while France reported 1026 within the same time period, see http://www.oie.int/en/animal-health-in-the-world/bse-situation-in-the-world-and-annual-incidence-rate/number-of-reported-cases-worldwide-excluding-the-united-kingdom/ accessed 15.08.18).

Risk factors:

The identification of the vast majority (178 of 231, see http://www.eurocjd.ed.ac.uk/surveillance%20data%201.html and https://www.cjd.ed.ac.uk/sites/default/files/figs.pdf both accessed 15.08.18) of vCJD cases among individuals resident within the UK for more than six months between 1980-1996 is attributed to the transmission of the BSE agent to humans between 1980 (when BSE is thought first to have begun circulating) and 1996 (when the final controls to protect animal and human food chains were put into place). The 53 non-

UK vCJD cases have been attributed either to exposure to BSE from UK exported cattle, meat or animal products – rather than necessarily representing infection outside the UK – or to the individual patient spending time in the UK during the period of probable BSE exposure (40, 41). The greatest risk to humans is thought to have been the dietary consumption of meat products which could have been contaminated with the BSE agent by the extraction techniques required in the production of mechanically recovered meat (42) or by contact with brain and other CNS material from infected animals during butchering (43), particularly prior to the UK ban in 1989 of certain tissues of cattle, sheep and goats (including brain, spinal cord and other material) considered likely to be infective. It is certainly noteworthy that no UK vCJD cases have been seen in people born after 1989, indicating the significant public health impact of the introduction of this ban.

Another important risk factor is age. Despite the fact that most BSE exposure occurred in the UK in the late 1980s and early 1990s, the median age at onset of vCJD (26 years, range 12-74 years) has remained the same, consistent with those born in the 1980s being infected later in the BSE epidemic when they were older, rather than at the beginning (see http://www.cjd.ed.ac.uk/data-and-reports/incidence-variant-cjd-uk, accessed 31.07.17). This may reflect lower age-related susceptibility to infection (44) or reduced exposure to BSE in food in the very young (45).

Codon 129 genotype is also important: until 2016, all neuropathologically confirmed cases of vCJD who were tested (119 tested of 123 confirmed cases) were found in methionine homozygous individuals. In 2016 the first pathologically confirmed case of vCJD in a methionine-valine heterozygous patient was identified (46). Whether this case represents the beginning of a second epidemic wave of vCJD amongst other genotypes, or is an isolated case remains to be seen. The incubation period between exposure to infection and vCJD symptom onset is estimated to be 15 years, however in non-methionine homozygous individuals this is likely to be longer (47). An earlier

case of suspected vCJD has been reported in a codon 129 methionine-valine heterozygote; the patient lacked the supportive investigations to be classified, according to World Health Organisation criteria, as either a definite or probable case (48).

Prevalence:

Despite early fears of a very large epidemic, far fewer vCJD cases have arisen than anticipated. The reasons for this are unclear. Given the extensive exposure of the human population to BSE, it is likely that there is a substantial species barrier, but other factors may also be involved, including effect of dose, age related- and genetic susceptibility and the presence of co-factors necessary for infection and the development of disease (49, 50).

However, a recent study of the prevalence of PrP^{Sc} deposition in appendix tissue in the UK has indicated 1 in 2000 among those born 1941-85, the age group considered most exposed to BSE, carry the abnormal convolutional form of the prion protein (51). The exact interpretation of this PrP^{Sc} deposition is uncertain, particularly given that it is observed at a frequency far higher than the observed symptomatic cases, but one interpretation is of an asymptomatic carrier state following BSE exposure. Moreover, such PrP^{Sc} deposition was found across all codon 129 genetic subgroups, with a higher proportion of valine homozygotes (25% of positive specimens) than would be expected in the normal population (11 % (18)). A subsequent UK study noted rates of PrP^{Sc} appendix deposition carriage consistent with the above, but in time periods outside the period 1980-1996, when the population has not previously been considered to be exposed to BSE (52). While such deposition is of unknown significance to the individuals' risk of developing vCJD, or as yet to interpreting the general population exposure to BSE, it is a significant public health concern.

Person-to-person spread:

In vCJD, PrP^{Sc} is deposited outside the CNS at significantly higher levels compared to other forms of CJD, most likely due to the route of exposure to BSE across the gut (53). This peripheral, predominantly lymphoreticular, pathogenesis outside the CNS in patients with vCJD has led to concerns regarding the potential for iatrogenic transmission of vCJD by procedures related to a wider range of (non-CNS) tissues than in other forms of CJD where pathology and PrP^{Sc} is largely limited to the CNS. This is a particular concern given the current absence in routine clinical and infection control practice of both decontamination methods that can fully remove PrP^{Sc} from surgical instruments, and of a validated early blood test for pre-clinical disease (see page 26 for further details).

There is no evidence of mother-to-child vertical transmission (54), or from case control studies of a risk of vCJD associated with occupation or past surgery (42). Dental instruments may provide a route of transmission, as animal studies have demonstrated that PrP^{Sc} can be identified in the dental pulp and gingival margin after small bowel challenge with infective material (55), although no dental risk factors were identified in an analysis of UK cases (56). Lookback studies have also not identified any associations between cases linked to tissue or organ transplantation (57), however depending on the mass of tissue transplanted, the site of transplant and infectivity of the tissues involved such procedures may carry a risk of passing on vCJD infection if the donor were to be infective.

Crucially, however, vCJD has been transmitted through blood. To date three such instances of vCJD transmission have been identified worldwide, all occurring in the UK, where a patient received a non-leucodepleted red cell transfusion from a donor who subsequently developed vCJD (2, 58). These three cases, two of whom received

blood from the same donor, became symptomatic at 6.5, 7.8 and 8.3 years after receiving the implicated transfusion; each shorter than the 15 year estimated mean incubation period of vCJD after dietary exposure to BSE (the exact range of incubation periods in patients with dietary vCJD is not known, as it is not clear when each individual patient was exposed, and many may have had multiple exposures over many years, but it took 10 years from identification of BSE to the detection of vCJD (59)). In addition, asymptomatic infection has been identified in the spleens of two patients who died of non-neurological illness. The first was a recipient of vCJD-implicated non-leucodepleted blood, transfused 5 years prior to the death of the recipient (60) and the other was treated with multiple UK sourced blood products, including batches of clotting factors derived from donors who subsequently developed vCJD (61). The second individual died 14 years after first exposure to vCJD implicated factor VIII, 12 years after a second exposure, and 10 years after 3 unit transfusion with non-leucodepleted red cells. It is considered statistically unlikely that these splenic deposits of PrP^{Sc} were acquired through dietary means and this is supported by the absence of detectable PrP^{Sc} in their tonsils or gut associated lymphoid tissue. Each of these individuals were codon 129 methionine-valine heterozygous; it is unknown whether, had they lived longer after the exposure, they might have developed clinical vCJD.

The occurrence of the blood/blood product-associated cases, together with laboratory evidence that indicates that the vCJD strain is not altered significantly following transfusion transmission (62, 63), suggests that transfusion of blood from donors who are in a presymptomatic phase of vCJD may be an efficient means of transmission. To try to prevent (the at that point theoretical) transmission of vCJD through blood transfusion, leucodepletion was implemented in 1999 in the UK, based on experimental data suggesting that lymphocytes were involved in early pathogenesis, and there have been no further reports of blood or blood product associated transmission of vCJD subsequently (1).

A direct detection assay was developed by the MRC Prion Unit to try to identify PrP^{Sc} in the blood of patients with vCJD; they published their work in February 2011 (64); further work by that group was published in April 2014, suggesting the test had sufficient sensitivity to have potential utility as a presymptomatic screening test for vCJD (65). This test appeared to detect PrP^{Sc} in blood of many vCJD patients, with a sensitivity when testing blood from patients who were symptomatic of vCJD of around 70%. It is unknown whether the observed sensitivity in symptomatic individuals would translate to those not yet symptomatic of vCJD as it is thought there would be smaller levels of circulating PrP^{Sc} early in the disease course. This test would require validation in the at risk population – those resident in the UK and exposed to beef products during the BSE epidemic – but such validation would prove problematic due to the extremely small numbers of new vCJD patients, with only a single individual seen in the UK between 2014 and 2018 inclusive (46) (see also https://www.cjd.ed.ac.uk/sites/default/files/figs.pdf, accessed 15.08.18). At least two other groups are also working in this field, both using protein misfolding cyclic amplification (PMCA), which appears to yield higher sensitivity and specificity (each approaching 100%) than the direct detection assay technique. In both studies (66, 67). Bougard *et al.* were also able to identify presymptomatic infection in 2 vCJD patients for whom blood samples were available prior to the development of clinical features of vCJD (those individuals had donated blood prior to developing the condition) (66).

Kuru

Kuru was first described in Western literature in 1957 (68) although according to local verbal histories it may have dated back to around 1920. Originally known as "the

shaking palsy" (the term Kuru is derived from the local word meaning "to shake with fear"), the disease was characterised by ataxia, tremor and other movement disorders, and emotional lability; it had a typical duration of 4 to 24 months from symptom onset to death.

Kuru affected individuals in the Fore region of the eastern highlands of Papua New Guinea, disproportionately affecting women (67% of Kuru patients) and children/adolescents of both sexes (23%); it was caused by ritual funeral endocannibalism. It is hypothesised that the epidemic began when an individual who died from sporadic CJD was consumed by their family members. Women were responsible for preparation of the mortuary feast, and women and children were significantly more likely to consume brain tissue during the ritual than men; furthermore, men tended not to eat materials from female relatives (69). The incubation period for the disease has proven to be widely variable, between around 5 and 50 years, with codon 129 heterozygotes having longer incubation periods and methionine homozygous individuals having earlier onset and more aggressive presentations (70). Following the prohibition of cannibalism in the late 1950s, the number of cases declined steadily, first in the younger age groups born after the alteration in practice, with no new cases identified in individuals born after 1959. The last reported patient died in 2005; as such Kuru is now regarded as an historical disease. The epidemic is therefore thought to have spanned 85 years, with more than 2700 deaths between 1954 and 2005 (70).

A genetic polymorphism at codon 127 of the *PRNP* gene, not reported in other populations, has been identified among survivors of the Kuru epidemic and appears to completely protect against prion diseases in animal studies, illustrating the very strong selection pressure exerted during the Kuru epidemic (71, 72).

Iatrogenic Creutzfeldt-Jakob Disease

Incidence and sources of infection:

More than 460 CJD cases worldwide have been acquired as a result of medical interventions, although the incidence varies from country to country due mainly to differences in the medical practices that exposed the patients to CJD infectivity (73, 74). The main routes of transmission comprise human pituitary derived growth hormone and dura mater grafts, produced from cadaveric tissues presumed to be derived from cases of sporadic CJD or asymptomatic carriers who had died from an unrelated illness. There have also been a small number of CJD cases acquired through other iatrogenic routes. All these cases of secondary transmission of CJD have been associated with parenteral exposure to contaminated materials – materials which were extracted from, or were in close proximity to, brain tissue (75).

Human pituitary derived hormones:

Starting in the late 1950s, intramuscular injection of human pituitary derived growth hormone was used to treat short stature in children. By 1985, when the first related CJD cases occurred (76-78), around 30,000 children had been treated worldwide. To date, around 230 cases have been identified worldwide, with deaths still occurring. More than half of these have occurred in France, where all of the cases received human pituitary derived growth hormone injections between December 1983 and July 1985, strongly suggesting that a batch of injections used in this time period was contaminated (79). Smaller numbers have been seen in other countries, mainly the UK and USA (73, 74). Human gonadotropin was also extracted from cadavers around the same time period and subsequently injected as a fertility treatment. There have

been 4 cases of CJD reported among recipients of this hormone in Australia, the first reported in 1990 (80), the most recent in 1995. The production of all human pituitary derived hormones involved pooling of thousands of donated cadaveric pituitaries, increasing the potential for contamination before extraction of the relevant hormone. The use of human pituitary derived hormones was stopped in 1985 in most countries, and since then has been replaced with synthetic treatments, making new exposures through this route unlikely to occur.

The clinical features of human pituitary derived hormone-related CJD differ from sporadic CJD cases, in that most cases typically present with a progressive cerebellar ataxic syndrome of duration 8-18 months, with dementia occurring infrequently and, if at all, typically late in the illness (75). Incubation periods estimated from the midpoint of treatment exposures until symptom onset in each individual range from 5 to 42 years and are widely variable between countries, reflecting inter-country variation in dosage regimes and durations of treatment, as well as the levels or dose of infectivity to which individual cases were exposed. The estimated average incubation period is 13 years in France, 20 years in the UK and 22 years in the US; the incubation period observed in the 4 recipients of gonadotrophin was from 12 to 16 years (73). As in other human prion diseases, codon 129 polymorphisms impact upon incubation periods and susceptibility to the condition; methionine-valine heterozygosity is associated with longer incubation periods, and methionine homozygosity at codon 129 is more common among growth hormone iatrogenic CJD patients than the population in general. However, differences in the codon 129 distribution of human pituitary derived growth hormone transmitted CJD have been also noted between the populations of different countries, which might be due to infection with different contaminating strains of the CJD agent (79).

Recent evidence of Aβ seeding in the brains of UK recipients of human pituitary derived growth hormone has raised concerns regarding the potential for transmission

of these proteins too through iatrogenic routes, which may include through surgery and other routes already shown to be means of iatrogenic prion transmission (81). Although there is no evidence to date that Alzheimer's disease itself has been caused in this way, research into the potential transmissibility of non-prion neurodegenerative disorders will continue to be important to clarify these issues in the future.

Cadaveric dura mater grafts:

The dura mater is a tough membrane which surrounds and protects the brain. Until 1992, dura mater obtained post mortem from human cadavers was commonly used as a patching material in neurosurgery, for example to repair cerebrospinal fluid leaks, and also in some other surgical domains. The first reported CJD death in a dura mater graft recipient occurred in 1979, and since then over 230 cases have been reported from 19 different countries worldwide (73, 74). The clear majority of these cases have been associated with a single brand, Lyodura® (for the remainder of cases, often the brand cannot be identified (82)), which was introduced in 1969. Most patients who developed iCJD after Lyodura® implantation were treated between 1981 and 1987, after which the production process was modified to include disinfection using sodium hydroxide, a processing step which may denature PrP^{Sc}, reducing infectivity. It is suspected that Lyodura® was contaminated by harvesting dura from a cadaver who died of sCJD (or perhaps died of some other cause in a presymptomatic phase of the disease), and that there was subsequent cross-contamination in the production process (75). Incubation periods for dura mater associated CJD from exposure to symptom onset have varied between 1 and 30 years, with a mean of 12. Most cases occurred between 1990-2000, but small numbers continue to be reported.

Worldwide, more than 60% of dura mater graft related iatrogenic CJD has occurred in Japan, where grafts were used more frequently than in other countries, particularly for neurosurgery relating to vascular disease, hemifacial spasm and trigeminal neuralgia (83). Lyodura® was used predominantly, while other product brands were used with greater frequency in other countries. Codon 129 methionine homozygosity is far more common in Japan than in many other studied populations, found in 92% of the population and appears to convey susceptibility to the development of human dura mater graft associated iatrogenic CJD. Cadaver derived dura mater usage was reduced substantially after 1991 with the development of synthetic substitutes, but may not have ceased until 1997.

Two distinct clinical and neuropathological phenotypes have been identified among the dura mater associated iatrogenic CJD patients (84). Two thirds of patients present similarly to sporadic CJD, but in some cases there is a prominent, isolated ataxic syndrome at onset and widespread plaque deposition on neuropathological assessment. It is thought that these differences might arise from differences in the strains of sporadic CJD that contaminated the grafts. The concept of strains is mentioned very briefly on page 15. In summary, more than one form of PrP^{Sc} has been identified in human (and other animal) prion diseases, and this, combined with codon 129 polymorphism status, is used to describe CJD strain (8, 13). Different strains convey slightly different clinical and pathological phenotypes, and can be suggested using neuropathological examination, identified using biochemical and genetic testing, and confirmed with animal transmission studies (8). The group of dura mater associated iCJD patients that mimic typical sporadic CJD presentations are suspected to have been exposed to dura mater grafts which were contaminated by a source of either codon 129 methionine homozygous type 1 PrP^{Sc}, or methionine-valine heterozygous type 1 PrP^{Sc} sporadic CJD; those with the atypical presentation are thought most likely to have originated from a valine homozygous type 2 PrP^{Sc}

subtype sporadic CJD donor. This suggests there was more than one exposure of dura mater graft production to sCJD contaminated material.

Other iatrogenic risk factors:

More rarely, cases of iatrogenic CJD have also been associated with corneal grafts, the use of infected neurosurgical instruments, and electroencephalogram (EEG) depth electrodes (73-75). The first evidence of iatrogenic transmission of CJD was reported in 1974 in the recipient of a corneal transplant. The cornea had been sourced from the cadaver of a person subsequently found to have died from sporadic CJD (85). The cornea recipient became symptomatic 18 months after grafting, and died 8 months later. A second case was reported in 1997, the recipient dying 30 years after the corneal graft (86). It is likely that the implicated grafts in both these cases included small quantities of retinal tissue, which is continuous through the optic nerve with brain tissue and can contain PrP^{Sc} in cases of CJD (87, 88). Typically, cornea is classified as an anterior eye tissue; such anterior tissues are not recognised to contain high levels of infectivity (89, 90). There have also been multiple reports of death by CJD in recipients of corneal grafts where it has not been possible to identify the cause of death of the cornea donors; these cases might represent further cases of iatrogenic transmission of CJD, but are more likely to reflect the relatively common occurrence of corneal transplantation as an ophthalmological procedure combined with the chance development of sCJD.

There have been a small number of iatrogenic CJD cases associated with the re-use of stereotactic EEG depth electrodes (2 cases, both linked to earlier use in a single patient later confirmed as having died of CJD, with subsequent animal transmission of CJD using the same electrodes adding support to this suggestion) (91) or neurosurgical instruments (4 cases) (92-94) previously used on patients subsequently discovered to have CJD. The full details of these 6 cases (EEG and neurosurgical iatrogenic

transmission) are covered in these references and are not repeated here. The instruments were likely to have carried infectivity, allowing direct intracerebral inoculation of the infectious agent to the recipient's brain. Consequently, these procedures have been associated with shorter incubation periods than for other forms of iatrogenic CJD: between 1 and 2 years: 16 and 20 months for the two cases of EEG electrode transmission, and between 12 and 28 months for the four reported cases of neurosurgical instrument-related transmission (73). Although PrP^{Sc} remains resistant to standard decontamination processes, there have been no new descriptions of surgical transmission through these routes reported since 1997, perhaps reflecting greater awareness of the potential for transmission, and efforts to minimise this risk through instrument quarantine after usage on potential prion patients, as well as increased usage of disposable surgical instruments in this group.

Genetic prion diseases

Incidence and geographic distribution:

Genetic forms of prion disease account for around 10 to 15% of human prion disease, and are caused by over 30 known mutations within the *PRNP* prion protein gene (95); they are often termed as familial CJD (fCJD), although genetic CJD (gCJD) is also used in the literature. These mutations alter the sequence of amino acids within the prion protein, resulting in an increased likelihood of conversion of PrP^{C} to PrP^{Sc} (96). They are associated with different clinical-pathological phenotypes of disease, for example affecting age at onset of symptoms and duration of disease. There are considerable differences in the prevalence of genetic prion disease between countries and regions (97, 98).

Clinical-pathological features:

There is significant variation in clinical phenotype and pathological findings at post mortem between the different forms of genetic prion disease (97, 99, 100). Familial CJD is most commonly associated with the E200K mutation and has a disease presentation and course indistinguishable from sporadic CJD without genetic testing, although it is typically associated with a slightly younger (usually 30 – 60 years) disease onset than would be typical in sCJD (these age ranges overlap considerably, meaning genetic analysis of the *PRNP* gene is essential if E200K mutations – and therefore fCJD – are not to be missed among presumed sCJD patients).

Gerstmann-Straussler-Scheinker syndrome (GSS, most frequently associated with P102L mutation) and Fatal Familial Insomnia (FFI, D178N paired with 129M within the mutated *PRNP* gene) can present at a marginally earlier age than most other forms of familial CJD (onset from 20-60 years for GSS, and 18-60 years in FFI), but with a slower disease course (2-5 years) (101). GSS presents as a slowly progressive cerebellar ataxia and pyramidal syndrome, with later dementia. FFI patients present with insomnia and autonomic failure, unusual for the human prion diseases. Sporadic Fatal Insomnia (sFI) is clinically and pathologically indistinguishable from FFI without genetic testing, but lacks any FFI-associated *PRNP* mutation and is thought to be a rare subtype of sporadic CJD.

Risk factors:

All the genetic forms of human prion disease are inherited in an autosomal dominant fashion, which means that if only one parent has the mutation there is a 50% chance of any offspring inheriting this mutation (97, 99). Despite this autosomal dominant inheritance, most clinicians involved in CJD surveillance are aware of fCJD cases identified in individuals with no known history in other family members (the figures quoted in the literature vary widely between 12% and 88% (97)), and even in those

families where the disease is known, it can appear to skip a generation. These occurrences of fCJD without a family history may occur for biochemical reasons which might allow to the relatives of a patient to carry a mutation, but not go on to develop fCJD (age-dependent penetrance, discussed below) or due to *de novo* mutation, or could be occur due to inaccuracies in family history information, for example due to incorrect diagnoses in deceased relatives, stigma around dementia preventing family discussion, undisclosed adoption, non-paternity, *etc.*

The concept of an age-dependent penetrance of the mutation requires some explanation. Any mutation which predisposes towards PrP^{C} instability may require time for such instability to occur as a chance event, meaning carriers of a mutation may not harbour PrP^{Sc} for the majority of their lifespan until some timepoint before they become symptomatic of the disease. For example, the E200K point mutation has penetrance of 0.45 by 60 years (*i.e.* 45% of E200K carriers will have developed clinical features of fCJD and may have died of the condition by the age of 60), increasing to 0.96 around 80 years (96% will have either died of fCJD or become symptomatic by the age of 80), meaning that individuals may die of other causes before they develop genetic prion disease (99). This reflects the fact that each mutation is a potent risk factor for the development of clinical disease, rather than causing onset of the pathology from birth or early childhood years, as might be seen in many other genetic conditions. Age-dependent penetrance varies for each mutation.

Blood relations of patients with genetic prion disease may be eligible for presymptomatic testing, depending on the practices of local genetics services, to identify whether they carry the mutation and are at risk of developing the condition subsequently. In the UK, any first order relative of a patient who has (or who has died with) fCJD could be referred to a genetics clinic for genetic counselling prior to presymptomatic testing; such testing can have significant psychosocial implications for the individual tested, including mental health complications, and implications for

future financial applications (life insurance, mortgages, *etc.*), as well as implications for other (untested) family members (102).

In the UK it is assumed that exposure in the medical setting to PrP^{Sc} in patients with, or potentially incubating (*i.e.* mutation carriers), genetic prion disease can result in a transmission risk, although no such cases are known to have arisen to date. The particular at-risk period is likely to occur in the window between the early formation of PrP^{Sc} and subsequent clinical development of the disease; prior to that point, an individual does not harbour PrP^{Sc} and there is no risk of onward transmission, and after that point, as the disease symptoms become increasingly evident, the symptoms should prompt investigation and diagnosis of the condition, with subsequent efforts to minimise onward transmission. Animal studies have identified transmissibility of fCJD PrP^{Sc} in primate and transgenic mice models for many mutations (103-105). Therefore, public health precautions are taken for individuals with a recognised risk of developing genetic prion disease later in life, namely those with a known *PRNP* mutation, or the presence of a *PRNP* mutation in a blood relative, or for other individuals who have not been tested, those with two or more relatives with any form of prion disease (106). The nature of the public health precautions for human prion diseases are discussed in Chapters 2, 3 and 4 in relation to blood transfusion, tissue and organ transplantation, and surgery respectively.

Animal prion diseases

This section is not intended to be a comprehensive review of prion diseases of non-human animals, since not all animal prion diseases have direct relevance to the potential iatrogenic transmission of CJD in humans, although there are areas of

general interest and of public health relevance. As such, below follows a brief overview of animal prion diseases deemed of relevance when considering human prion diseases and their onward transmission.

Scrapie:

Scrapie cases were first reported in the 1730s among sheep in England, and the disease has since been found in most countries where sheep are farmed, except Australia and New Zealand (107). It is an acquired disease, associated with transmission through direct contact with infected animals and environmental contamination, where infectivity can persist for years. The classical form of the disease presents with intense pruritis, leading to rubbing and scraping which damages the wool, and provides the name of the disease. The disease has an incubation period of over 2 years and is fatal within 2 weeks to 6 months of onset. Atypical scrapie differs on clinical, epidemiological and neuropathological features and is not known to be transmissible among sheep through environmental routes. Further details about the epidemiology of scrapie in sheep, and concepts about disease outbreaks can be read in work by Hoinville (108) and by Matthews *et al.* (109). The Matthews *et al.* paper discusses the concept of the basic reproduction number, R_0, a measure of how many secondary cases follow each infected individual. If R_0 is less than 1, an infection will fail to reproduce itself, and the number of infected individuals will dwindle; if R_0 is greater than 1, an outbreak will replicate and multiply, at least as long as the number of hosts is not limiting.

Work by Cassard *et al.* on transgenic animal models overexpressing human PrP^C has demonstrated transmission of scrapie to those animals, perhaps indicating the potential for onward transmission to humans, and if that was to occur, the authors stipulate such transmission might appear similar to sCJD (110). This study was reviewed by the European Food Safety Authority who comment that the results "do

not provide evidence that transmission can or does take place under field conditions" (111). Epidemiological evidence against scrapie transmission to humans includes the observation of similar incidence of sCJD in countries without scrapie – such as Australia – compared to countries where scrapie has been endemic (suggesting that scrapie is unlikely to be a significant causative factor in human prion disease) (see http://www.health.gov.au/internet/main/publishing.nsf/content/cda-cdi4003k.htm accessed 25.08.18); and the observations of an Icelandic study, showing lower incidence of sCJD in Iceland compared to most countries with surveillance systems despite a significant scrapie burden (112). Nevertheless, public health and veterinary health authorities remain vigilant to the possibility of scrapie transmission.

Bovine Spongiform Encephalopathy:

BSE was first reported as a new disease in 1986 in the UK, the first case coming to the attention of a veterinary surgeon in December 1984 and dying in early 1985, shortly before further cows in the same herd developed similar signs (37, 38). The disease was noted to have similar neuropathological features to scrapie, Kuru and CJD, causing spongiform changes in the brain and spine of affected animals. The animals displayed an altered mental state, becoming fearful and aggressive, they also developed exaggerated startling to minor stimuli, and gait ataxia.

The disease is thought to have been spread among cattle through dietary supplementation (particularly given to young calves of dairy herds) using feed containing contaminated meat and bone meal (MBM), a protein rich product derived from cattle carcass waste products and used in intensive agriculture from the 1940s (113). The incubation period of the disease was judged to be around 5 years (114), estimated in part by the delayed peak number of cases occurring in 1992-3 following the removal of MBM from cattle feed in 1988; it is suggested that MBM may have been first contaminated in the late 1970s. A north-south gradient was observed during the

epidemic, with more BSE cases seen in cattle from the southern counties of England; Stevenson *et al.* comment that this may relate to regional variations in the rendering process causing "differential rates of amplification of the infectious agent" in the MBM (113). The initial source of contamination remains unknown; hypotheses include contamination of MBM by either scrapie material or a sporadic, atypical occurrence of BSE, before subsequent amplification by further recycling of affected carcasses.

Following the identification of BSE, several steps to try to control the epidemic were introduced by the UK government and livestock farming industry. These included the withdrawal of cattle feed containing mammalian protein in 1988; the removal from animal and human food chains of tissues of cattle, sheep and goats likely to be infective, in particular tissues of the CNS and other "specified risk materials" in 1989 (the impact of this ban is discussed in the vCJD section of this chapter, page 19; see also https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_ssc_out22_en.pdf, accessed 29.08.18); and the ban on sale for human consumption of any cow older than 30 months in 1996. Similar measures followed worldwide, and the totality of changes resulted in significant reductions in disease transmission both to animals and humans.

Some details about the scale of the BSE epidemic, and the spread of BSE outside the UK have already been covered earlier in this work in the section concerning vCJD (page 21). To summarise, one million cattle are thought to have been infected during the BSE epidemic, with over 400,000 of these animals estimated to have entered the human food chain (39). BSE did not occur in isolation in UK cattle herds, as it is thought that MBM or infected cattle were exported to other EU countries, but the numbers of infected cattle were far smaller outside the UK, as can be seen in Figure 1.3 on page 20, with the EU peak occurring 10 years after the UK BSE epidemic peak.

Atypical forms of BSE in cattle (called H- and L-type BSE) have been identified. They are believed to occur sporadically, are typically found in older cattle and are associated with different clinical and neuropathological findings. They have been found in most countries with BSE surveillance and account only for a very small number of total BSE cases. They may represent a zoonotic risk to human health, although this is unclear (115).

Chronic Wasting Disease:

Chronic wasting disease (CWD) was first identified in north Colorado in 1967 among a captive population of a single species of deer (116). Subsequently it has been identified in both wild and farmed animals across a number of cervid species throughout North America and elsewhere, with cases identified in South Korea in 2002 (117), and more recently Norway in 2016 (118).

The first origins of CWD are not known. However, it has since spread through translocation of infected animals and animal products by farmers and hunters. Outbreaks appear to be self-sustaining, with transmission likely to occur in the presence of live infected animals or as a result of the infectious agent persisting in the environment. The disease presents with slowly progressive weight loss, mobility and behavioural changes (including a loss of fear of humans), excess salivation due to difficulty swallowing, and thirst, leading to urinary frequency (119). Animals often die of aspiration pneumonia, as a complication of the dysphagia, combined with the thirst. Incubation periods based on inoculation of mule deer are thought typically to be in the 2-4 year range (120), but the disease has been identified clinically in a 17 month old animal, suggesting the lower limit may be shorter than 2 years. Most animals survive for a period of weeks up to 4 months from the point of diagnosis. PrP^{Sc} (sometimes more specifically called PrP^{CWD} in the literature) is found in high levels in CNS and lymphoid tissue, as well as being detectable in other body tissues

and fluids, even in presymptomatic animals. Moreover, infected animals express PrP^{Sc} in their saliva, blood and urine resulting in environmental contamination (121).

Once in the environment, the PrP^{Sc} binds to soil and vegetable matter and may be consumed by other animals (122). Any attempts to contain the disease by culling cervid populations in North America would be extremely costly due to the reclusive nature of healthy wild cervids, the remoteness of their habitats, and the wide distribution of the disease. Such a cull would cause extensive ecological damage, due to cervids' key role as a large land mammal in their ecosystems, as well as not addressing the environmental contamination, which could lead to reinfection of migrating animals which moved into the region cleared by the cull, meaning eradication in North America is unlikely (119). In contrast, in Norway, due to the much smaller scale of the outbreak there, a cull of wild reindeer was completed around February 2018; as reindeer return to the area, close surveillance for reinfection from environmental PrP^{Sc} contamination will be critical. Efforts are being made to restrict the movement of those cervids controlled by human activity – *i.e.* those that are farmed or herded. For a summary of ongoing surveillance and control activities in North America, and efforts to limit the spread of CWD to Europe, see pages 24-33 of https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_ssc_out324_en.pdf, accessed 30.08.18.

In addition to concerns related to the impact of CWD on local ecology and the environment, CWD may pose a risk to human health. Hunting may help in spreading disease (as some hunters import and use deer urine as an attractant), but is also a concern because many enthusiasts butcher and/or consume the animals they kill. The recent occurrence of CWD cases in Norway (118) is of particular concern in Europe and moreover includes the first instances in reindeer, which, unlike previous naturally affected cervid species, live in huge herds, migrate across vast areas of land and form the staple diet of many indigenous people. It is highly likely that humans

have already consumed CWD prions, raising concern that we may witness another zoonosis (in the manner of vCJD) in the years ahead. Animal transmission studies have shown that CWD may be transmitted to squirrel monkeys (123), but other studies suggest the presence of a significant species barrier for interspecies transmission of this prion disease (122). To date, no transmission to humans has been identified, although it remains to be seen whether the species barrier will be sufficient to protect humans (124).

Conclusions

Although human prion diseases are rare, their long incubation periods, transmissibility, and the risk posed by asymptomatic carriage of PrP^{Sc} in the general population, with the possibility that further cases of vCJD may occur in the future due to past food-borne infections in mostly unaffected codon-129 genotypes or as a result of secondary person-to-person spread, caution against complacency (49). Compounding these concerns is the absence of a validated test that can be used to identify subclinical infection, and the lack of effective instrument decontamination methods for use in routine surgical practice that can fully remove the prion agent from instruments (74, 125, 126), although progress continues to be made in both these areas. In the meantime, in the UK and many other countries, a range of public health measures have been put in place to protect the human and animal food chains, and to reduce the risk of person-to-person transmission in medical settings. These measures include universal precautions to better safeguard the supply of blood, organs and tissues for transplantation; to mitigate against the risk of transmission through surgery; and also potential occupational exposure. Further precautions may be adopted for high risk situations in relation to the handling of surgical instruments

and the identification and notification of those with a specific exposure to infection through surgery, through treatment with blood or blood products or with a genetic risk (106).

The possibility of animal sources of future human prion disease also cannot be excluded. There is no evidence that either scrapie or atypical scrapie in sheep is transmissible to humans, but we remain vigilant to this possibility. While the BSE epidemic is at an end, atypical cases may represent an ongoing zoonotic risk to human health, although this is unclear (115). The possibility of a widespread epidemic of CWD in cervids has elevated concerns for human health as well as impact this may have on animal health and the environment (118, 121, 122, 124).

Sporadic forms of human prion disease have varied phenotypes which, as demonstrated by VPSPr (29), are challenging to diagnose in rarer or atypical cases. It is possible that some of these rarer forms thought to be of idiopathic origin may, in fact, reflect transmission of atypical or novel infections from a yet unknown source, or that iatrogenic transmission through means not previously reported may be missed, or misclassified as a sporadic form of prion disease (something addressed in the chapters that follow), even though there is no evidence to date that this is the case.

Continued scrutiny of clinical-pathological phenotypes and epidemiological risk factors for prion disease in the general population and at-risk groups, as part of national prion disease research and surveillance initiatives, is essential to help address the uncertainties and inform the subsequent management of risk.

Chapter 2

Evidence for Blood and Blood Product Transfusion Transmission of sCJD

Chapter 2 Introduction

This chapter comprises two papers which have been published, as reported in the Declarations on pages 1 and 2 (1, 3). Some adaptations have been made, including adaptations of regional spelling changes (the second paper was published with American English spellings), standardising abbreviations and nomenclature as used elsewhere in this thesis, and adjusting the references to fit into the thesis reference collection. Since publication of the first paper, I reviewed the first codon 129 MV heterozygote patient who subsequently died of neuropathologically confirmed variant CJD; this individual's presentation and epidemiological significance was published as a case report (46). As such, some aspects of the first paper are no longer accurate in this regard, so corrections have been made where necessary. Each paper includes its own abstract, introduction and conclusion, but in addition, I have included a chapter introduction and conclusion.

The first successful transfusion of blood was reported in 1666 between two dogs (127). Early efforts to transfuse humans were hampered both by issues around sterility and bacterial contamination, and by transfusion reactions, which were reduced substantially following the work by Karl Landsteiner in 1902 in identifying the first three blood groups, A, B, and O (then called C), for which he was awarded the Nobel Prize for Medicine in 1930. The UK National Blood Transfusion Service (NBTS) was created on 26 September 1946. At this point, around 200,000 donations were collected each year. Prior to the creation of NBTS, blood donation was organised on a local level. Blood donations have increased over time, and in 2016, the 70th anniversary of the NBTS, around 900,000 donors made around 1.6 million donations. On average, each donation is used in the creation of blood products used by three recipients (for more information, see https://www.blood.co.uk/news-and-campaigns/news-and-

statements/70-years-of-life-saving-blood-donations/ accessed 25.07.18). Unfortunately, blood transfusion has resulted in iatrogenic transmission of HIV (first reported in 1982) (128) and Hepatitis (129), with the problem concentrated among patients with clotting disorders many of whom were transfused with pooled clotting factors derived from several thousand donors and contaminated with viruses including Hepatitis C and HIV (130); this risk was later substantially mitigated using donor viral screening.

The possibility of blood transfusion transmission of CJD was considered in light of known transmissibility of other prion diseases, including scrapie, and known iatrogenic transmission of CJD through neurosurgery and other means (73). vCJD transmission by blood transfusion has been detected in 3 patients (2), while a 4th individual was found to have PrP^{Sc} deposition in splenic tissue at post mortem, and is thought to represent a preclinical transmission (60); this transmissibility of vCJD by blood is thought likely to have occurred due to the increased deposition of PrP^{Sc} in peripheral tissues in this form of prion disease when compared to other forms.

The potential for transmission of sCJD by blood transfusion has been explored using animal models, at first using human prion diseases adapted to the experimental animal species (131), and later using transgenic mice which express human PrP^{C} (132). Epidemiological evidence has been collected in the form of case control studies and lookback studies. One case control study found blood transfusion more than 10 years before onset of clinical symptoms was more common in the sCJD group than in non-CJD controls (22), but similar work in the UK did not replicate those findings (57), and look back studies have not shown any support for sCJD transmission by blood (23, 133). The paper which comprises the first half of this chapter is the update to the Transfusion Medicine Epidemiological Review (TMER) (2), a lookback study utilising NCJDRSU patient details and the UK Blood Services records of blood products.

The threat of transmission of CJD by pooled, fractionated blood products is of particular concern due to the potential for one contaminated donation to infect multiple recipients. These fractionated blood products are used primarily by the clotting disorder patient cohort, a group who have already suffered considerably through iatrogenic HIV and Hepatitis C transmission, as discussed above. Lookback studies have found no evidence of sCJD transmission among haemophilia patients (134, 135). The fact that no cases of sCJD had been seen among patients with clotting disorders has been previously used as evidence of safety of these products in relation to sCJD (136). The second half of this chapter, a case report of two patients with clotting disorders, who were both treated with pooled plasma products, and who both subsequently developed sCJD is therefore of significant public health interest.

In light of the concern regarding blood product transmission of CJD, several public health steps were implemented in the UK to minimise the risk of onward transmission, in particular of vCJD (137). These steps include leucodepletion, implemented by autumn 1999, and following which there have been no further transfusions implicated in blood product transmission of vCJD. This is a process to remove the buffy coat, a fraction of whole blood containing the majority of leucocytes (white cells) which can be separated by centrifugation; this step is likely to have reduced the infectivity of any donated blood contaminated with PrP^{Sc}.

From 1998, UK derived fractionated plasma production ceased, following recommendations that non-UK sourced product should be used for individuals born after 1995 (who would not have been exposed to BSE from contaminated meat products), imported plasma products have been used thereafter. A parallel development reducing the risk from clotting disorders has been the move towards recombinant clotting factors, preventing the risk of any human to human iatrogenic

transmission for patients requiring these products. Recombinant Factor VIII products were licensed for haemophilia A in 1992, while factor IX products for individuals with haemophilia B followed in 1997 (see http://www.hematology.org/About/History/50-Years/1524.aspx accessed 01.09.18)

Finally, in part since importation of fresh blood products such as packed red cells or platelets was not feasible, and thus there was still a need for UK sources for these products, restrictions were placed on blood donors so that anyone deemed at increased risk of CJD would no longer be allowed to donate blood. This includes individuals diagnosed with CJD, those considered at increased risk of developing CJD (including those 2 or more relatives with CJD, or those known to be at risk of fCJD, recipients of dura mater grafts, human pituitary hormones, or corneal grafts, and those informed they have been put at risk through medical procedures). To prevent potential onward propagation of CJD through blood transfusion, any individual who has received a blood transfusion may no longer donate blood. The government statement concerning these interventions can be read at https://www.gov.uk/government/news/measures-currently-in-place-in-the-uk-to-reduce-the-potential-risk-of-vcjd-transmission-via-blood - accessed 01.09.18, while additional details are located in the UK Blood Services Joint Professional Advisory Group Position Statement on vCJD, which can be read at www.transfusionguidelines.org/document-library/documents/jpac-position-statement-vcjd-may-2015/download-file/position statement on vcjd may 2015.pdf - accessed 01.09.18.

Outside the UK, many other countries have introduced similar measures, with the additional step of preventing those resident in the UK during the BSE epidemic from donating blood. For example, the FDA published their recommendations to reduce the risk of CJD transmission by blood products, which can be read at

https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM307137.pdf accessed 01.09.18.

Creutzfeldt-Jakob disease and blood transfusion: updated results of the UK Transfusion Medicine Epidemiology Review Study

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RGW, PEH, CAL and JMM all contributed to the research design, acquisition and analysis of data, PU contributed to data acquisition and data analysis and wrote the first draft of the paper. All authors contributed to drafting the paper and all approved the final version.

Abstract

Background and Objectives

This paper reports the results to 31 May 2015 of an ongoing UK study to look for additional cases of variant Creutzfeldt–Jakob disease (vCJD) transmission by blood transfusion, and to seek evidence whether other subtypes of Creutzfeldt–Jakob disease (CJD) may be transmissible via blood components.

Materials and Methods

All vCJD cases of appropriate age and any sporadic CJD (sCJD) or familial CJD (fCJD) cases with a history of blood donation or transfusion are notified to the United Kingdom Blood Services (UKBS). Donation records are sought, and the usage of all donations is determined by look back. Death certificates are obtained for all donors

to patients with CJD and recipients of transfused components from patients with CJD who are deceased.

Results

The study identified 29 sCJD blood donors, of 370 reported, with transfusion to 211 recipients. Five of these recipients were reported to have died with or of dementia, but were not believed to be cases of CJD. The vCJD arm found 18 vCJD blood donors who had donated blood which was issued for clinical usage, of 24 traced donors from 177 UK vCJD cases. To date, 3 cases of vCJD have occurred in 67 recipients identified in this recipient group, and one recipient had post-mortem confirmation of abnormal prion protein deposition in the spleen (all previously reported).

Conclusion

The results of the ongoing TMER study show no new cases of transfusion-associated vCJD since 2007 and no evidence of transfusion transmission of sCJD.

Introduction

Creutzfeldt–Jakob disease (CJD) is an untreatable and invariably fatal member of a group of neurodegenerative conditions known as prion diseases or transmissible spongiform encephalopathies (TSEs). Prion diseases are recognised in both humans and other mammals and have a number of aetiologies including sporadic, acquired or familial forms. Despite this apparent heterogeneity, there is a unifying hypothesis linking all prion diseases: the "protein hypothesis" described by Prusiner (4), which proposes that a post-translational change occurs in the normal prion protein (PrP^{C} – cellular) forming the infective form of the prion protein (PrP^{Sc} – Scrapie). PrP^{Sc} essentially replicates by catalysing further transformation of PrP^{C} into PrPSc.

The variant form of Creutzfeldt–Jakob disease (vCJD) is the zoonotic form of bovine spongiform encephalopathy, a prion disease in cattle, which entered the human food chain in the UK between 1980 and 1996. vCJD has been transmitted by blood transfusion on three occasions (2), as well as one non-symptomatic transmission (60). The most recent UK primary case of vCJD at the time of publishing this paper had symptom onset in 2012 and died in 2013; subsequently, I reviewed the first neuropathologically confirmed MV heterozygote patient with vCJD in 2015, before his death in February 2016 (46). Surveillance to look for further cases of vCJD continues. In contrast, sporadic Creutzfeldt–Jakob disease (sCJD), the most commonly occurring human subtype, is believed to be a spontaneous illness with no identified causative event or exposure. There has been one epidemiological study, which has suggested blood transfusion may be a risk factor for the development of sCJD (22), but this has not been supported by a similar study in the UK (24) or through look-back studies (2, 23, 138). The familial form of CJD (fCJD) is caused by a mutant copy of the PRNP gene, encoding a form of endogenous PrP^{C} prone to spontaneous conversion to PrP^{Sc}. fCJD is inherited in an autosomal dominant pattern, but family history may not be present in some cases due to loss of contact, non-paternity, variable penetrance, *etc*.

This study updates the 2006 Transfusion Medicine Epidemiology Review (TMER) paper (2) and describes the results of the UK study on blood transfusion and the development of CJD, for all CJD subtypes.

Study design and methods

CJD surveillance:

The National Creutzfeldt-Jakob Disease Research & Surveillance Unit (NCJDRSU) was founded in Edinburgh, UK, in 1990 to identify all cases of CJD in the UK and to look for evidence of a link between BSE in cattle and CJD in humans. The methodology of the surveillance process has been described previously (139) and includes referral of suspected cases to the Unit from clinicians from a number of professional backgrounds, including neurologists, psychiatrists, other physicians and neuropathologists. The referred cases are seen, where possible, by a neurologist from the Unit, who carries out a detailed interview with the family of the patient and reviews the specialist investigations. The interview includes details about past medical history, blood transfusion and donation. Cases are categorised according to WHO diagnostic criteria (140).

TMER:

The Transfusion Medicine Epidemiology Review (TMER) was created in 1997 as a collaboration between the NCJDRSU and the United Kingdom Blood Services (UKBS) to try to seek any evidence that CJD had been transmitted via blood transfusion in the UK.

Notification of CJD cases with a history of donation:

sCJD and fCJD cases with a reported history of blood donation, including cases where a family may be uncertain, are notified to UKBS retrospectively, following the visit by the NCJDRSU clinician. All vCJD cases old enough to be a blood donor are notified to UKBS at diagnosis irrespective of whether they have a reported history of blood donation. Following this notification, all computer and any archived paper records

are searched at blood centres for evidence of the documented donation – using name, date of birth and address at time of donation as identifiers. If available, information about dates and places of donation is used to target the search. If donor records are identified, a list is generated of all components issued for clinical usage. The outcome for each component is determined from hospital transfusion laboratory records, with the names of recipients of these components cross-checked against the NCJDRSU database of known CJD cases and flagged with NHS Digital (formerly the Health and Social Care Information Centre (HSCIC), and prior to that the Office of National Statistics (ONS)) to collect data from death certificates regarding cause and date of death.

Notification of CJD cases with a history of transfusion:

Where relatives have indicated any patient with fCJD, sCJD or vCJD is suspected to have received blood or blood components, the information collected is passed to the relevant blood service, which contacts the hospital transfusion laboratories to confirm, if possible, details of the transfusion. The transfused components are identified from records and details passed back to the blood centre for attempted identification of the donors. As above, the donor details are checked against the NCJDRSU database and flagged with NHS digital.

Further information:

UKBS and hospital transfusion records prior to 1980 are extremely limited, making such historical searches frequently unrewarding; records are still poor until after 1990. For cases where NHS Digital data list potentially relevant diagnoses on the death certificate (e.g. dementia, Alzheimer's disease) but the individual concerned has not been seen by the NCJDRSU clinician, we have sought further information where possible regarding the nature of this illness, from either general practice records or hospital notes.

Results

sCJD blood donors:

A total of 370 sCJD cases (from the 1597 sporadic CJD patients seen by the NCJDRSU between 1990 and end of December 2014) were reported to have been blood donors, with 204 of these believed to have donated after 1980. In only 29 of 204 cases were these individuals traced as blood donors; blood components from these donors were transfused to 211 recipients. The sCJD donor (and recipient) figures are represented in Figure 2.1 overleaf.

Fate of recipients from sCJD blood donors:

To date, 143 individuals (67.8%) of the 211 recipients identified in this study have died, 44 (20.9%) were alive, and 24 individuals (11.4%) were of unknown status due to insufficient information to identify the individual, or relocation of that individual abroad. For each of the 143 who had died, death certificates are available. The underlying causes of death for all cases are listed in Table 2.1, and these figures are represented in the flow diagram below: Figure 2.1. Five of 143 had dementia (including Alzheimer's disease) listed on their death certificates but are not thought to represent cases of CJD. These five cases are represented graphically in Figure 2.2. They had mean age at death of 88 years, and in each case, dementia was not listed as the primary cause of death. In one of these five cases, dementia was considered a relevant comorbidity, rather than the underlying cause of death on the certificate; hence, only four dementia deaths are listed in Table 2.1. The first case received whole blood donation about 21 years before becoming symptomatic of dementia; and had a 6 year, slowly progressive probable neurodegenerative illness, dying 26 years after receiving the transfusion. The donor became symptomatic of CJD nearly 21 years after

the donation. The second dementia case received red cells (non-leucocyte depleted) 10 months prior to death, while the donor became symptomatic of sCJD almost 4 years after donation (more than 3 years after the recipient died). The third case received red cells (non-leucocyte depleted) 8 years before death; the donation occurred 4 1⁄2 years before the donor became symptomatic of sCJD. The fourth case received fresh frozen plasma 28 months before death; the donor became symptomatic 5 1⁄2 years after donation. The fifth case received red cells (non-leucocyte depleted) 18 years before death; the donor became symptomatic 15 ¾ years after donation.

Figure 2.1. sCJD cases with blood donation and blood transfusion histories, and the recipients and sources of those components.

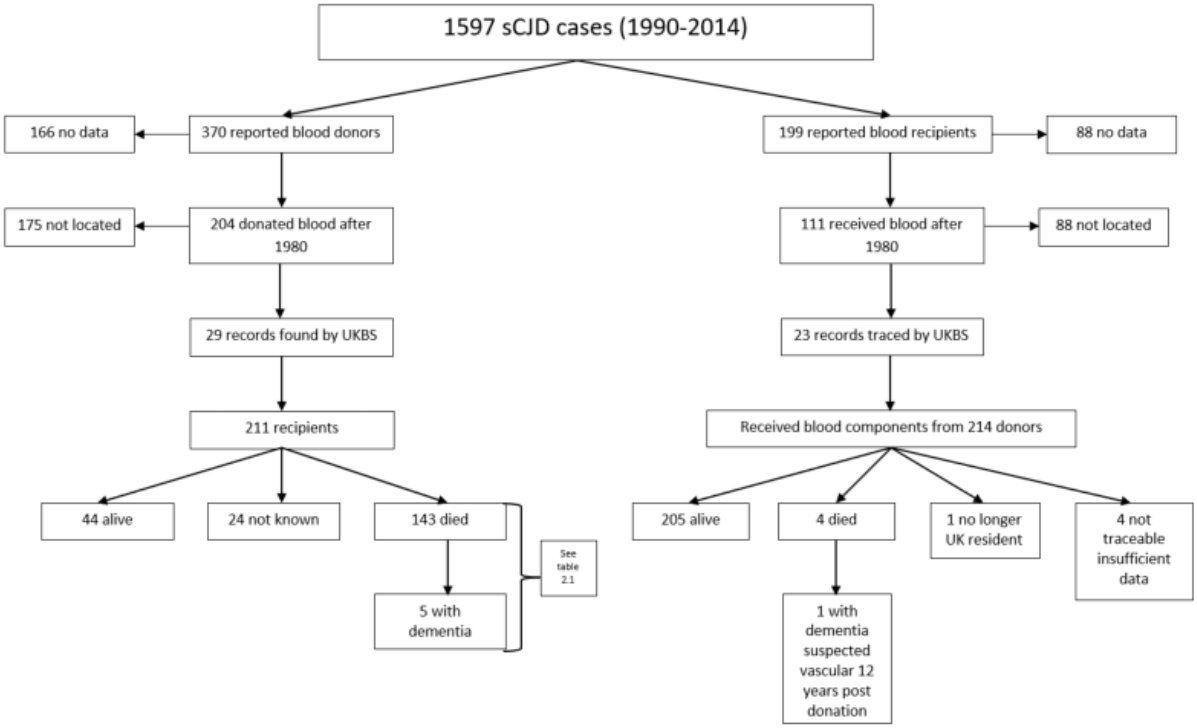


Figure 2.2: Recipients of blood products from sCJD donors who subsequently died with dementia

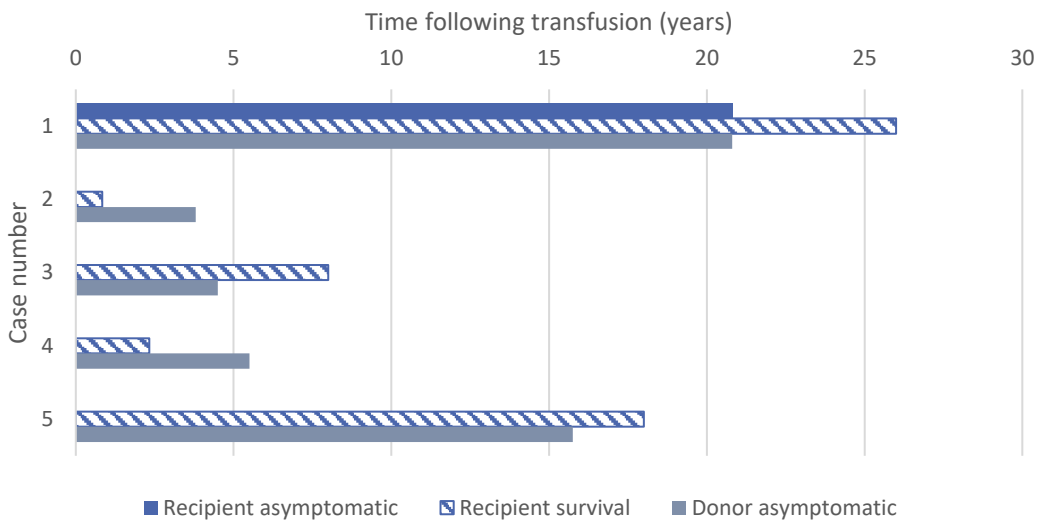


Figure 2.2 illustrates the five cases who died of dementia and are discussed above, showing time intervals from the point of blood transfusion (at year 0), to the onset of dementia symptoms (at the end of the solid medium blue bar, data for case 1 only), or survival after donation (striped blue bar), and to the development of sCJD symptoms in the donor (solid grey bar).

Table 2.1: Underlying causes of death in recipients from sCJD donors

Cause of Death	Number
Cancer (non-haematological)	39
Leukaemia, myeloma, myelodysplasia, myelofibrosis, sideroblastic anaemia	34
Ischaemic heart disease, other cardiac disease	25
Pneumonia	10*
Stroke	7
Liver disease	5
Dementia (including Alzheimer's)	4
Abdominal aortic aneurysm	3
Chronic obstructive pulmonary disease	2
Old age	2
Renal failure	2
Atherosclerosis (not otherwise specified)	1
Diverticulosis and bowel angiodysplasia	1
Haemorrhage	1
Multiple sclerosis	1
Peritonitis	1
Polytrauma	1
Pulmonary embolism	1
Septicaemia	1
Small bowel obstruction	1
Vasculitis (and thrombotic thrombocytopaenic purpura)	1

*In one case, Alzheimer's was recorded as a co-morbidity

At least three other recipients of blood from sCJD donors listed in Table 2.1 could be regarded as "of interest", even without a listing of dementia on the death certificate: the deaths from Atherosclerosis (not otherwise specified), Multiple Sclerosis and Vasculitis. It is interesting that the death certificate for the unspecified atherosclerosis individual did not specify which organ was affected by the atherosclerosis – where mentioned elsewhere, certificates listing atherosclerosis indicated either cerebral or cardiac. No further details are available for this individual, but if this was a cardiovascular process, this would be unlikely to represent a misdiagnosis of a symptomatic sCJD presentation. By extension of the same interest in that patient, the other 7 patients with stroke might also be of concern. Stroke-like presentations of sCJD are well recognised, are the subject of numerous case reports (e.g. (141)), and are one of many potential early misdiagnoses in patients not yet known to be symptomatic of sCJD. These usually present as acute, or sometimes subacute, presentations of focal cortical deficits, rather than causing death immediately, and then subsequently progress after the first presentation; this progression usually follows the more typical rapidly progressive dementia and would be expected to prompt further investigation and hopefully the correct diagnosis being reached, although this cannot be assumed always to occur, and it is certainly possible that some cases of sCJD are misdiagnosed as successive stroke episodes, particularly in individuals with either prior stroke or extensive other cardiovascular disease.

The death certificate for the individual who died from vasculitis also listed thrombotic thrombocytopaenic purpura (TTP) as a secondary cause of death; the two conditions can co-occur and be interrelated (142, 143). This detail was removed from the TMER publication as it was considered superfluous, but such detail may in fact be helpful in explaining why this individual is not thought likely to represent a missed diagnosis of sCJD. While both vasculitis and TTP can present with neurological symptoms, given this individual's TTP, other systemic features must have been identified, which would not be seen in sCJD. These features usually include

fever, microangiopathic haemolytic anaemia, thrombocytopaenic purpura and renal involvement (144). Neurological manifestations of vasculitis can be very variable (145), but the majority have different clinical features and investigation findings when compared to sCJD (146). It is considered unlikely this diagnosis represented a missed case of sCJD, but using only death certificate data, it is not known how extensively the vasculitis was investigated.

No further details are available for the patient with multiple sclerosis (MS), but with ready access to contrast MRI brain scanning in the UK (as well as the possibility to look for oligoclonal bands in the cerebrospinal fluid (147)), it would be unusual to mistake MS for sCJD. The rapidity of progression in patients with sCJD would not be expected in MS patients, and should prompt further investigation, as in the stroke-like presentations. White matter involvement on standard clinical magnetic resonance imaging sequences is very rare in sCJD (148), in contrast to those MR sequences used in research, such as diffusion tensor imaging (149). White matter disease in sCJD is seen in the panencephalopathic subtype (150), but this is exceptionally rarely seen outside Asia, and the white matter appearances in panencephalopathic sCJD would be highly atypical for MS, while oligoclonal bands would be not be positive in sCJD. It is unlikely that the MS patient represents a missed sCJD diagnosis, but there no data are available regarding the accuracy and clinical certainty in this diagnosis.

Eighty-eight of 143 (61.5%) recipients of blood from sCJD donors died less than 1 year after transfusion, 25 (17.5%) between one and 5 years after transfusion and 28 (19.6%) more than 5 years after transfusion (range in this group 5.77– 26.10 years, median 8.97); for two recipients, the transfusion date was unknown.

Of the 44 recipients still alive as of May 2015, all have survived more than 9 years from the date of transfusion. Twenty-two (50%) of these recipients received donations from eight donors who donated less than 5 years before they became symptomatic of sCJD (range 0.23 – 4.92 years, median 2.21). None of these living recipients have developed sCJD and been referred to the NCJDRSU.

vCJD blood donors:

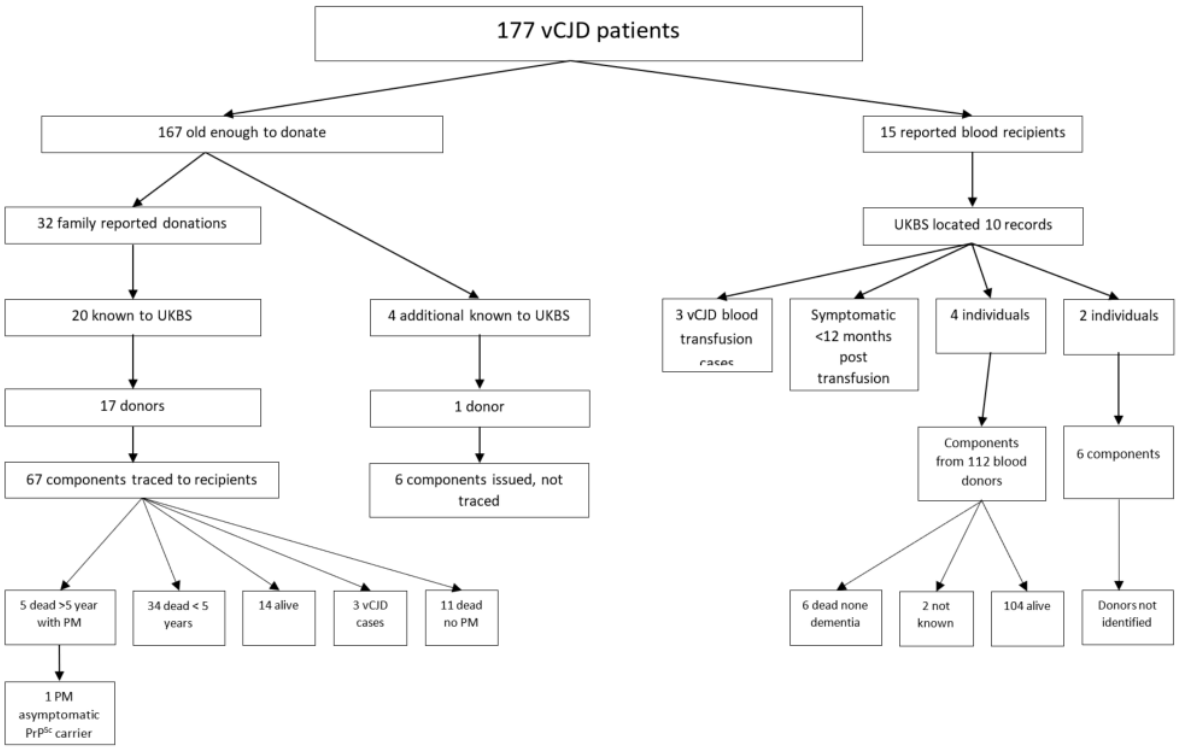
Of the 177 UK cases of vCJD, 167 were old enough to have been blood donors. In 32 of the 167 cases, family reported to the NCJDRSU clinician a possible history of blood donation. A further four cases not reported by the families as donors appear on UK databases, but only one of these four had donated. In total, 24 of the 167 cases old enough to have donated had records with the UKBS, but only 18 of these 24 had donations which were subsequently used clinically. Sixty-seven blood components from these 18 donors were traced to identified recipients; a further six components known to have been issued could not be traced. These figures are represented in a flow diagram in Figure 2.3 below.

Fate of recipients from vCJD blood donors:

Thirty-four (50.7%) of the 67 successfully traced recipients died within 5 years of their transfusion – none were thought to have died from CJD, but none of these cases had post-mortem examination to look for PrP^{Sc} deposition. Three cases (4.5%) of vCJD have already been reported from this cohort of 67 (2); these three developed vCJD between 6 ½ and 8 years 4 months after their transfusion. Five (7.5%) of the 67 died more than 5 years after transfusion and had post-mortem examination including examination for PrP^{Sc} – only the single case already reported tested positive (60), with PrP^{Sc} deposition in the spleen. A further 11 recipients who died more than 5 years after transfusion did not have post-mortem examination to look for PrP^{Sc} deposition. To date, 14 of the 67 recipients remain alive. At the time of first publication, it was

believed that one recipient had moved abroad, and that the fate of that individual was unknown; this was inaccurate, as two individuals have now moved overseas, and even those individuals overseas are followed up periodically by Public Health England; these two are included in the 14 living patients. These 14, including the two overseas, have now survived more than 10 years after receiving transfusion from vCJD donors. There have been no new cases of vCJD identified by the NCJDRSU among the recipients of blood from vCJD donors. These figures are represented in a flow diagram in Figure 2.3 below.

Figure 2.3: Flow diagram of vCJD patients showing those who donated and received blood and blood products.



<u>fCJD blood donors:</u>

Of the 17 familial/genetic cases reported to have been donors, four were traced by the UKBS, one with a D178N mutation, one with an E200k mutation and two with

octapeptide repeat insertion mutations. Fifteen recipients were traced, and of these eight have died, all of a non-neurological disorder, other than one with a history of stroke. Four are alive and three could not be identified. Blood transfusions took place between 1977 and 2002, and four recipients are alive more than 13 years after the transfusion. None of the recipients appear on the NCJDRSU database as CJD cases.

Transfusion history in sCJD recipients:

A total of 199 sCJD cases (from the 1597 sporadic CJD patients seen by the NCJDRSU between 1990 and end of December 2014) were reported to have received blood or blood component transfusion, 111 of these after 1980. The records were traced in 23 (20.7%) of these 111 cases, with 214 donors identified. This implies 9.3 donors per sCJD transfusion recipient and is considered in the discussion. I do not have access to further details concerning the distribution of donor exposures, although this may be of interest and could be explored when the TMER is next updated. These 23 cases received their first blood or blood components between 0.3 and 14.2 years before becoming symptomatic of sCJD (mean 3.89, median 2.61). These figures are represented in Figure 2.1 earlier in this chapter.

Fate of donors to sCJD transfusion recipients:

To date, 205 (95.8%) of the 214 donors are still alive, four (1.9%) have died, and five (2.3%) were of unknown status due to insufficient data (four) or relocation abroad (one). These figures are represented in Figure 2.1 earlier in this chapter. The surviving donors ranged from 25 to 82 years of age (median 56). Three of the four deceased donors died of causes other than dementia (intracerebral tumour, liver disease and suicide in an individual who had suffered depression for decades), but for one individual, dementia was listed on the death certificate; this donor died almost 12 years after the donation, aged 76, and was thought likely to have vascular dementia,

rather than CJD. The other three donors died aged 54, 59 and 63 years, respectively 4, 10 and 12 ½ years after their donations.

Transfusion history in vCJD recipients:

Fifteen of the 177 UK vCJD cases were reported to have received blood or blood components. Transfusion laboratory records were traced in 10 of these 15 cases (corresponding to the 10 cases discussed in Checchi *et al.* 2016 (151)), which include the three cases of transfusion-associated vCJD previously published (2) and listed earlier in this study). One of the 10 recipients had onset of symptoms less than 1 year after transfusion and is unlikely to represent possible transfusion-associated vCJD given the timings of the three known cases. Four of the 10 received blood components from 112 donors; the remaining two recipients received a total of six blood components, but it was not possible to identify the donors in these cases. These figures are shown in the flow diagram Figure 2.3 above.

Fate of donors to vCJD transfusion recipients:

Six of these donors have died of causes unrelated to CJD (Table 2.2), 104 are currently alive, and the fate of two is not known (one having moved abroad). These figures also are shown in the flow diagram Figure 2.3 above.

Table 2.2: Cause of death in six donors to vCJD recipients:

Donor	Cause of Death
1	Haemorrhage due to abdominal aortic aneurysm
2	Hypertensive heart disease
3	Pulmonary Embolus/deep vein thrombosis/ischaemic heart disease
4	Bronchopneumonia/disseminated sigmoid colon carcinoma
5	Complication of heart valve surgery
6	Bronchopneumonia/atrial fibrillation/ischaemic heart disease

Transfusion history in fCJD recipients;

None of the familial human prion disease cases who were reported to have had a history of having received a blood transfusion were traced by UKBS. fCJD patients are not seen by the NCJDRSU clinicians (these families are diagnosed and cared for by the National Prion Clinic); I do not have access to the figures of reported blood transfusion in this group. Potential reasons for lack of tracing by UKBS are considered in the discussion section.

Discussion

This study has not identified any new cases of transmission of vCJD by blood transfusion, with only four documented infections to date, as described in the earlier TMER publication in 2006 (2). The possibility that there are significant numbers of

missed transfusion cases is judged to be unlikely, not least because the great majority of vCJD cases have no history of blood transfusion (2, 152). It is surprising that there have been no further transfusion-transmitted cases in view of the estimated prevalence of abnormal PrP^{Sc} positivity of 1/2000 in the general UK population, derived from an anonymised survey of routine appendix tissue (51). At the time of first publication, all clinical cases of vCJD with data on genotype had been methionine homozygotes at codon 129 of the PRNP gene, but, since then, the first confirmed MV heterozygote case has been identified and published (46). Analysis of the codon 129 distribution in the UK population indicates that 44% are MM homozygotes, with 45% MV heterozygotes and 11% VV (valine homozygotes) (18). It is possible that individuals who are either heterozygotes or valine homozygotes may experience a longer pre-symptomatic phase before developing clinically evident vCJD and all codon 129 genotypes were represented in the positive appendix samples in the recent prevalence study. It is more than 20 years since the onset of symptoms in the first case of vCJD and only a single definite case of vCJD in a non-MM homozygous genotype individual has been identified in the UK (46); no other MV heterozygote cases have been reported to date, and no VV homozygous individuals have been identified. It remains to be seen whether more non-MM homozygous cases will present in the future.

The codon 129 genotype is known in 19 of the 67 recipients, including the three vCJD cases (MM homozygotes) and the preclinical infection (MV heterozygote). Four deceased recipients with no evidence of abnormal PrP in brain or peripheral tissues have been genotyped. These four individuals had survived for 6.3–15.9 years post-transfusion, and the intervals from donation to the onset of clinical symptoms in the donors was between 2 months and 6.8 years. Two were MV heterozygotes and two were MM homozygotes. Two further cases without post-mortem examination were MM homozygotes. Nine recipients who are currently alive have been genotyped. Five are MV heterozygotes and four are MM homozygotes. One of the MV heterozygote

recipients had a tonsil biopsy, which showed no evidence of abnormal PrP deposition. This recipient received a transfusion from an individual from whom earlier donations were implicated in two of the three known transfusion-transmitted cases, who were, as previously stated, MM homozygotes. It is of interest that in three surviving asymptomatic MM homozygotes, red cells had been leucodepleted, a policy introduced in the UK as a vCJD risk-reduction measure in 1999. In addition, the fourth surviving asymptomatic MM homozygote had received cryo-depleted plasma.

The high proportion (50.7%) of blood transfusion recipients who died within 5 years of transfusion reflects the underlying conditions which led to blood transfusion, and any comorbidities. Extrapolating from other acquired prion diseases, kuru and non-CNS exposure iatrogenic CJD, the minimum incubation periods are at least 4.5 years (73, 153) (the incubation period ranges are shorter for dura mater grafting, neurosurgical and EEG depth electrode usage, but these do not reflect transmission through peripheral tissue contamination and are not a direct comparison), and it is unlikely that this group would have manifest symptoms of vCJD prior to death, even if infected. The observed interval from transfusion to symptom onset in the identified transfusion cases was 6 ½ – 8 years, 4 months. It is also likely that there may be variable levels of infectivity in blood from vCJD donors relating to the proximity of the time of the donation to symptom onset in that individual and early donations might have a lower level of infectivity, which could be associated with a longer pre-symptomatic phase in recipients.

Recipients of any blood transfusion are now deferred from themselves donating blood, which prevents the potential propagation of a transfusion vCJD epidemic, which is important if some donors have a subclinical infection. It is of note that laboratory transmission studies using splenic tissue from the subclinically infected

vCJD case have confirmed the presence of infectivity and this case was a codon 129 heterozygote (63).

In contrast to the vCJD group, as yet there have been no cases of sCJD with definite epidemiological evidence to support a transfusion link. Evidence of an increased risk through blood transfusion in sCJD with a lag period of more than 10 years in an Italian study (22) was not replicated by a similar analysis of UK data (24). In our study of sCJD, there has been a total of 1194 patient-years survival following transfusion from a sCJD donor with no evidence of transmission via blood transfusion. The absence of any observed cases supports the hypothesis that blood infectivity, should it be present at all, is lower in sCJD than vCJD. This would be compatible with the extensive PrP^{Sc} deposition in lymphoreticular tissues in vCJD, which contrasts with sCJD where there is comparatively much less peripheral PrP^{Sc}. Nevertheless, animal studies using transgenic mice overexpressing human PrP^{c} have suggested there can be infectivity in sCJD blood (132) and work is ongoing to attempt to use amplification techniques, such as real-time quaking induced conversion (RT-QuIC), to identify a positive signal in blood in sCJD. The identification of positive findings in sCJD blood using highly sensitive techniques may be difficult to interpret in relation to actual risk, and epidemiological data remain important in assessing risks for public health.

As in the recipients of blood from the vCJD donor group, early mortality among recipients of blood transfusion is high in the sCJD study and it is possible that some of these recipients could be in the pre-symptomatic phase of sCJD infection at time of death. Post-mortem uptake is low in the UK, and, unless explicitly looked for, the changes of early sCJD may be missed. The combination of these factors raises the possibility of as yet undetected transmission of sCJD by blood transfusion, although this is unlikely given the negative data in this study and similar findings from other studies (2, 23, 138). The cumulative data from look-back studies in sCJD suggest that transfusion transmission of sCJD is a rare event, should it occur at all.

It is interesting to note that 23 sCJD recipients received blood components from 214 donors. This reflects several factors: it is rare to transfuse only a single unit of packed red cells, or any other blood component, many individuals requiring transfusion will continue to need transfusions in the future for the same conditions; also, many blood components are pooled from multiple donors, such as platelets, where each unit to be transfused is pooled from the platelet fractions of (typically) 4 individuals' donations (for more information, see https://www.transfusionguidelines.org/transfusion-handbook/3-providing-safe-blood/3-3-blood-products accessed 15.08.18).

The data on fCJD show no evidence of transfusion transmission and, although the data are very limited, there is some evidence of restricted peripheral pathogenesis in hereditary forms of human prion disease similar to sCJD.

The study has some limitations. Both the sCJD and fCJD arms depend on relatives reporting blood transfusion or donation at the time of the NCJDRSU clinician interview; if the relative was uncertain or believed a patient may have possibly donated blood or received a transfusion (e.g. intraoperatively), this was still flagged to the UKBS. Despite this it is likely that some patients with sCJD and fCJD who had either donated blood or received transfusion were not identified by the current methodology. By comparison, all vCJD cases of donation age are flagged to UKBS, reducing potential under-reporting. Investigation of all sCJD and fCJD cases, whether or not they have been reported to be blood donors or recipients, has been considered, but follow-up of cases is labour intensive, and investigating these cases regardless of the transfusion history is unlikely to provide much additional information.

A further limitation is that no transfusion or donation records have been identified for some cases. In some instances, this may be because the possible donors or recipients as reported by the family had never donated or received blood, but these cases are in the minority. The lack of centralised computer records, particularly prior to 1980, and the tendency of many hospital trusts to destroy old, unused, medical records means a large pool of potentially useful data has been lost. This problem is compounded in the non-vCJD group, as these patients tend to be older and are therefore more likely to have had their contact with the UKBS prior to the improvement in record keeping. This missing data therefore is more prevalent in transfusions earlier in timepoint prior to clinical disease. Given the fact that peripheral infectivity with PrPSc is substantially lower in sCJD, if sCJD was to be transmitted by blood transfusion, this might result in a smaller dosage of PrP^{Sc} transferred, and potentially a longer incubation period. The missing data is likely to be "missing not at random", and this may introduce a bias against the detection of sCJD transmission by blood, were such a thing to have occurred. It may be possible to perform a multiple imputation assessment of the blood dataset, but this would require direct access to the UKBS data, and a statistician, both no longer possible at this stage; this further analysis could be considered as an additional possibility for future research (154).

The reliance on data derived from death certificates is also a limitation as the final diagnostic classification of identified cases relies on the conditions listed on the death certificate. There have been widely variable estimates of inaccuracy on death certificates. An Office for National Statistics survey suggested inaccuracy in 22% of certificates, with under-reporting of common underlying conditions including heart disease and cancer (155). It is likely that dementia of any cause is also under-reported, and CJD may not be diagnosed in life, with symptoms attributed to an alternative cause of dementia. Review of death certificate data on CJD since 1990 at the NCJDRSU suggests that the sensitivity and specificity of a correct diagnosis on death certificates

is about 80%. It is also likely that not all UK cases of CJD are referred in life or identified through death certificates or post-mortem findings.

Despite these caveats, the data presented in this paper has provided no evidence that cases of sCJD have developed the condition as a result of prior blood transfusion.

The unexplained mismatch between the observed data in vCJD and prevalence estimates in the general population and the recent experimental evidence of infectivity in sCJD blood underline the importance of continuing the epidemiological studies of blood transfusion in all forms of human prion disease.

Sporadic Creutzfeldt-Jakob Disease in 2 Plasma Product Recipients, United Kingdom.

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Author contributions:

PU reviewed both patients, identified their importance and obtained permission from one of the two patient families for publication (KT obtained permission from the other family) and wrote the first draft of the paper. RGW supervised PU with this work. All authors contributed to drafting the paper and all approved the final version.

Abstract

Sporadic Creutzfeldt-Jakob disease (sCJD) has not been previously reported in patients with clotting disorders treated with fractionated plasma products. We report 2 cases of sCJD identified in the United Kingdom in patients with a history of extended treatment for clotting disorders; one patient had haemophilia B and the other von Willebrand disease. Both patients had been informed previously that they were at increased risk for variant CJD because of past treatment with fractionated plasma products sourced in the United Kingdom. However, both cases had clinical and investigative features suggestive of sCJD. This diagnosis was confirmed in both cases on neuropathologic and biochemical analysis of the brain. A causal link between the treatment with plasma products and the development of sCJD has not been established, and the occurrence of these cases may simply reflect a chance event in the context of systematic surveillance for CJD in large populations.

Introduction

Human prion diseases are a group of rare and fatal neurodegenerative diseases that include idiopathic (sporadic), genetic (inherited), and acquired (infectious) disorders (4). All are associated with the accumulation of an abnormal isoform of the prion protein (PrP^{Sc}) in the central nervous system (4). The most common human prion disease is the sporadic form of Creutzfeldt-Jakob disease (sCJD), which occurs worldwide with a relatively uniform incidence of 1–2 cases per million population per year, a peak incidence in the 7th decade of life, and a median duration of illness of 4 months. The relatively consistent mortality rates associated with sCJD, the overall random spatial and temporal distribution of cases, and the absence of any confirmed environmental risk factor have led to the hypothesis that sCJD occurs because of the spontaneous generation of PrP^{Sc} in the brain (4). In contrast, variant Creutzfeldt-Jakob disease (vCJD) (32) is an acquired disorder that is most likely caused by the consumption of meat or meat products contaminated with the bovine spongiform encephalopathy agent. The median age at death in vCJD is 28 years, with a median duration of illness of 14 months (15). Most cases of vCJD have occurred in the United Kingdom, which has had the largest epizootic of bovine spongiform encephalopathy in the world. Of the 178 UK vCJD cases, 3 have been identified as cases of secondary transmission caused by the transfusion of non-leucodepleted red blood cell components from vCJD-infected blood donors.

Lookback studies have shown no evidence of transmission through blood transfusion in sCJD (1, 23), despite the identification of PrP^{Sc} in some peripheral tissues (156), and experimental evidence, which demonstrated infectivity in blood (132) by using intracerebral inoculation of highly sensitive transgenic mice. The absence of clinical cases causally linked to past treatment with fractionated plasma products has been used as evidence of the safety of these products in relation to sCJD (136). These

products are generally manufactured from the pooled plasma from several thousand donors; production using UK plasma was discontinued in 1999.

We describe 2 cases of sCJD in patients who had previously received treatment with UK plasma–sourced plasma products; both patients had been informed that they were at increased risk for vCJD because of that treatment. The clinical features and investigations in these cases were typical of sCJD; the neuropathologic diagnosis in both cases was sCJD (subtype MM1).

The Investigation

The UK National CJD Research and Surveillance Unit has been carrying out systematic epidemiologic studies of CJD since 1990. The methodology of this study has been published previously (139). In brief, patients with suspected CJD are referred by clinicians and visited by a research registrar, who obtains details of the clinical history and investigations, information on a range of possible risk factors, and past medical history. The Transfusion Medicine Epidemiology Review study investigates potential links between donors and recipients of labile blood components and, in cases of sCJD, investigates patients who have a history of blood donation or having received a blood transfusion.

Coordinated surveillance of CJD has been undertaken in the European Union since 1993 (157). National surveillance programs for CJD also are in place in several other countries, including Australia, Canada, Japan, and the United States.

Case 1:

In 2014, a 64-year-old woman suffered a rapidly progressive dementia with deterioration in driving skills and balance disturbance, then limb coordination deficits with handwriting impairment. In the second month, her gait deteriorated, becoming shuffling and unsteady, she struggled to dress herself, and she had onset of daytime hypersomnolence. She became distractible, had visual misperceptions, emotional lability, and spatial memory problems. She was hospitalised at the beginning of the third month of her illness and had onset of cortical blindness, myoclonus, and akinetic mutism. She experienced rapid decline and died after a total illness duration of 3 months.

An electroencephalogram performed during the final stages of illness showed background slowing and runs of periodic complexes, and a magnetic resonance imaging (MRI) brain scan showed high signal in the caudate heads with posterior cortical ribboning. A cerebral spinal fluid (CSF) 14–3–3 assay and real-time quaking-induced conversion test for PrP^{Sc} both were positive. Prion protein gene (*PRNP*) sequencing showed no mutations with methionine homozygosity at codon 129.

Post mortem examination of the brain showed widespread spongiform encephalopathy of predominantly microvacuolar type. Immunocytochemistry (a common laboratory technique used for anatomical visualisation of a specific protein (158)) for prion protein gave a widespread positive reaction in a granular/synaptic pattern in brain tissue (Figure 2.2). No plaques or plaque-like structures were identified. Results of immunocytochemistry for disease-associated prion protein were negative in peripheral nerve, liver, lymph node, appendix, and spleen. Western blot analysis of frontal cortex and cerebellum confirmed the presence of protease-resistant prion protein with a type 1A isoform. For an overview of pathological and

biochemical features of CJD subtypes, please see Ritchie *et al.* 2015 to which I contributed as third author (159).

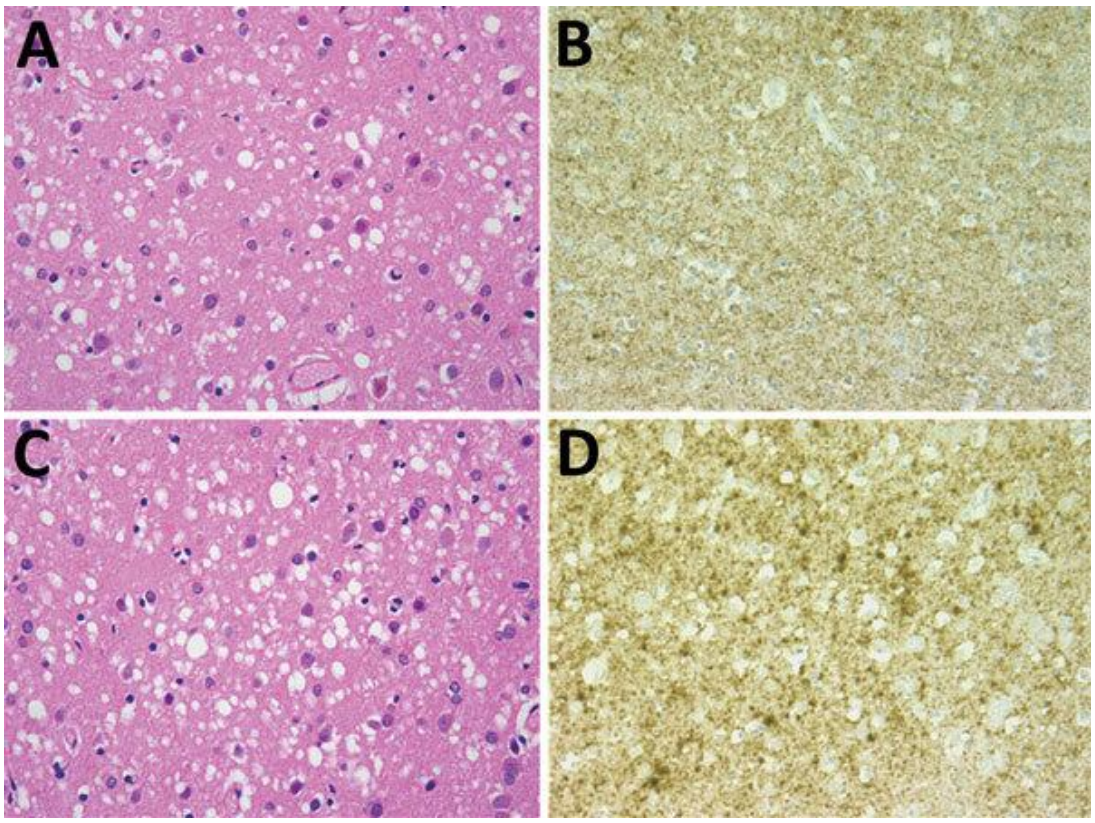
Figure 2.2: Results of neuropathologic examinations of the brains of the 2 patients with sporadic Creutzfeldt-Jakob disease, United Kingdom, 2014.

A) Microvacuolar spongiform change in the frontal cortex (case 1). Haematoxylin and eosin stain; original magnification ×400.

B) Fine granular/synaptic accumulation of abnormal prion protein in the cerebral cortex (case 1). 12F10 antiprion protein antibody; original magnification ×400.

C) Microvacuolar spongiform change with neuronal loss and gliosis in the frontal cortex (case 2). Haematoxylin and eosin stain; original magnification ×400.

D) Focally intense granular/synaptic accumulation of abnormal prion protein in the cerebral cortex (case 2). 12F10 antiprion protein antibody; original magnification ×400.



The patient had been diagnosed with von Willebrand disease in childhood. Her early therapies include numerous transfusions of red blood cells and platelets; in more recent years, she received plasma-derived and recombinant factor VIII and additional blood component transfusions at times of haemorrhage. Factor VIII was administered on 4 occasions in the 1990s and during 2000–2004, and von Willebrand factor/factor VIII (Haemate-P) during 2001–2013. Because of her history of exposure to UK-sourced plasma products, for public health purposes she had been informed that she was at risk for vCJD, although she was not known to have been exposed to factor VIII derived from a batch including a vCJD donation. She had no history of potential iatrogenic exposure to CJD through recognised routes (*e.g.* no history of dura mater grafting, human growth hormone usage, *etc.*) and no family history of CJD.

Donors for all blood or platelet transfusions since 2001 have been identified. Of the 107 donors, 106 are still alive, with a median age of 55 years (range 27–80 years). (Table 2.3). One donor of leucodepleted platelets, which were transfused 12 years before clinical onset in the recipient, died in 2013 at 76 years of age, and the diagnoses on the death certificate were vascular dementia and bladder cancer; it has not been possible to review the case notes for this individual. Identification of donors for transfusions before 2001 has not been possible, and due to the lack of available records, particularly from childhood, any attempt to estimate the number of transfusions and donors would be highly insubstantial.

Table 2.3: Selected characteristics of blood donors to the patient with sporadic Creutzfeldt-Jakob disease described in case 1, United Kingdom, 2014*

Interval from transfusion to onset, y	Component	No. donors	No. donors alive	No. donors dead
3	RBC LD	4	4	0
6	RBC LD	6	6	0
7	RBC LD	19	19	0
9	RBC LD	3	3	0
10	RBC LD	4	4	0
12	Whole blood LD; RBCLD; platelets LD	2; 27; 42	2; 27; 41	0; 0; 1

*LD - leucodepleted; RBC - red blood cells. Median age of donors: 56 years (range 27–80 years).

Case 2:

In 2014, a 64-year-old woman reported day/night reversal of sleep patterns and, 3 months later, excessive tearfulness, for which she was started on antidepressants. She then had onset of writing problems, followed during the next few days by increasing language problems that led to expressive dysphasia. She deteriorated rapidly thereafter, requiring assistance with her activities of daily living and having coordination and memory problems, jerking movements suggestive of myoclonus,

and itching in both arms. She was admitted to the hospital and experienced a probable focal seizure with secondary generalization. She had onset of a homonymous hemianopia and limb rigidity and then became bedbound and mute, dying 7 months after the onset of symptoms.

An electroencephalogram performed during the final stages of illness showed widespread slowing, more evident on the left. An MRI brain scan showed left-sided caudate head and anterior putaminal high signal. Diffusion weighted imaging showed areas of cortical high signal. Results of a CSF 14–3–3 assay and real-time quaking-induced conversion tests were positive. Consent for full sequencing of the *PRNP* was not obtained; methionine homozygosity at codon 129 was identified.

Post mortem neuropathologic examination of the brain showed a widespread spongiform encephalopathy with microvacuolar spongiform change, neuronal loss, and gliosis. Immunostaining for prion protein showed widespread positivity with a granular/synaptic pattern (Figure 2.2). No amyloid plaques were identified. Western blot analysis confirmed the presence of protease resistant prion protein with a type 1A isoform. There was no evidence of abnormal prion protein accumulation in spleen and appendix either on immunocytochemistry or high sensitivity Western blot analysis.

The patient was known to have haemophilia B since 1964 and had received plasma-derived and recombinant factor IX during 1984–2012. For public health purposes, she had been informed that she was at risk for vCJD and in 1991 had received factor IX derived from a pool containing plasma from a donor who subsequently developed vCJD. She had no history of potential iatrogenic exposure to CJD and no family history of CJD.

In 1985, the patient received 6 units of fresh frozen plasma (FFP). Tracing of donors has not been possible.

Discussion

This report describes 2 cases of sCJD in patients with a history of treatment with UK-sourced plasma products, 1 with a history of haemophilia B and 1 with von Willebrand's disease. To our knowledge, no previous case of sCJD in a person with a history of extended exposure to plasma products has been reported. It is clearly of concern that there have been 2 such cases in a relatively short period in the UK, where many plasma product recipients have been informed that they are at increased risk for vCJD. However, a causal link between the treatment with plasma products and the onset of sCJD has not been established, and the occurrence of these cases may simply reflect a chance event in the context of systematic surveillance of CJD in large populations.

Both patients had been informed that they were at increased risk for vCJD, and considering the evidence for the type of CJD in the 2 cases is important. Both patients had a clinical phenotype suggestive of sCJD, including a short duration of illness, typical early symptoms, a suggestive MRI scan, and, in 1 patient, a typical EEG. (The typical clinical features of each type of CJD are discussed in the thesis introduction Chapter 1; details regarding diagnostic criteria can be read in Appendix 2 and details about investigations are covered in the references (160, 161) and can be read at https://www.cjd.ed.ac.uk/sites/default/files/investigations.pdf accessed 25.08.18.)

Notably, both patients had a positive real-time quaking-induced conversion (RT-QuIC) test result for PrP^{Sc} in CSF (Table 2.4) (162); this test has not been positive in any case of vCJD evaluated in the NCJDRSU laboratory (RT-QuIC performed at the NCJDRSU has been optimised and validated for detection of sCJD; this work is covered extensively elsewhere (163-165)).

Neuropathological examination was critical; it showed appearances typical of sCJD in both patients and no evidence of peripheral pathogenesis on immunostaining of lymphoreticular tissues, a feature that is observed in all tested specimens of vCJD patients to date (90). Furthermore, both patients had a type 1A isoform PrP^{Sc} on Western blot consistent with a diagnosis of sCJD subtype MM1 (166). Neither patient had a history of potential iatrogenic exposure or a family history of CJD, and for the case for which sequencing of the *PRNP* was performed, no mutations were detected. In both cases, an MM genotype occurred at codon 129 of *PRNP*, which does not distinguish between sCJD and vCJD; methionine homozygosity is seen in 44.1% of the UK population, and 59.5% of UK sCJD patients (18), as well as all but one vCJD patients (46). Laboratory transmission studies to provide evidence of agent strain in the cases have not been possible.

Table 2.4: Selected characteristics and clinical features of the 2 patients with sporadic Creutzfeldt-Jakob disease described in cases 1 and 2, United Kingdom, 2014

Characteristic/clinical feature	Case 1	Case 2
Patient age, y/sex	64/F	64/F
Symptoms/signs	Ataxia, cognitive impairment, visual impairment, myoclonus	Somnolence, depression, dysphasia, cognitive impairment, myoclonus, ataxia
Magnetic resonance imaging	+	+
Electroencephalogram	+	Slow activity
Cerebrospinal fluid 14–3–3 assay	+	+
RT-QuIC*	+	+
Genotype	MM	MM
Diagnosis	Definite sCJD	Definite sCJD
Duration	3 months	7 months

*RT-QuIC, real-time quaking-induced conversion.

One patient had received multiple transfusions of blood components over an extended period, and the other had received 6 units of FFP 19 years before clinical onset, raising the possibility that these cases could have resulted from secondary transmission through blood components. In the case of the patient with von Willebrand disease, 107 donors have been traced, and none appear in the register of cases of CJD kept at the National CJD Research and Surveillance Unit. However, it has not been possible to obtain information on blood transfusions for this patient before 2001 nor on the FFP transfusions for the patient with haemophilia B. This missing data could potentially contain donors who did subsequently develop sCJD, and might have passed on sCJD iatrogenically, but it is impossible to be certain; I am not able to quantify the amount of missing data. If further work was to be performed in relation to these cases, this would certainly be a topic to explore with a statistician. As in the TMER, the missing data is more prominent for earlier blood products, and this may introduce bias. Any contamination of pooled product with PrP^{Sc} would convey only a tiny dose, and such a small dose would be expected on the basis of dose dependence to convey a prolonged incubation period (167, 168), meaning those earliest products might be the most relevant. The implications of leucodepletion (introduced in 1999) for the potential for infectivity from pooled plasma products are not clear; pooled plasma products are prepared from plasma after removal of all whole cells, including the leucocytes (otherwise removed by leucodepletion, which was primarily a step to reduce infectivity of fresh blood products). Animal studies have indicated that leucodepletion may have significantly reduced infectivity of vCJD blood (169), but there is no similar data concerning sCJD.

Lookback studies in the United States and United Kingdom have provided no evidence of transfusion-transmission of sCJD (1, 23), and although 1 study suggested an increase in risk after a lag period of 10 years (22), this finding was not confirmed in another study (24). The balance of evidence indicates that, if sCJD is transmitted by

blood transfusion, it must be a rare event, if it happens at all, and transfusion transmission is probably not the explanation for the 2 cases we describe.

Systematic surveillance for CJD, including a coordinated study in Europe (98), has been carried out in many countries over the past 25 years and is continuing. Many of these studies obtain information on potential risk factors, including details of past medical history. To date, no case of sCJD has been reported in a person who has received treatment for a clotting disorder. In fact, the absence of such a case has been used to argue against the possibility that plasma-derived products pose a risk for sCJD transmission (136). CJD surveillance centres are aware of the relevance of this issue, and sCJD patients with a history of treatment with plasma products probably would have been identified and reported if they occurred. Although it is surprising that 2 cases of sCJD have been identified among a population of 4,000–5,000 patients in the UK who have been treated for clotting disorders with fractionated plasma products, the total population under surveillance for CJD in Europe and internationally exceeds 500 million. Assuming an annual incidence rate of sCJD of 1.5–2.0 per million population (170), the occurrence of 2 cases of sCJD in this total population may not imply a causal link between the treatment and the occurrence of the disease. The 2 cases were identified over a period of months, and no further cases have been found since 2014; however, continuing to search for such cases through CJD surveillance programs is essential.

As an approximate calculation, the combined prevalence worldwide of the 4 most common clinically symptomatic hereditary clotting disorders (Haemophilia A, Haemophilia B, Haemophilia C and clinically significant Von Willebrand) is approximately 268.5 cases/million population (171). “Clinically significant Von Willebrand” disease (VWD) is specified to reflect the spectrum of severity of VWD, and only to include those who are symptomatic of the condition to the point they may require blood products. This value may be a slight underestimate, as the prevalence

figures for Haemophilia A and B are given in the literature for males only, as they are X-linked conditions, however, as case 2 highlights, both are seen on occasion in females, although this is not considered for the purposes of this calculation; this calculation also does not consider the extremely rare subset of patients with multiple clotting disorders, which may to some degree offset the earlier omission of female patients with Haemophilia A and B. Within the population under surveillance for CJD (exceeding 500 million), this indicates approximately 134,250 patients are likely to have clotting disorders, although it is less clear whether all of these patients would have received treatment using pooled blood products. Using the upper limit of sCJD incidence at 2 cases/million per year, we might expect to see as many as 0.2685 cases of sCJD among the clotting disorder community each year. As a further very crude estimate, with surveillance ongoing since 1990, more than 27 years have passed, and we might have expected to have seen around 7 patients in this time window; of course, not all CJD surveillance systems worldwide were established by 1990, so the multiplication for observation over 27 years is not entirely valid, but nevertheless given earlier clotting disorder iatrogenic epidemics (including HIV and Hepatitis (172)), the clotting disorder patient cohort have been under closer scrutiny by haematology doctors for longer than routine CJD surveillance. As such, this crude estimate of around 7 cases indicates that the two reported sCJD cases among the clotting disorder patient group may not be all that unexpected as a chance occurrence of sCJD.

Further consideration using rare event statistical modelling may help to explore the significance of the detection of these two patients within a short time period within the UK, but on discussion with my supervisors and co-authors, and consultation with statisticians while preparing this work for publication, it was agreed that such work is beyond the remit of this thesis (and indeed of the publication). Any such further work would benefit from careful input from a statistician, particularly if additional cases are detected in the future.

Chapter 2 Discussion

The two papers presented in this chapter attempt to address the epidemiological evidence acquired by the NCJDRSU pertaining to potential acquired transmission of CJD by blood transfusion, and both feature their own discussion sections. The TMER update covers all forms of human prion disease where blood transfusion is believed to occur, and the aspects of that paper pertaining to vCJD are of relevance given this is the only form of CJD known to have been transmitted by blood product transfusion. Overall, there is no evidence that sCJD has been transmitted by blood transfusion from the TMER lookback, but there are individual cases where accessing further information beyond that provided by death certificates would be interesting; the limitation of accessing death certificate information only is clear.

The two individuals with clotting disorders are both believed to have developed sCJD by chance, with no data either on pathological examination or on lookback of the products they have received to suggest that blood products were responsible. Crude estimates about sCJD prevalence and the number of patients under observation would suggest we may see other patients in this cohort with sCJD in the future, and perhaps we even should have seen more by this stage. Given the scrutiny this cohort of patients face following earlier iatrogenic viral exposures, it seems unlikely that other prion cases might have been identified but not published. It may be possible to perform statistical modelling to explore the temporally close presentation of these two patients, but this was not performed in this thesis.

Both studies are limited considerably by data availability of the medical records tracing blood products, substantially reducing the ability of either study to identify

potential linkages, and potentially causing bias, as earlier blood records are more likely to be missing, particularly so prior to 1990. As the quality of availability of blood donation and transfusion records has increased over time, repeating the analysis for the TMER in future years, as new CJD patients are reviewed by the NCJDRSU may provide more complete data. As clotting disorder patients age, if we do see further CJD cases, the improved quality of blood product data may not be as helpful as with the TMER, since these patients may only have been exposed to human derived plasma products earlier in their life, with the roll out of recombinant factors. Should a future individual patient who has received only recombinant clotting factors (and no transfusion or other blood products) subsequently develop sCJD, this case would clearly not be caused by blood transmission.

A development of great interest since the two publications from this chapter is the development of a blood test which has identified PrP^{Sc} in stored blood from vCJD patients, including two preclinical samples from patients who subsequently developed vCJD (66, 67) – this test is mentioned in Chapter 1 in the vCJD section. This test is based on protein misfolding cyclic amplification (PMCA), another protein amplification technique with some similarity to RT-QuIC (also mentioned very briefly in the case report section of this chapter), and appears to offer high sensitivity and specificity for the detection of PrP^{Sc} in vCJD. The test has not identified PrP^{Sc} in blood from sCJD patients, which may be considered further evidence that blood is of low – if any – infectivity in sCJD. It is not yet clear whether it will be possible to scale this test up to the point it could be used to screen UK blood donors and other donors from countries with prior vCJD cases, nor whether at that stage the test would be affordable. With vCJD cases appearing to have dwindled, and given the disappearance of CJD from the public eye (on the basis of family reactions to my visits to see patients while working in the NCJDRSU), there may neither be a strong public health case, nor a political will to encourage adoption of such measures; however, a pilot prevalence study (similar to the appendix studies discussed in later chapters

(51)) of UK, French and/or Irish blood donors, if performed, might provide additional evidence as to whether scaling up the test would be potentially beneficial on public health grounds.

Chapter 3

Evidence for Organ and Tissue Transplant Transmission of sCJD

Introduction

Background:

While sporadic CJD is believed to occur as a result of a spontaneous protein misfolding event (4), the possibility of person-to-person transmission cannot be ruled out. Iatrogenic transmission of CJD has been recognised in recipients of cadaveric dura mater or human growth hormone and in a few instances related to surgery, EEG depth electrodes and corneal transplantation (73). It remains possible that at least a small number of apparently sporadic CJD cases may in fact represent unrecognised iatrogenic transmission through tissue or organ transplantation due to infectivity intrinsic to the transplanted material. The possibility of transmission by blood or blood product transfusion is addressed in Chapter 2, while surgical instruments are considered as another potential means of transmission in Chapter 4.

Transplant may convey a greater risk of transmission of PrP^{Sc} than surgery not involving transplantation due to the prolonged contact with the material from the donor/source affected by sCJD, and the greater quantity of PrP^{Sc} implanted with the organ or tissue, when compared with whatever small quantity could have adhered to a surgical instrument and remained despite sterilisation procedures; both factors would increase the dosage of PrP^{Sc} implanted into the recipient, and a dose dependent model of transmission has been postulated in explaining some observed prion transmission characteristics (167, 168).

Approximately 4000 whole organ transplants are performed in the UK each year; for example, between 1 April 2016 to 31 March 2017, 1413 individuals donated organs, resulting in 3675 transplant surgeries, involving 4025 organs (173) (see Figures 3.1 and 3.2 below). The discrepancy between these figures can be explained for a number of

reasons, including that one donor (in the case of non-living donations) may donate more than one organ, and the fact that multiple organs may be transplanted during a single surgical procedure, such as in combined liver, kidney and pancreas transplantation. Other chains of procedures are possible such as domino transplants – where a recipient with a lung pathology receiving a combined heart and lung transplant may themselves donate their unaffected heart to another individual. In addition, non whole organ, human derived materials are used as a variety of tissue grafts, including for corneal grafts, tendon, bone, skin and heart valves; these result in numerous graft procedures, including 3564 corneal transplants in the UK between 2010-2011 (174) (see Figures 3.3 and 3.4 below).

The rates of whole organ donation have fluctuated over the years, but the overall trend is increasing. Full details of UK donor activity can be read on the NHS Blood and Transplant website https://www.nhsbt.nhs.uk/what-we-do/transplantation-services/organ-donation-and-transplantation/ accessed 02.09.18, but I have extracted two graphs showing the frequency of deceased and living whole organ donors in the UK into Figures 3.1 and 3.2, overleaf. Figure 3.1 shows HB (heart beating), NHB (non-heart beating) and living donors, while Figure 3.2 uses the terms DBD (deceased donors after brain death), DCD (deceased donors after circulatory death) and living donors. The change in terminology reflects contemporary parlance and the importance of communicating brain death to families prior to consideration of organ harvest. Living organ donation has increased almost 5-fold over 21 years, while there have also been modest increases in deceased organ donation of around 2/3 over the same time window, with the increases seen predominantly in the deceased donors after circulatory death category (formerly non-heart beating). Both living and deceased donor organ donation from an individual in a presymptomatic phase of sCJD might convey a risk of transmission of sCJD, but the opportunity subsequently to detect transmission from an asymptomatic donor would be greater from a living

donor, as that individual may survive to the development of clinical symptoms, and subsequent referral to the NCJDRSU.

Figure 3.1: **Number of deceased and living donors in the UK, 1 April 1996 - 31 March 2006**

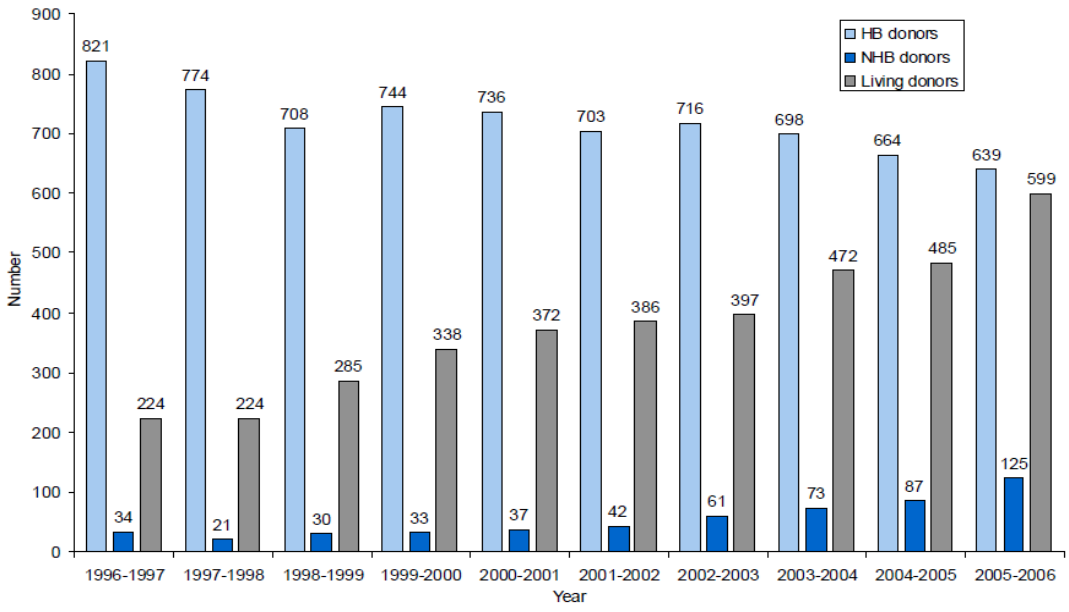


Figure 3.1 shows the trends in UK organ donation from 1996-2006 inclusive. Copied from: NHS Blood and Transplant: Transplant Activity in the UK, 2006 (175).

Figure 3.2: **Number of deceased and living donors in the UK, 1 April 2007 - 31 March 2017**

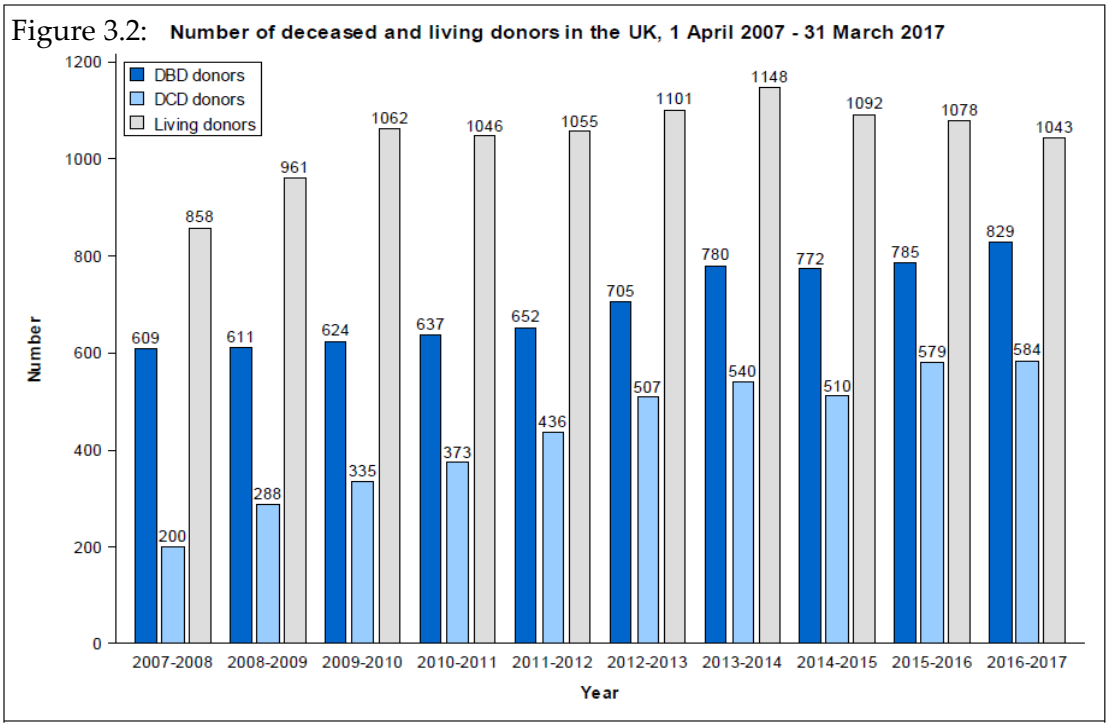


Figure 3.2 shows trends in UK organ donation from 2007-2017 inclusive. Copied from: NHS Blood and Transplant: Overview of Organ Donation and Transplantation, 2017

(173). Note that living donors are grey on both charts, while the colours for HB/DBD and NHB/DCD are reversed comparing the two graphs.

Separate from whole organ donation is the category of tissue donation, mentioned on Page 90. This comprises several different tissues from multiple different organs. Corneal grafts are considered first, as they are a recognised means of transmission of CJD. Corneal grafts are used in ophthalmology to replace diseased or damaged corneas for indications such as keratoconus, failure of the corneal endothelium (as in Fuchs' dystrophy, or following cataract surgery), infection, injury, ulceration, or corneal opacification; corneal grafts are usually sourced from cadavers, as it is infrequent for an eye to be removed from a living patient unless it is sufficiently diseased that usually its tissues would be unsuitable for donation (174). Figure 3.3 and 3.4 (overleaf) show those corneal graft donations and implantations in the UK by year which are reported to the UK Transplant Registry (not all cases are, so the complete figures will be higher, but the figures nevertheless show an indicative trend); rates of corneal donation and implantation are increasing over the observed 21 year period. The UK Transplant Registry figures for 2015-2016 record 3,045 corneal donors, 2,675 of these donated corneas only, and 370 donated corneas and solid organs (173). In that time period, 25% of solid organ donors after brain death, and 30% of solid organ donors after circulatory death also donated corneas.

Figure 3.3 **Cornea programme in the UK, 1 April 1996 - 31 March 2006**
Number of corneas donated and corneas grafted

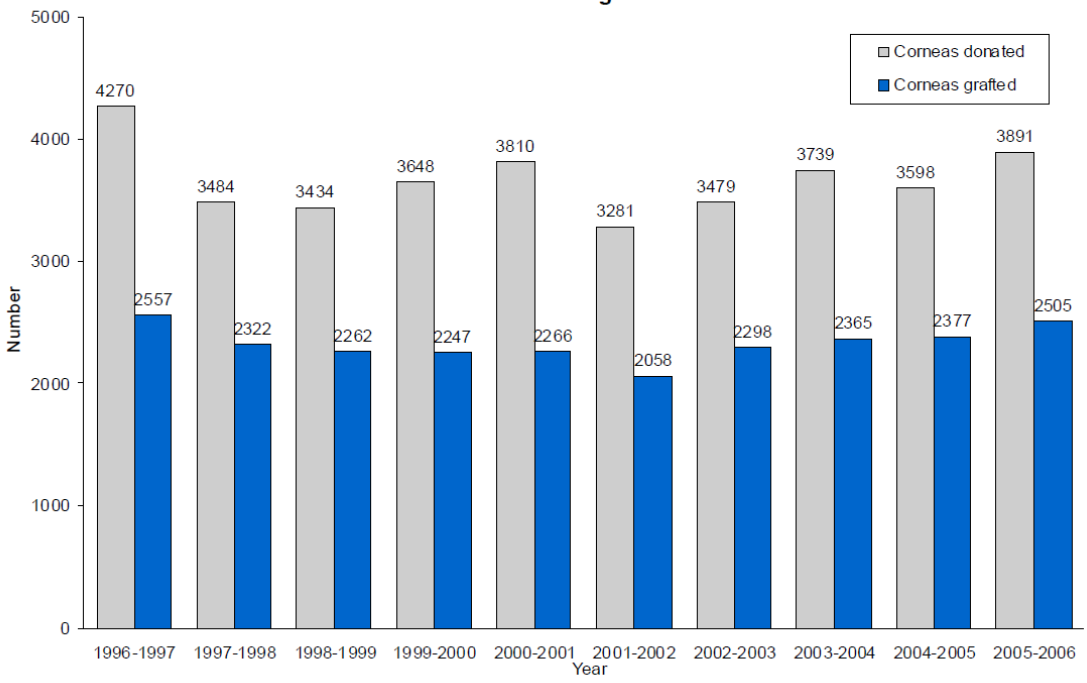


Figure 3.3 shows the trends in UK cornea donation and implantation from 1996-2006 inclusive. Copied from: NHS Blood and Transplant: Transplant Activity in the UK, 2006 (176).

Figure 3.4 **Cornea programme in the UK, 1 April 2006 - 31 March 2016,**
Number of corneas donated and corneas grafted

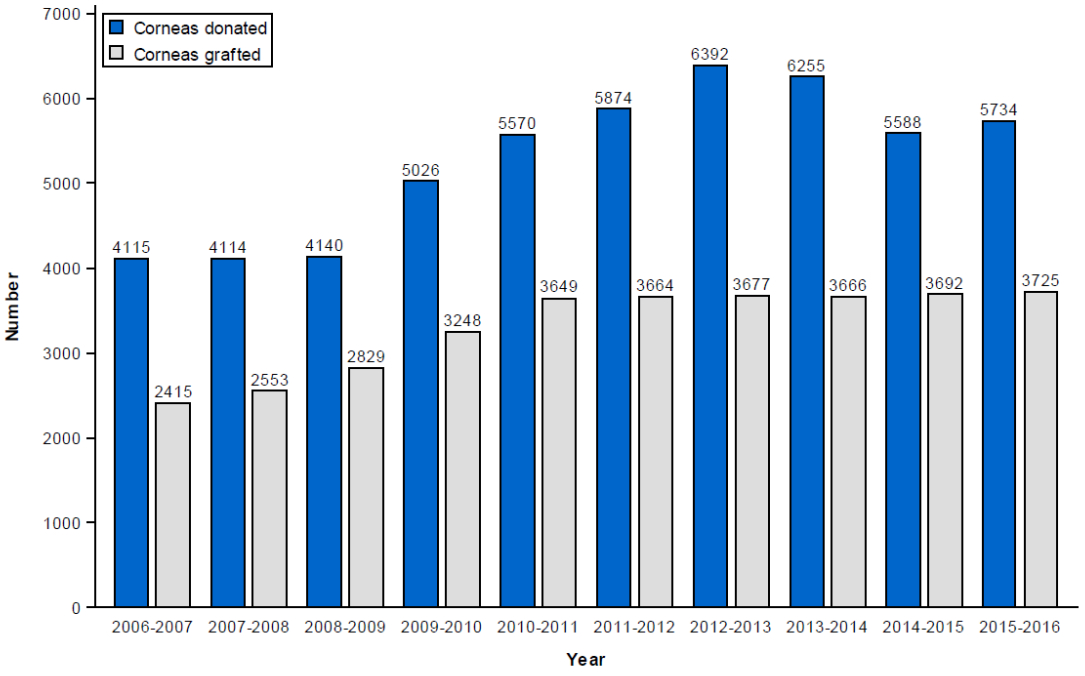


Figure 3.4 (on preceding page) shows trends in UK organ donation from 2007-2017 inclusive. Copied from: NHS Blood and Transplant: Overview of Organ Donation and Transplantation, 2017 (173). Note the reversal of the colour scheme compared to Figure 3.3.

The first documented case of corneal transplant transmission of CJD was described in 1974 by Duffy *et al.*, from a donor who died of pathologically confirmed CJD; the recipient became symptomatic 18 months after the transplant (177). A second case was published in 1997 by Heckmann *et al.* of a patient who died of CJD 30 years after receiving a corneal transplant from a donor who died of an illness with typical clinical and neuropathological features of CJD (86). It is not clear exactly how many cases of corneal transmission of sCJD have occurred, with differing totals within the literature. In 2006, Brown *et al.* reported that there were 3 additional possible cases in addition to those already described above (as a supplementary one line comment attached to a table within the paper) (178). Maddox *et al.* wrote in 2008 that there had been 6 such cases in total, referencing the Brown *et al.* paper, and also Hammersmith *et al.* – the latter's paper explains that they were unable to locate any records relating to the donor of the potentially implicated cornea (179, 180). The Maddox paper also described an additional four patients who received corneal transplantation and subsequently died from CJD, each of whom is suspected to have had sporadic CJD without any association with their transplant, reflecting a coincident development of the rare neurodegenerative disease among a growing population of transplant recipients (the larger the pool of transplant recipients becomes, the more likely that a disease which occurs sporadically is likely to be represented and potentially reported). The disparity in reported numbers reflects the difficulty of proving causality when an individual received a transplant and subsequently developed CJD. The two cases (published by Duffy *et al.* and Heckmann *et al.* and discussed above) widely accepted to represent corneal transmission of CJD had incubation periods of 1.5 and 30 years between exposure and onset of symptoms, and neuropathological

confirmation of sCJD in the donor. A summary of the 6 cases reported by Maddox et al. to represent suspected corneal transplantation transmission of sCJD is below (Table 3.1).

Table 3.1: Corneal Transplantation-associated CJD cases. Data from Maddox *et al.* (179).

Publication	Year reported	Country	Incubation period for each case (years)
Duffy *et al.* (85)	1974	USA	1.5
Uchiyama *et al.* (181)	1992	Japan	1.25
Heckmann *et al.* (86)	1996	Germany	12 and 30
Rabinstein *et al.* (182)	2002	USA	2, 4, and 6
Hammersmith *et al.* (180)	2002	USA	14.5 and 23.67
Heinemann *et al.* (183)	2005	Germany	13

Other, non-corneal graft materials (not associated to date with CJD transmission) are listed below. Tendon grafts are primarily used in orthopaedic surgery, such as for repair of ruptured ligaments (commonly cruciates, less commonly pectoralis major, biceps or triceps), and also for management of joint instability at the knee and elbow. Tendon grafts are usually donated from deceased donors, and the harvested materials are often processed to reduce the likelihood of rejection after grafting, using freezing, freeze-drying or cryo-preservation, as well as irradiation; since the graft contains mostly proteinaceous material, and the desired outcome of the graft is retention of its mechanical properties after implantation, the steps involved in processing are designed to avoid too much protein damage, which would otherwise weaken the graft, but such a restriction on processing would reduce the likelihood of inactivation of any infectious prion particle (184).

Bone grafts are used in orthopaedic, spinal and maxillofacial surgical procedures, usually to replace large areas of bone removed or damaged by infection, neoplasm, radiotherapy, etc. and thought unlikely to regrow; bone grafts are also used for fixation procedures, such as vertebral fusions, and as a cement to stabilise joint prostheses and encourage host bone regrowth. Bone grafts can be prepared in a number of ways, similar to those methods used in preparing tendon grafts, but again, intact protein structure is integral to bone strength, meaning such processing cannot cause too much protein degradation, and removal or inactivation of prion is unlikely. Bone grafts are harvested from cadaveric donors, but also from living donors such as from the head of the femur, removed from patients undergoing total hip replacement (185-187). Bone grafting was considered one possible risk factor for vCJD transmission, in part due to probable contamination on any bone graft with blood, and several health protection measures were introduced during the vCJD epidemic to try to protect the population, including no longer manufacturing pooled bone grafts from multiple donors, and also the exclusion of blood transfusion recipients from bone graft harvest (188).

Skin allografts are used in patients with severe skin damage (usually burns) who have insufficient unaffected skin to allow for harvest and use of autologous grafts. The grafts are cadaveric in origin, and are gamma irradiated and freeze dried to reduce immunogenicity (PrP^{Sc} is resistant to both treatments); they may only be used temporarily until autologous skin grafting is possible, or may be sloughed off once sufficient wound granulation has occurred under the allograft, allowing wound healing by secondary intention (189, 190).

Iatrogenic transmission of sporadic CJD has been recognised in recipients of cadaveric dura mater grafting (where the process in manufacturing the graft

materials was contaminated with PrP^{Sc}) and corneal transplants (where individual to individual transmission has occurred), both of which are procedures with transplantation of tissue, as well as in recipients of cadaveric pituitary derived hormones (growth hormone and gonadotropin), and cases of reuse of contaminated neurosurgical instruments and EEG depth electrodes (73). Cases associated with dura mater grafting and growth hormone continue to be reported.

Some case report publications in the field of suspected tissue and organ transplantation of CJD demonstrate a failure of understanding, such as Hashoul *et al.* (191). The authors describe a patient with a probable diagnosis of a subtype of sporadic CJD, who had received a bovine bioprosthetic heart valve; they attempt to make a connection between the bovine valve (which might conceivably be regarded as means of transmission of BSE prion) and the patient's illness, perhaps confused by the terminology "Heidenhain variant", which is a subtype of sporadic CJD where the patient presents with isolated cortical visual symptoms, rather than indicating variant CJD. Such papers highlight the need for caution in the interpretation of case reports.

Other (non-transplant) associated transmission of CJD is described in the introduction to this thesis (Chapter 1). At least 238 cases of dura mater graft related transmission of CJD (iatrogenic CJD) have been identified worldwide as of 2015 (74), with a mean incubation period of 12 years (range 1.3 – 30 years) (73). It is important to recognise that Lyodura® (the brand of cadaveric dura mater graft processed and sold by B Braun Melsungen AG and predominantly associated with such transmission) was not solely used in neurosurgical procedures, but also in other domains of surgery, including gynaecological, ENT (ear nose and throat), orthopaedic, dental, urological and cardiac procedures, fields not associated with iatrogenic transmission of CJD to date (192). Coincidentally, there have also been at least 238 identified worldwide cases of cadaveric-derived pituitary Human Growth Hormone associated transmissions of iCJD, with mean incubation of 17 years (range

5-42 years). Four cases of cadaveric-derived Gonadotropin iCJD transmissions are reported, with mean incubation 13.5 years (range 12-16 years) (74).

Although PrP^{Sc} is found in the highest concentrations in central nervous system (CNS) tissues and the pituitary gland in sCJD patients, the pathological protein is not restricted solely there, and has been identified in tissues other than the CNS. The Advisory Committee on Dangerous Pathogens Transmissible Spongiform Encephalopathy subgroup (ACDP TSE) published a useful summary on tissue infectivity in CJD in 2003, updated in 2012 (89), and summarised in Table 3.2, below. The high risk tissues are brain, spinal cord, cranial nerves, cranial ganglia, the posterior segment of the eye and pituitary gland. Nevertheless, other tissues can also express lower levels of PrP^{C}, and have been found to contain PrP^{Sc} in sCJD patients, and, as such, spinal ganglia and olfactory epithelium are considered medium risk, while other tissues are classified as low (rather than no) risk. The ACDP data is largely derived from the WHO, which in turn collated the results of multiple studies using both Western blot detection of PrP^{Sc} and animal transmission studies (in primates and mice) (5). The data in relation to vCJD are included in Table 3.2 for completeness and comparison.

The development of highly sensitive *in vitro* amplification techniques such as RT-QuIC has allowed identification of seeding activity in a variety of non-CNS peripheral tissues which are not recognised as high risk in CJD (5, 193, 194). Rubenstein and Chang suggested that non-CNS PrP^{Sc} distributions in sCJD patients may not be vastly different to those seen in vCJD, where lymphoreticular involvement is common (195). It should be noted that the mere presence of PrP^{Sc} does not necessarily imply infectivity of these tissues; however, animal studies have indicated the potential for infectivity of at least some non-CNS tissues, including recently bone marrow (196). The early recognition of corneal transplant transmission of CJD identified a route of CJD transmission in humans other than exposure to CNS materials, although the

possibility remains of contamination of corneal graft tissue at the time of tissue harvest with retina (which arguably should be considered a CNS tissue).

Table 3.2, overleaf, is sourced from the ACDP TSE Subgroup 2012 publication (89), with minor adjustments for formatting only. The original can be viewed at https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/444243/Annex_A1_update.pdf accessed 05.09.48.

Table 3.2 (89): **Distribution of TSE infectivity in human tissues and body fluids**

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

Tissue	Presence of abnormal prion protein and level of infectivity			
	CJD other than vCJD		vCJD	
	PrP^{TSE} detected	Assumed level of infectivity	PrP^{TSE} detected	Assumed level of infectivity
Brain	**+ve**	High **P**	**+ve**	High **P**
Spinal cord	**+ve**	High **P**	**+ve**	High **P**
Cranial nerves, specifically the entire optic nerve and only the intracranial components of the other cranial nerves	**+ve**	High	**+ve**	High
Cranial ganglia	**+ve**	High	**+ve**	High **P**
Posterior eye, specifically the posterior hyaloid face, retina, retinal pigment epithelium, choroid, subretinal fluid, optic nerve	**+ve**	High **P**	**+ve**	High
Pituitary gland	**+ve**	High (?)	**+ve**	High (?)
Spinal ganglia	**+ve**	Medium	**+ve**	Medium **P**
Olfactory epithelium	**+ve**	Medium	**NT**	Medium
Dura mater	**-ve**	Low	**+ve**[4]	Low
Tonsil	**-ve**	Low	**+ve**	Medium **P**
Lymph nodes and other organised lymphoid tissues containing follicular structures	**-ve**	Low **P**	**+ve**	Medium **P**
Gut-associated lymphoid tissue	**-ve**	Low	**+ve**	Medium
Appendix	**-ve**	Low	**+ve**	Medium
Adrenal gland	**-ve**	Low	**+ve**	Medium
Spleen	**+ve**	Low **P**	**+ve**	Medium **P**
Thymus	**-ve**	Low	**+ve**	Medium
Anterior eye and cornea	**-ve**	Low	**-ve**	Low
Peripheral nerve	**+ve**	Low	**+ve**	Low
Skeletal muscle	**+ve**	Low	**+ve**	Low
Dental Pulp	**-ve**	Low	**-ve**	Low
Gingival Tissue	**NT**	Low	**-ve**	Low
Blood and bone marrow	**NT**	Low	**-ve**	Low
CSF	**-ve**	Low **P**	**-ve**	Low
Placenta	**-ve**	Low	**-ve**	Low
Urine	**-ve**	Low	**-ve**	Low
Other tissues	**-ve**	Low **P**	**+ve**[4]	Low

There are many unknown factors about the early stages of sCJD. For example, it is not known how long it may take from the first spontaneous conversion of a molecule of PrP^{C} into PrP^{Sc} to the clinical development of the disease, nor do we know at what stage in the disease process PrP^{Sc} may be identified in peripheral tissues; furthermore, while it may be hypothesised in sCJD that the first spontaneous conversion to PrP^{Sc} would occur in CNS tissues, where the host PrP^{C} is found in highest concentrations, this is not certain. As such, there is the potential that a presymptomatic individual may die of a cause unrelated to sCJD and their organs be used for transplantation, or they may donate organs or tissues in life prior to becoming symptomatic of sCJD, but after the biochemical onset of the disease. Even if symptomatic of sCJD, due to the disease's variable and rapid presentations, and the potential to mimic other more common neurological illnesses, it is possible that patients may die without a diagnosis and their organs still be used for transplantation – the features of dementia and other neurodegenerative diseases are not in themselves sufficient to exclude patients as donors once they die. The Joint UK Blood Transfusion and Tissue Transplantation Services Professional Advisory Committee requirements concerning organ and tissue donation stipulate:

> "Must not donate if:
>
> 1. Diagnosed with any form of CJD, or other human prion disease.
>
> 2. Identified at increased risk of developing a prion associated disorder. This includes:
>
> a) Individuals at familial risk of prion-associated diseases (have had two or more blood relatives develop a prion-associated disease or have been informed following genetic counselling they are at risk).
>
> b) Individuals who have potentially been put at increased risk from surgery, transfusion or transplant of tissues or organs.

c) Individuals who have been told that they may be at increased risk because a recipient of blood or tissues that they have donated has developed a prion related disorder.

d) Recipients of dura mater grafts.

e) Recipients of corneal, scleral or other ocular tissue grafts.

f) Recipients of human pituitary derived extracts.

g) Since January 1st 1980: Recipients of any allogeneic human tissue."

Reproduced from www.transfusionguidelines.org (197).

The Advisory Committee on the Safety of Blood, Tissues and Organs (SaBTO) provides similar, but more detailed guidance in their publication "Guidance on the Microbiological Safety of Human Organs, Tissues and Cells used in Transplantation", including a table titled "Exclusions from organ and/or tissue donation based on possible TSE exposure", which I have copied into Appendix 3. The full guidance can be read at https://bts.org.uk/wp-content/uploads/2016/09/Guidance-on-the-microbiological-safety-of-human-organs-tissues-and-cells-used-in-transplantation.pdf accessed 16.09.18.

Aims:

I have looked for evidence of tissue or organ transplantation associated transmission of sporadic CJD in the UK among sCJD patients seen by the NCJDRSU between 1st January 2010 and 31st December 2015 inclusive.

The method described below looks for a link between known transplant recipients. As explained above, any patient known or suspected to have CJD is prohibited from donating tissue and organs. If this study is to identify transplant associated

transmission of CJD, then the donor of any organs (and therefore source of PrP^{Sc}) must not have been known to be suffering from the disease – either they might be in a presymptomatic phase, or their organs were donated after becoming symptomatic, but before the diagnosis was considered. If in a presymptomatic phase, transmission would be suspected if the donor and recipient both subsequently developed sCJD and were referred to the NCJDRSU. If the donation occurred at the end of the donor's life and sCJD was not diagnosed, to demonstrate evidence of tissue or organ transmission, that donor would need to donate organs to more than one individual, and at least two of the recipients of those organs would themselves need to go on to develop CJD and be referred to the NCJDRSU. Given the likelihood that transplant transmission of sCJD is a rare event (with only 2 cases of corneal transmission identified), it is very unlikely that this method will identify a connection between two recipients of organs from the same donor. However, if such a connection was identified, this would be highly suspicious for iatrogenic CJD transmission.

Other dementias are not regarded as an exclusion for organ donation, and CJD may be misdiagnosed either as another dementing illness, or other neurological disease (*e.g.* stroke). If a hypothetical donor was indeed in a preclinical phase of sporadic CJD, then the highest concentration of PrP^{Sc} would be expected to be located in the CNS. Other than dura mater grafts and pituitary hormones, no other CNS material is intentionally transplanted. The lower concentrations of PrP^{Sc} in other, non-CNS organs, and lack of exposure of the transplanted material direct into the recipient's CNS (as is often performed in animal transmission studies) is likely to result in longer incubation periods than seen in established transmission of iatrogenic CJD.

Methods

UK CJD Surveillance Process:

UK Clinicians are asked to refer to the NCJDRSU any patients in whom CJD is a possible diagnosis. Such clinicians come from multiple different specialties, including Neurologists, Psychiatrists, Geriatricians and General Physicians. These clinicians may refer for many reasons, and in doing so, they obtain expert diagnostic advice and support, as well as access to the national cerebrospinal fluid analysis laboratory (within the NCJDRSU) for 14-3-3, S-100b and RT-QuIC assays (which are vital parts of the CJD diagnostic process).

NCJDRSU data collection:

For patients in whom CJD is considered a possible diagnosis, an NCJDRSU clinician travels to assess the patient, interview their relatives, and to review the hospital notes and diagnostic tests. The visiting NCJDRSU clinician completes a standardised questionnaire with information obtained primarily from the patient's family (in rare circumstances, also from the patient themselves – regrettably, by the point of referral, it is unusual for patients to be able to communicate clearly or recall accurately). Where possible, consent is obtained from the family (or patient) to access further information from the General Practitioner medical records. Patients were classified according to standardised diagnostic criteria (see Appendix 2); these criteria have been adapted over the years as new diagnostic tests have been developed and validated. Throughout the study period, the 2010 "Rotterdam modification" to the 1999 "WHO diagnostic criteria for CJD" (198, 199) was applied; in 2017, these diagnostic criteria were updated as the "Euro CJD diagnostic criteria" with inclusion of RT-QuIC on CSF, a highly specific diagnostic test (164, 200, 201) (also included in

Appendix 2); this updated classification criteria has not been retrospectively applied to the cohort under study in this thesis as it was not in use when I saw these patients, when the data was extracted, and when the analysis was performed. Retrospective application of the updated criteria would generate a new, slightly larger cohort involving more patients, and requiring a complete reanalysis for both this chapter and Chapter 4. The updated classification will be applied retrospectively to the entire NCJDRSU database by those staff still employed there, and any future NCJDRSU studies will include these updates when formulating their cohorts.

NCJDRSU case classifications for individual patients are, however, updated as tests which are included in the diagnostic criteria in use at that point become available. For example, a case seen in life by the NCJDRSU clinician might be classified as an uncertain diagnosis, possible or probable sCJD, or (exceptionally rarely) as definite sCJD if a brain biopsy had been obtained; those cases not classified as definite are recoded as definite sCJD after neuropathological data is acquired if this confirms the diagnosis – usually following a post mortem examination. Similar changes in coding would occur if results relating to a patient who had already been seen – at that stage without positive diagnostic testing – later became available; such delays were not uncommon while I worked in the NCJDRSU in relation to 14-3-3 CSF testing, which was performed only once weekly, and required transport of CSF while frozen from the referring hospital to the NCJDRSU reference laboratory, but were rare for MRI scans or EEG tracings. These changes are unlikely to have impacted on the cohort under study, as sufficient time (11 months) passed after December 2015 before final data extraction to ensure that neuropathological results were available for every patient who had undergone post mortem.

The standardised family questionnaire includes details about any surgical procedure which the family (or patient) can recall the patient undergoing. The visiting NCJDRSU clinician collects information about the name of the hospital where the

procedure was performed, and the name and year of the procedure. We try to include even minor procedures, including removal of minor skin lumps or bumps, endoscopies, and stitches – such as after a laceration or episiotomy tear.

For those cases where we obtain consent to access the GP records, it usually takes weeks to months to access these. Typically, we wait until after the death of the patient, for completeness of this record, and on occasion this can add an additional complication, as the physical notes for deceased patients are often moved from an individual GP practice to a central storage location. Once the notes are obtained (either the originals, which are promptly processed and returned, or photocopies), they are reviewed (either by one of the NCJDRSU clinicians, other senior NCJDRSU staff with appropriate backgrounds, or by trained medical students completing a research project in the NCJDRSU) and compiled into a standardised GP history form. The data extracted are then entered into the NCJDRSU clinical surveillance database by administrative staff members. For each operation, the surgical procedure is recorded as a free text field, along with the name of the hospital, location (town), and year of the procedure. Both the GP history form and family questionnaires are available online as part of the NCJDRSU protocol document, https://www.cjd.ed.ac.uk/sites/default/files/NCJDRSU%20surveillance%20protocol-january%202017.pdf accessed 01.09.18. The family questionnaire is titled "Clinical and epidemiological review" and can be found from page 23 onwards, while the GP history form is located from page 62 onwards of the protocol document.

For each completed questionnaire, the operations are categorised into the following surgical types: neurosurgery, eye, ear, abdominal, orthopaedic, gynaecological, tonsillectomy, carpal tunnel, spine/disc, appendicectomy, transplant, cardiology/cardiovascular, stitches, nose/throat, varicose vein, dermatology/minor lumps, vasectomy/testicular, breast, plastics, dental surgery, urological, and other (including endocrine, salivary gland surgery, lymph node excision not covered by

other categories, bone marrow aspiration, nail surgery, and thoracic surgery). A copy of the guide for procedure classification used at this stage is included in Appendix 1. The process of operation coding is suggested at the time of data entry onto the family questionnaire or GP notes form, as the form requires entries to be written into the appropriate box; but this coding is formalised by administrative staff in the NCJDRSU using the procedure classification guide. The guide for operation coding was first developed in the NCJDRSU for use in case-control studies, and still has significant similarity to that used in the 2006 variant CJD case-control paper (42). The guide document has developed iteratively since then, as, over the years of ongoing surveillance, for any surgical procedure which the administrative team found difficult to fit into an established category, this would then be queried with a clinician as to how best to classify that procedure. These decisions are recorded, and the document has gradually become more comprehensive. The recording of these decisions ensures that operations should be classified consistently, reducing the risk of variability of individual interpretation impacting on this aspect of data entry.

For all patients referred to the NCJDRSU between January 2010 and December 2015 inclusive who were classified as either definite or probable sporadic CJD, all surgical procedures identified from either the GP records or family questionnaire were extracted from the surveillance database to a Microsoft Excel spreadsheet. This data set was also used for analysis of surgical instrument transmission of CJD, described in Chapter 4. The efforts required to improve data completeness and to identify and correct errors are also set out in Chapter 4, as they are more pertinent to the subsequent analysis of time-place associations, covered in that chapter. In brief, efforts were made to ensure as many records as possible from both family questionnaires and GP records were completed and included in the data set. All data obtained by 1st December 2016 were included in the analysis.

Examination of the extracted data for suspected tissue and organ transplantation:

The spreadsheet was sorted by operation category in the first instance; all surgical procedures listed in the transplant category were highlighted for review. The remaining categories were assessed for the possibility of use of tissue or transplant, first by scrutiny of the surgical procedure to identify possible operations of interest, and thereafter by accessing the hospital records and any supportive information from the GP notes for every procedure deemed likely to include tissue usage or transplantation. This step was performed to allow for the possibility that some procedures might have been miscoded (i.e. not coded as transplantation despite this being the primary purpose of the surgery), or coded appropriately but still included tissue usage (such as skin cancer excision where a skin graft might have been used). This was performed in the same manner as the analysis of transplant procedures, i.e. considering the operation type, and further reference to any other available data sources for any procedure identified as "of interest".

Processing the extracted tissue and organ transplant procedures:

With all procedures identified which were deemed likely to include tissue or organ transplantation, these were then classified as to whether they included artificial graft materials only, autologous transplantation only (i.e. the patient themselves was the "donor" of all tissues used), xenograft usage (for grafts derived from animal tissues, including bioprosthetic grafts), or allografts (where tissues or organs were donated from other individuals). In operations where the procedure name entered into the NCJDRSU surveillance database did not stipulate the nature of the graft used, the original GP notes and hospital notes (where available) were consulted to see whether any further detail could be extracted to allow clarification.

Exploring potential links with other sCJD tissue and organ recipients:

In order to consider whether there may be any potential connection between those patients identified within the 2010-2015 cohort who underwent transplant procedures and any other UK sCJD cases, a second data set was extracted from the NCJDRSU surveillance database of all patients classified as definite or probable sCJD who had been reported to have had transplant surgery. To avoid missing any potential miscoded procedures (particularly given the iterative improvements and adaptations to the procedure coding document, and the period prior to the 2006 case-control study from which it was developed), all ophthalmological, cardiac, abdominal and urological procedures were also extracted, and the procedure names were scrutinised to look for any procedures involving transplant. These additional transplant procedures were then compared to the transplants identified from the studied cohort, to look for any potential links where multiple organs might have been harvested from the same donor, indicating the possibility of potential transplant associated transmission of CJD.

For patients who received relevant tissue or organ transplantation, where available, I review the clinical presentations and neuropathological findings, where available, to try to identify any features which might be considered atypical for sCJD, such as those described in a subset of the Japanese dura mater graft associated iCJD cohort (202-204).

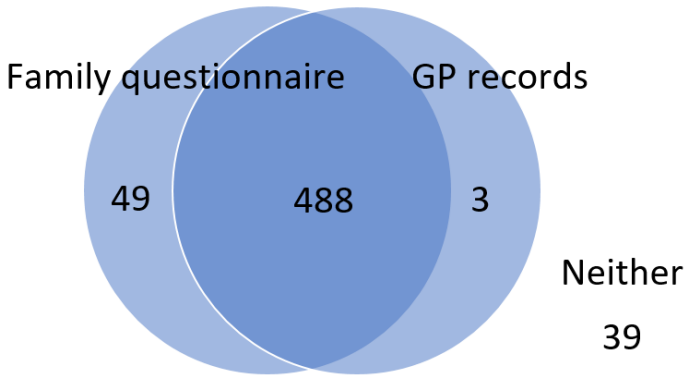
Results

Data availability:

579 cases of definite or probable sporadic CJD were reported to the NCJDRSU between January 2010 and December 2015 and were included in this study.

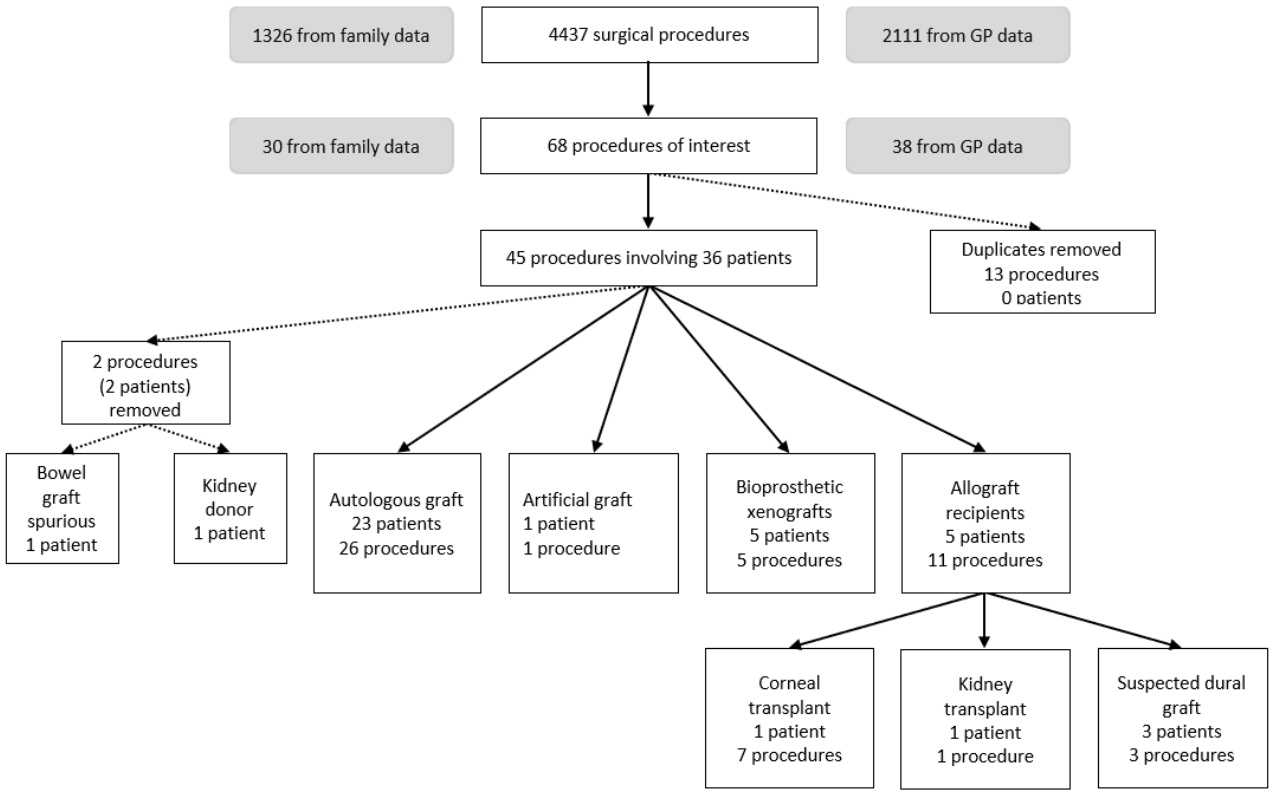
Both family questionnaire and GP record data were collected for 488 patients (84.3%), family questionnaire data only for 49 patients (8.46%), GP record data only for 3 patients (0.518%) and no data for 39 patients (6.74%). One, or both, data sources were available for 540 (93.3%) of the cases (illustrated below in figure 3.5).

Figure 3.5: Data sources for the 579 definite or probable sCJD cases 2010-2015



1326 surgical procedures were recorded in the family questionnaire data and 3111 in the GP record data, giving a total of 4437 surgical procedures when combining the two; this total includes any duplicates. These figures are represented in flow diagram Figure 3.6 below.

Figure 3.6: Flow diagram illustrating the surgical procedure data, and the identification of relevant procedures where tissue or organ transplantation may have occurred.



Identifying transplant procedures:

From the 4437 procedures, 68 were identified as "of interest" – deemed likely to involve tissue or organ transplantation. 30 of these came from the family questionnaire entries and 38 from the GP records. There was considerable duplication between the flagged procedures from the family questionnaire and GP record data sources. After removal of these duplicates, this left 45 reported tissue or transplant related procedures, involving 36 of the 540 patients for whom we had data, described in Table 3.3 and below. These figures also are illustrated in Figure 3.6, above.

Donors:

One patient donated a kidney, 30 years before onset of symptoms of sporadic CJD; the donor is of course at no risk of acquiring CJD from this organ since that individual was the source of it. The details of the recipient are not known to the NCJDRSU, but the only recipient of a renal transplant known to have developed sCJD in the UK is the other patient in this cohort, for whom the source of the organ is known and was not from this donor (furthermore, the two surgical procedures were separated by 29 years). No other non-autologous donation surgeries were identified.

Transplant recipients:

One patient was reported to have undergone a "bowel graft" in the late 1960s, according to the family questionnaire. No corresponding entry was identified from the GP notes, with only a variety of minor gynaecological procedures listed a few years either side of the year that this procedure allegedly occurred; none of the gynaecological procedures are likely to have included any tissue graft or organ transplant. The procedure was performed 55 years prior to symptom onset, and there are no extant hospital records. Bowel transplantation was first performed in humans only 4 years before the reported procedure, and was not survivable until the development of ciclosporin and other immunosuppressants to prevent organ rejection, around two decades later (205). While it is not clear exactly what this reported "bowel graft" surgery represents (some bowel resection and/or anastomosis might be plausible), it is more likely there is some inaccuracy in the reported procedure name. Overall, it is highly unlikely that the procedure represents any tissue or organ transplantation; this report was judged to be spurious and was removed from further analysis.

After removal of duplicate entries where procedures were listed in both the GP records and family questionnaire, most patients (30 of the 34 remaining patients after

exclusion of the kidney donor and "bowel graft" patient) underwent only a single procedure involving tissue or organ transplantation. 4 patients were listed in the data set with more than one procedure (details of these can be found below).

The procedures were divided by type of organ or tissue used. The largest category is for patients who received either autologous grafts or harvest procedures relating to (potential) subsequent autologous reimplantation: 23 patients and 26 procedures were identified. One patient underwent a dental procedure to relocate a tooth that had erupted in the wrong location. Two patients each underwent two autologous bone marrow related procedures (one patient had a bone marrow harvest and subsequent reimplantation, and the other underwent two harvest procedures, but there is no record of reimplantation). Eleven patients underwent coronary artery bypass grafting (CABG) without any paired procedure (see the paragraph concerning xenograft materials below). One patient underwent a tendon graft procedure; this is believed to have been an autologous graft based on the limited details available. Six patients underwent seven skin graft procedures; although full details are not available concerning the nature of the graft for six of the seven procedures, they are presumed all to be autologous, as the grafts were performed for small surgical wounds (such as skin cancer removals), rather than for extensive burns (where allografting has occasionally been used). One patient underwent a bone graft following a fracture; review of the notes suggested this was autologous. One patient underwent a cycle of *in vitro* fertilisation and had reimplantation of the fertilised eggs; it is arguable whether or not this represents a transplant, but it is removal of material from the body, with subsequent reimplantation and is included in the autologous category for the purposes of this study.

A single patient was identified who had undergone an entirely artificial graft, with a metallic heart valve inserted.

Five patients underwent procedures involving bioprosthetic xenografts. Four of these patients underwent combination coronary artery bypass grafting (autologous) with bioprosthetic valve insertions in the same procedure; three were porcine valves, and one valve was constructed from bovine pericardium. These patients became symptomatic at 0, 15, 65 and 135 months after their surgical procedures (mean 53.75 months). One patient underwent a non-valvular cardiac surgical procedure – resection of an atrial myxoma – with bovine pericardium used as a patch material, 24 months prior to CJD symptom onset. Both patients who received bovine pericardial materials had post mortem examinations, with findings typical of sporadic CJD.

Five patients were identified who are believed to have received allografts. One patient received seven corneal transplants, between three and 20 years prior to symptom onset. One patient received a transplanted kidney, two years prior to onset of symptoms; the organ was donated by a living relative, who has not to date been referred to the NCJDRSU. Three patients were identified who underwent procedures during which it is suspected dura mater graft material may well have used; two of these were neurosurgical procedures, the third was a skull base surgery performed under the ENT (ear, nose and throat) surgical speciality. These three procedures were performed 21, 26 and 40 years before symptom onset. The procedures were recorded as "transanthral ethmoidectomy and repair of CSF leak" (surgery to repair CSF leaks commonly used graft material); "left modified radical mastoidectomy (graft used)"; and "excision of frontal laceration, suturing of bones, artificial dura". Further clinical and (where available) pathological details about each of these patients are provided below.

Table 3.3: NCJDRSU sCJD 2010-2015 Cohort. Tissue and transplant procedures, organs or tissues involved, data sources, patient numbers, year range between procedure and symptom onset

Type of tissue/ transplant	Organs/ tissue involved	Number of patients	Number of procedures (GP records)	Number of procedures (family questionnaire)	Number after removal of duplicates	Year range procedure to symptom onset (mean, median)
Artificial recipient	Metallic heart valve	1	1	1	1	14
Autologous recipient	Assorted (see text)	23	19	18	26	1 – 60 (15.8, 9)
Bioprosthetic (xenograft) recipient	3 Porcine and 1 bovine valves, 1bovine pericardium	5	5	3	5	0 – 11 (3.98, 2)
Allograft recipient	7 Cornea grafts, 3 suspected dura mater grafts, 1 kidney	5	12	6	11	2 – 40 (13.4, 10)
Kidney donor	Kidney	1	1	1	1	30
Bowel graft (considered spurious)	Unclear	1	0	1	1	54

Additional transplant connections:

On searching for additional transplant procedures undergone by sCJD patients referred to the NCJDRSU between 1990 and 2016, but not included within the 2010-2015 cohort, who were classified as either definite or probable cases, four additional whole organ transplant or tissue allograft recipients were identified. One liver transplant, one heart transplant, one renal transplant and one corneal graft. Several autologous grafts were also identified, but these are of no relevance to this work. On comparing to the dates of the corneal transplant procedures for the individual from the 2010-2015 cohort, there were no occasions when any other sCJD patient received another organ which might have been sourced from the same individual donor within the same or adjacent years.

Clinical phenotypes of the three suspected dura mater graft recipients:

The first suspected dura mater recipient was 57 when she became symptomatic. She had suffered a head injury aged 31 and underwent neurosurgery with a transanthral ethmoidectomy and repair of a CSF leak. She recovered well, and had no other past medical history other than a short depressive illness 3 years prior to clinical symptoms, and from which she made a full recovery. She experienced a two month neuropsychiatric prodrome with anxiety, paranoia, social withdrawal, followed by a rapid cognitive decline with dysphasia, myoclonus and visual hallucinations. She died 4 months after onset of symptoms. When reviewed by the NCJDRSU clinician late in the disease course, she had developed some cerebellar findings on examination. EEG, MRI and CSF examination were all typical of sporadic CJD, as were neuropathological changes at post mortem.

The second suspected dura mater recipient was not seen in life by the NCJDRSU clinician due to the death of the patient before arrangements could be made to visit. MRI brain in life showed changes suggestive of sCJD with typical basal ganglia DWI high signal. The family agreed to meet the NCJDRSU clinician as a "late visit" after post mortem results became available confirming findings typical of sCJD. The patient was 67 when he became symptomatic of a rapidly progressive cognitive decline with memory and language deficits. He developed myoclonus and ataxia, and died 5 months after symptom onset. He developed mastoiditis 21 years prior to symptom onset and underwent a mastoidectomy with an unspecified graft material used.

I reviewed the third suspected dura mater graft recipient shortly after starting in the NCJDRSU. She was a 63-year-old woman who experienced a 10 month progressive cognitive decline, presenting with frontal behavioural changes, apathy and hypersomnolence. She developed dizziness around the fifth month, followed by ataxia, myoclonus and dystonic posturing. Around the ninth month of her illness, she deteriorated acutely with unilateral stroke-like motor deficits, and was found to have developed two acute intracerebral haemorrhages on CT brain. She progressed rapidly from that point towards akinetic mutism, before her death. At the age of 22, she sustained a skull fracture in a road traffic collision, for which she underwent neurosurgery including the usage of artificial dura mater graft. Investigations in life included a non-specific EEG with encephalopathic changes, MRI evidence of high basal ganglia DWI and FLAIR signal, as well as gliosis in the region of her neurosurgery, and areas of microhaemorrhage suggestive of amyloid angiopathy. CSF markers were typical for sCJD. Post mortem examination revealed changes typical of sCJD, as well as coexisting amyloid angiopathy with intracerebral haemorrhages.

Clinical phenotype of the corneal transplant recipient:

I met the family of the corneal transplant recipient after the patient's death; she had not been referred to the NCJDRSU in life. She was a 72-year-old woman who experienced a 3 ½ month rapidly progressive dementia which presented with dysphasia, frontal behavioural changes, and shortly afterwards ataxia and cortical visual impairment. She deteriorated rapidly, developing myoclonus, dysphagia, and subsequent akinetic mutism. EEG and MRI were both typical of sCJD. CSF was not analysed, and there was no post mortem examination. Her corneal transplants were initially for Fuchs' corneal dystrophy, but she had problems with corneal graft rejection and infection, leading to successive reoperations.

Clinical phenotype of the kidney transplant recipient:

The renal transplant patient was a 67-year-old woman who experienced an 8 month progressive illness with early hypersomnolence. Around 3 months into the illness, more overt neurological symptoms manifested, with unsteadiness, dizziness, followed a month later by falls, overt ataxia, low mood and visual blurring. By month 6 she became paranoid, obsessive and confused, and bedbound, with choreiform movements. She developed perseverative behaviours and a sweet tooth, as well as visual hallucinations and misperceptions, and continued to deteriorate rapidly. She had received a renal transplant from her daughter 2 years prior to symptom onset for an IgA nephropathy. Her daughter (the donor) was present at the visit by the NCJDRSU clinician. Investigations in life included positive CSF 14-3-3 protein, and MRI changes suggestive of frontal cortical ribboning. She underwent post mortem examination which demonstrated typical sCJD changes only.

Neuropathology:

9 of the 11 recipients of either allografts (including the cases discussed above) or xenografts underwent post mortem examination. Each post mortem identified typical changes for sCJD, including on immunocytochemistry. There were no unusual post mortem features identified to indicate any prion pathology other than sCJD. In addition, amyloid angiopathy and intracerebral haemorrhages were seen in the third probable dura mater graft recipient as described above. For those patients in whom genetic analysis was performed, their codon 129 status correlated with the neuropathological phenotype, as would be expected in sCJD (100).

Discussion

Consideration of data quality and completeness:

In comparison to the (other, predominantly non-transplant) surgical procedures examined in Chapter 4, the GP record and family data reported similar numbers of procedures (for reference, for non-transplant surgery, GP data reports almost four times as many procedures as the family questionnaire). This may have occurred because of the memorability of transplant procedures (when compared to other surgical procedures). Most families try to rationalise as to why their relative developed CJD and would consider the implications of the donated organs.

The two data sets did not show total concordance; the most obvious discrepancy occurred in the allograft category, where the family questionnaire data reported only half as many procedures as were identified from the GP record data of the same patients. Two of the "missed" procedures in the family data occurred for the patient who received the corneal transplants, as her family had found it very difficult to keep track of how many grafts the patient had received; during the interview, they produced their best guess, and they were reassured that we would seek confirmation from the GP record. Such omission is unsurprising. Due to the coding strategies developed for the surgical category classification process, one operation was split into two entries on the GP data set (which is why there were 11 procedures after removal of duplicates despite 12 GP record reported procedures). The other three "missed" procedures in the family data were the three suspected dura mater graft recipients. For the two suspected neurosurgical uses of dura, the family reported neurosurgery, but were not aware of, or did not recall, the graft usage; the situation was similar for the patient who underwent skull base surgery, as the family were aware of the

surgery, but not graft usage. The variations between the two data sources supports the usage of both to try to minimise omissions.

Chapter 4 uses the same data set, and discusses the methods used to try to improve the completeness of the data set, and some reasons why a small proportion of data was not available. Discussion of this topic is more relevant to that chapter, and it is not repeated here. For those patients where no family or GP data was available, it is possible we may have missed a patient who had undergone relevant transplant surgery; it is also possible that a transplant recipient might have not been diagnosed with sCJD, and never been referred to the NCJDRSU. Given the small numbers of potential overlapping recipients of other organs from the same donor, missing even a single relevant case would prevent this study from identifying a positive connection. Incomplete case acquisition is unavoidable, while incomplete data acquisition might become addressed in the future if health records ever become digitised, centralised, and stored indefinitely – however, the past failures of large scale NHS IT projects do not inspire much confidence that this will occur in the near future.

Summary of key results:

Among the 2010-2015 cohort, 43 relevant procedures were identified with reported (or suspected) tissue or organ transplantation, involving 34 patients. This study identified five patients among the cohort who are believed to have received transplanted organ or tissue from human sources, including one patient who received a kidney transplant, one who received seven corneal transplants, and three patients who are suspected to have received dura mater grafts. Five patients received xenografts during cardiac surgery, four of these tissue heart valves (three porcine, one bovine), and one a bovine pericardial patch. These cases are considered further below.

Several types of procedures which are of no risk of sCJD transmission were also extracted, including autologous transplants, artificial grafts, and spurious data; these cases are not discussed further. The kidney donor's organ was harvested in life, 30 years prior to onset of symptoms – the recipient has not been referred to the NCJDRSU and given the protracted time interval between donation and symptom onset, it is unlikely that the donor was infective at that stage.

Allograft recipients – suspected dura mater grafts:

The records for the three patients who are suspected to have received dura mater graft materials in two neurosurgical and one ENT procedures are limited, and it was not possible to obtain information to determine whether Lyodura® or another brand of cadaveric dura mater graft was used, or whether a non-cadaveric derived substitute graft material was used, for example a purely artificial (206, 207) or autologous substitute. Although the term "artificial dura" was located in the GP notes for one patient, without being able to determine exactly which graft material was used, we cannot be certain that this guarantees this was not a cadaver-sourced graft, only that it makes autologous grafting unlikely (208). The term "artificial dura" has been used to describe processed graft materials which were still originally cadaver-derived, and those cadaver-derived dura grafts were in widespread usage around the time of this procedure. As was seen in the xenograft results, bovine tissues were sometimes used for grafting requiring connective tissues (such as repairs to the dura), with bovine pericardium either used directly or produced following extraction of collagen from the pericardium and manufactured into dura graft substitutes; such grafts may also be termed "artificial dura" (209). Typically bovine pericardium was used in cardiac surgery, but it has been used in neurosurgery (210). The choice of graft material was dependent on the surgeon and material availability.

These three suspected dura graft procedures were performed 21, 26 and 40 years before symptom onset, in 1991, 1984 and 1973 respectively. In 1987, B Braun Melsungen AG instituted an additional sterilisation step in the production of Lyodura®, immersing the graft material in sodium hydroxide, reducing the infectivity of any PrP^{Sc} in the material. There have been fewer cases of iatrogenic CJD associated with Lyodura® formulated after the addition of this step. Lyodura® usage stopped around 1992 (192, 211). Although it might seem that one of the procedures occurred after the addition of sodium hydroxide, the shelf life of Lyodura® was 5 years (and indeed some appear to have been used beyond this) (82). It is possible, therefore, that all three suspected dura mater recipients may have received Lyodura® grafts manufactured prior to the implementation of sodium hydroxide processing, and if so, they would then represent iatrogenic CJD, albeit transmitted through a recognised means of transmission.

One of these three patients had the surgery outside the UK, and while we attempted to access health records internationally through communication between the NCJDRSU and the local surveillance system, our international colleagues were unable to locate any operation notes. In one of the UK cases, the hospital notes had been destroyed in a flood, and the (still retained) theatre log books did not contain any details about the graft materials used. For the remaining case we were also unable to obtain further details, and it is believed the records have been destroyed. After careful discussion of each case with Professor Will in the NCJDRSU (two cases by colleagues prior to my employment at the NCJDRSU, one by me), it was felt given the lack of definite evidence of cadaveric dura mater grafting for each of the three patients, that they should remain coded as sCJD, rather than being recoded as iCJD (see Appendix 2 for diagnostic criteria, including iCJD). If any of these cases did represent iatrogenic transmission, two would have occurred towards the upper end of the known spectrum of the incubation period following CNS exposure to PrP^{Sc},

and the third (where artificial dura was used) occurred 40 years after usage of this material, outside the range recognised for dura mater iCJD (73, 82).

As explained in Chapter 1, more than 60% of dura mater graft iCJD has occurred in Japan (83). The Japanese CJD surveillance system have invaluable experience within this subset of iCJD, and have described particular clinical and pathological phenotypes in a subset of dura mater graft CJD cases which would be unusual in sCJD. These features include a prolonged isolated cerebellar ataxic syndrome at onset, the detection of florid plaques at post mortem, and non-concordant features when comparing the patient's codon 129 polymorphism on genetic testing to expected codon 129 status on the basis of neuropathological phenotype at post mortem (202-204, 212). Were any of these features to be present in any of these three patients, this would have provided some evidence to suggest they represent dura mater related iCJD; however, none of the three patients had any of these features. The work in Japan does indicate that many dura mater iCJD patients have more typical sCJD-like presentation and pathology, so the absence of such features in these three individuals is inconclusive.

During the 2010-2015 period, I visited a fourth patient who was believed to have been exposed to dura mater grafting during ENT surgery on the middle ear. This individual was not included in the data set (or results) for this study, as the case was classified as possible CJD on the diagnostic classification in use at the time (rather than the probable or definite classification required to be included when the cohort was generated – see Appendix 2). If the recent revision to the diagnostic classification was used in this thesis, incorporating RT-QuIC results (the Euro CJD classification, as discussed above on page 106, rather than the 2010 modified WHO diagnostic criteria), then this patient would be classified as a probable sporadic case, and would then have been included in this cohort. This patient's family reported that he received some graft material in 1980 as part of a reconstructive process to repair damage to his

middle ear, 34 years prior to symptom onset, and within the time window where Lyodura® was used, prior to the sodium hydroxide preparation. The surgery was not performed in the UK, and the CJD surveillance system of the country where the surgery occurred were unable to locate any operative details, so again, unfortunately we cannot be certain whether or not dura mater graft was used in this surgery, as other (non dura mater) materials were also used in similar procedures in this time window. This individual did not have a clinical course similar to that reported in the Japanese iCJD subset, and did not have a post mortem.

Allograft recipients – corneal transplants:

The patient who received 7 corneal transplants is of considerable interest. It is worth noting that the temporal spacing of the 7 procedures indicates the corneal grafts must have been from 7 different donors. As discussed in the introductions of both this thesis and this chapter, corneal graft transmission of CJD has been reported in at least 2 patients (179). The family of the corneal transplant patient reported during the interview that the Ophthalmologist who operated had commented that one of the later transplant surgeries required a deeper resection of the prior, failed graft, and a more extensive graft than would be typical for such procedures. Due to the anatomy of the eye, the anterior structures do not come into contact with retina, but the deeper the graft, the greater the chance of including contamination by posterior eye material, such as retina; regarded as a high risk tissue for transmission of CJD (89).

Corneal grafts are usually used within four weeks of harvest (213). Other solid organs must be used more rapidly. These time constraints allowed for comparison of the year entries for when this individual underwent each graft against the list of organ transplants received by other patients seen by the NCJDRSU since 1990 to look for other transplant being performed in the same year, or adjacent years (in case the transplant surgery occurred near the beginning or end of calendar year, as the

NCJDRSU surveillance database only contains whole year values (rather than specific dates) about surgical procedures). The process for this comparison is explained extensively in Chapter 4, as it is very similar to the space-time association assessment. Harvested organs and tissues are distributed geographically widely, so it was not reasonable to filter the transplant procedures on the basis of location. There was no evidence of any connection between the corneal transplant recipient and any other sCJD organ recipients, for each of the 7 graft procedures that this individual underwent.

As was mentioned in the introduction to this chapter, the majority of corneal donors do not donate other organs (88%) (173). This reduces the potential to identify a connected case of a whole organ recipient being identified. If a similar proportion of this patient's corneal grafts came from donors who donated only corneas, the population of other recipients of organs from the same donors might be only approximately 7 other cornea recipients (from each donor's other eye), and perhaps one of these donors might have also donated whole organs, which are, on average, donated to 3 recipients. The number of "co-recipients" is likely to be reduced further by the observation that only around ⅔ of harvested corneas are implanted. With such small numbers, combined with the rarity of transplantation transmission of CJD (with only two accepted cases – dura mater and pituitary hormone cases aside), it is likely that the sensitivity of the method used in this study in detecting pairs of iatrogenic transmission is extremely low.

The work by Maddox *et al.* suggested that in the US, they should expect to see one case of sCJD developing by chance in a corneal transplant recipient approximately every 18 months (179). This figure was calculated on a sCJD incidence of 1 case/million population/year. The incidence of sCJD in UK data is thought to be around 1.8 cases/million/year. Corneal transplant procedures in the UK number around 10-fold less than the US, and as a crude approximation, therefore, we might

see a similar case of sCJD in the UK every 8 ⅓ years. A single observed case in this cohort is not unexpected, therefore. The identification of this patient offers little more than "unproven association", to quote Hogan *et al.* in their correspondence (214) in relation to Rabinstein's corneal transplant CJD case report (182) (listed in Table 3.1).

Allograft recipients – renal transplant:

This individual's kidney was donated by her daughter, who was present at the visit by the NCJDRSU clinician. Her daughter was asymptomatic of any neurological disease at the time of the interview, and has not since developed CJD and been referred to the NCJDRSU. This organ transplant is not related to the subsequent sCJD development by the recipient.

Xenografts recipients:

There is no known naturally occurring (or intensive farming induced) porcine prion disease (although animal studies have indicated that pigs may be susceptible to BSE PrP^{Sc} inoculation, particularly direct injection to the brain (215), there was no known dietary transmission of BSE to pigs), and therefore the porcine valve recipients are highly unlikely to have xenograft associated transmission of any form of CJD. Bovine tissues could conceivably be contaminated by BSE prion, although both patients who received bovine pericardium underwent surgery considerably after the cessation of the BSE epidemic in the UK, and bovine pericardium is sourced from non-UK cattle (216). Both these patients had post mortem examinations, with findings typical of sporadic CJD and their codon 129 polymorphism status correlated with the neuropathological features as would be expected; there is no suggestion that they could have had variant CJD. Neither bovine nor porcine material is a potential vector for tissue transplantation transmission of sporadic CJD between human patients.

Limitations of the method:

The process reviewing the extracted database of named surgical procedures is an inherently flawed means of identification of all transplanted materials, particularly for non-whole organ tissue grafting. Neither the GP record nor family questionnaire collects detailed information about the nature of any surgical procedure than the name of the operation, and the classification of surgery type. On occasion, the GP record includes whether autologous material was used, but in the cases of spinal and orthopaedic surgery, for example, the operation name never includes what material may have been used, for example, to help to cement a prosthetic joint in place, or fix two vertebrae. We lack sufficient data, therefore, to know whether synthetic cements or donated bone graft may have been used, meaning any patient undergoing such procedures could be exposed to allograft material, and this would not be recorded in the NCJDRSU database. Similarly, tendon graft materials may be missed. Even in cases where grafts are recorded, the details of type of those grafts often are not available, as was evident in the suspected dura mater cases. There is no "gold standard" data set to compare to quantify any missed graft procedures.

It is far less likely that we missed any other whole organ transplantation among the patients for whom data was obtained, as mentioned above, these tend to be highly memorable procedures and the name of the surgical procedure explains the transplanted organ, meaning the recording of such procedures in medical records (and onward coding to the NCJDRSU database) is also likely to be reliable. Some xenografting may have been overlooked if not listed in the procedure name as part of the data extracted from the NCJDRSU database, or in a procedure where such grafting may not be commonplace. Bovine pericardium was used in fields outside cardiothoracic surgery (the surgical category covering all identified uses found among the cohort in this study); some procedures performed by specialities including gynaecology, ophthalmology and ENT surgery (216), particularly in reconstructive

procedures, but no non-cardiothoracic usages were identified within this patient cohort.

Potential improvements and future work:

This slightly circuitous way we have looked for transplant or tissue transmission of CJD could be avoided if it became routine practice to perform brain biopsy or CSF analysis on all non-living donors, but any such change in policy requiring further samples could potentially reduce the uptake of organ donation. On observation of family behaviours around the topic of post mortem in patients suspected to have sporadic CJD, many families are very resistant to the idea of any procedure which requires accessing brain tissue, even when their loved one may have expressed a desire to be an organ donor. Many families and individuals are very protective against any intrusion to the face or head after death, more so than other body regions – this is reflected by the choice of many UK organ donors to opt out of corneal harvest while accepting other organ donation (author's personal observations on discussion with friends, colleagues and family). Any such compulsory post mortem analysis would come with ethical difficulties in the event of a positive result – the timeframe for turnaround of neuropathological and biochemical tests on brain or CSF would mean any harvested organs would already have been transplanted into their recipients before results were available. A positive result would force the recipients to be informed of their new "at risk" status for the future development of CJD, and some might request that their organ be removed – at least for those individuals where organ replacement therapy (such as dialysis for renal transplant recipients) was available, or an alternative organ donor could be found. Indeed, this has already occurred in the UK, with a patient developing symptoms of sCJD shortly before death, symptoms which were incorrectly believed to represent brain metastases prior to later neuropathological confirmation of sCJD (217). These two factors mean any change to current donation practice would seem unlikely to be accepted, unless it had a positive protective benefit for those individuals who were about to receive

potentially contaminated organs. Future improvements in assays such as the RT-QuIC (which currently offers high sensitivity and specificity for the detection of sCJD, but requires at least 5 days to produce a result in the NCJDRSU laboratory) to improve speed of production of results, and access of alternative tissues such as the olfactory mucosa (218), potentially avoiding the need for brain tissue or lumbar puncture, might not only begin to offer more timely screening, but also be better accepted by families around the time of organ harvesting. The group of donations where this would be likely to have the earliest benefit would be the heart beating brain-dead donors, where suitable swabs and samples could be obtained prior to organ harvest.

To try to identify other allograft and xenograft recipients would require review of the operative notes for every surgical procedure for each patient thought to have sporadic CJD, rather than the current approach of identifying procedures thought likely to include graft materials. When hospital records are accessed by the NCJDRSU, these are usually those from the hospital where the individual was diagnosed and referred. Patients that have moved from one region to another many have had operations in multiple other hospitals, and on moving, any earlier notes might become inactive and over time be destroyed – unlike the medical records from General Practice, which tend to follow patients. An alternative would be a prospective register of graft recipients, with that information passed to the NCJDRSU to cross check against our patient group. Such a register would probably require support from the graft/implant manufacturers, and would risk breaching patient confidentiality, but there would be an arguable public health benefit from better record access.

There exists no formal process for the NCJDRSU to look into potential transplant transmission of sCJD, in contrast to the ability to explore high risk surgical procedures (through the public health/health protection teams) and blood transfusion (through the TMER (1, 2). Such a formal structure would need to review the source of all

materials transplanted into any sCJD patient, to determine the recipient of any material donated by any sCJD patient, and thereafter to access and review the other party's medical records to look for evidence of sCJD, follow them up for subsequent symptom onset, and also to identify any other at risk individuals who may have received potentially contaminated tissues or organs. A prospective follow up of this type would improve the likelihood of identifying any potential organ or tissue transplant associated transmission of CJD compared to the method in this study. A look back study of this sort has previously been performed, but only of a single case of a vCJD patient who received a liver transplant (57); this would be a useful direction of further work for the cornea transplant patient seen in this study.

Implication of increasing donors/donation:

As illustrated in Figures 3.1 and 3.2 earlier in this chapter, organ donation in the UK has doubled over the last two decades. As the number of recipients is likely to follow a similar trend, there will be an increasing population of individuals at exposed to potential organ transplantation associated CJD transmission, and more opportunities to identify connections between recipients if they indeed exist. At the same time, with a larger population of organ recipients, the number of chance occurrences of sCJD in this group will also increase. It may be difficult to disentangle any future organ transmission of CJD from the increased noise of any chance occurrences.

Future directions in organ transplantation:

It seems that induced pluripotent stem cells have been hailed as a panacea for almost every medical condition over the last decade. Something that was once a science fiction dream – growing an individual a cloned replacement organ which would not require immunosuppression – may start to become reality in coming decades (219, 220) At that stage, organ harvest from donors may no longer be required. Until that point, the potential for iatrogenic transmission of prion diseases remains.

Conclusion

There is no robust evidence of tissue or transplant associated transmission of CJD among the sporadic CJD cohort from 2010 to 2015 inclusive. Three patients are suspected to have received dura mater graft, but due to limitations in retained data, we are not certain for two of the cases whether dura mater grafts were definitely used, while for the third we know only that "artificial dura" was used, but not whether this was cadaveric derived, purely artificial or potentially even a xenograft. The recipient of artificial dura became symptomatic 40 years after the neurosurgery, which is outside the range reported with dura mater iCJD (73, 82). A fourth individual is mentioned in the discussion, who was not included as part of the studied cohort, may also have been exposed to dura graft material for middle ear surgery. It is impossible to know how to interpret each of these cases, due to the lack of contemporary operative records to indicate the materials used. They may each represent missed iCJD cases, incorrectly classified as sCJD, or the procedures they have undergone may not be relevant.

The recipient of 7 corneal transplantations could potentially also represent corneal transplant associated iCJD, but the only means of exploring this with NCJDRSU data is to look for other patients who might also have received transplant materials from the same donor among the sCJD cohort. No such other patients were identified. Additional studies to access the health records and death certificates of the donors would be of benefit in determining their cause of death, and whether any had neurological symptoms; it would also be of interest to track the recipients of any other organ or tissue recipients from the same donor. These adaptations to the study would mirror the TMER process (as described in Chapter 2). At present, there is no such structure to facilitate this style of look back study. An estimate of the incidence of

sCJD among corneal graft recipients indicates this patient could represent such a chance development.

The cases identified at most represent circumstantial evidence that some iCJD transmission may be missed among the sCJD cohort. The NCJDRSU will continue to monitor for future transmission through organ transplantation, but the methods used are unlikely to be particularly sensitive for what is thought to be a rare occurrence.

Whether or not transplanted materials continue to transmit CJD and are hidden among suspected sCJD cases, a quote from Dr Peter Bennett's work concerning risks of vCJD transmission in bone and tissue (188) is of equal relevance to sCJD and expresses an appropriate sentiment on which to close this chapter:

> "such transplants are carried out with good reason. Many procedures are life-saving, and the rest are substantially life-enhancing. Any response to the theoretical risk of [CJD] transmission must be set against the clinical need for such procedures. Many tissues and organs are already in short supply, shortages that are often compounded by the need to match would-be recipients with suitable donors. There is often no alternative to the use of donated tissue: even where artificial alternatives are available (as with heart valves, for example) these are not always clinically suitable. In this complex area, there is thus the necessity to balance the potential risk of [CJD] transmission against the clear and immediate risk of not being able to undertake essential procedures."

Chapter 4

Evidence for Surgical Transmission of sCJD

Introduction

Background:

Sporadic CJD is the most common human prion disease. It is believed to occur as a result of a spontaneous protein misfolding event of the PrP^{C} host protein into PrP^{Sc}. The PrP^{Sc} then propagates by causing remaining PrP^{C} to undergo the same misfolding, as per Prusiner's prion hypothesis (4). This leads to neuronal loss, symptom onset and subsequent death. The abnormally folded PrP^{Sc} protein is believed to be the infectious particle that transmits CJD between individuals in iatrogenic CJD and Kuru (transmitted by implantation of contaminated material, either by medical instruments and tissue or human derived hormones, or in the case of Kuru, by endocannibalism as a funeral rite) (75). These known vectors of transmission of CJD raise the possibility of person-to-person transmission as a potential cause for a small number of cases currently believed to be sporadic, through interventions such as surgery.

The recognised causes of iatrogenic transmission of CJD include dura mater grafting (with Lyodura® being the most implicated brand), cadaveric pituitary derived human growth hormone, cadaveric gonadotropin hormone, and a few instances related to neurosurgery, EEG depth electrodes and corneal transplantation (73); the evidence for tissue and transplant transmission is covered in Chapter 3, while the EEG and neurosurgical cases are considered below. Additionally, variant CJD has been transmitted via contaminated blood from donors who were in a pre-clinical phase of variant CJD (58); further details about blood and blood product transmission of CJD can be found in Chapter 2.

In the cases of known surgical instrument transmission of sCJD, EEG depth electrodes were used in an individual symptomatic of CJD, and reused in two individuals 2 and 3 months later, who subsequently themselves developed CJD (91). Will and Matthews provided further details relating to non-EEG electrode neurosurgical transmission of CJD which occurred between two individuals operated on the same day of a neurosurgical list, with the second individual becoming symptomatic 17 months after the surgery; and also in two other individuals, who both underwent operations 2 weeks after surgery on two other CJD patients, before becoming symptomatic 19 and 40 months later (94). There are also numerous reports where patients with CJD have undergone neurosurgical procedures in close proximity, but where causality has not been definitely proven, such as Stricof *et al.* who reported two patients who underwent neurosurgery, using the same drill, but different drill bits – the first patient was symptomatic at the time of surgery, while the second patient was diagnosed with CJD 6.5 years later (221). This may be similar to the cases of corneal transplantation in sCJD patients, discussed in Chapter 3 and by Maddox *et al.* (179).

Other than blood transmission of vCJD, and corneal transplantation, the other recognised transmission routes for iatrogenic CJD are all associated with central nervous system (CNS) contact or contact with pituitary hormones (while not strictly a CNS tissue, the pituitary is located within the skull vault, in very close anatomical proximity to CNS). This reflects the distribution of PrP^{Sc} in affected individuals – the host PrP^{C} is mainly located in the CNS, and the PrP^{Sc}, after transformational change, largely remains there. The Advisory Committee on Dangerous Pathogens Transmissible Spongiform Encephalopathy (ACDP TSE) subgroup summary on tissue infectivity in CJD has already been discussed in Chapter 3 (89): see Table 3.2. To recap a little, the high risk tissues are brain, spinal cord, cranial nerves, cranial ganglia, the posterior segment of the eye and pituitary gland. Other tissues can also express lower levels of PrP^{C}, and have been found to contain PrP^{Sc} in sCJD patients, and as such spinal ganglia and olfactory epithelium are considered medium risk,

while other tissues are classified as low (rather than no) risk. It is known that PrP^{Sc} can adhere to surgical equipment, and it may be highly resistant to traditional sterilisation techniques (74, 125, 126). The combination of non-CNS PrP^{Sc} and this resistance to sterilisation means it could be theoretically possible for sCJD to be transmitted between patients by contaminated surgical instruments.

Prior epidemiological studies of sporadic CJD have produced mixed results with respect to surgery as a risk factor; most of these have been case-control studies and a review of these follows. Those with positive results have implicated differing sets of surgical procedures as potentially being associated with surgical transmission of sCJD.

Collins *et al.* found in their Australian case control study there was an association between surgery in general and sCJD development, but no specific association between specific anatomical operation sites and sCJD (222). Ward *et al.* found that in the UK sCJD cohort, a history of having undergone surgery was associated with increased risk of sCJD, but that this association was only found in the miscellaneous category of surgical procedures, including skin stitches, removal of lumps and bumps, and nose and throat operations (19). Mahillo-Fernandez *et al.* used the Danish and Swedish sCJD cohort and found an association between surgery ≥ 20 years earlier and sCJD. Their surgical category data indicated peripheral vessels, the gastrointestinal tract and gynaecological surgery were associated with increased sCJD risk. Hamaguchi *et al.* assessed the Japanese cohort and found no association between surgery and sCJD; the authors also comment that 4.5% of sCJD patients underwent operations after onset of clinical symptoms without any special precautions, but with no identified transmission of sCJD related to these procedures (223).

de Pedro-Cuesta *et al.* reported different positive risk factors for sCJD development among surgical categories in specific time windows (224). For example, retinal and optic nerve surgery was a risk factor at ≥ 1year, and peripheral nerve and skeletal muscle surgery between 10 and 19 years prior to disease onset. Two later papers from the EUROSURGYCJD Research Group (again from Danish and Swedish registry data) add interesting nuance and complexity to the understanding of surgery as an epidemiological risk factor in sCJD. Cruz *et al.* suggest that particular surgeries are more common in sCJD patients around the time of (and shortly prior to) disease onset – potentially exposing others to contaminated instruments (225). de Pedro-Cuesta *et al.* suggest that there may be an age-dependent transmission in sCJD: *i.e.* a young age at exposure to surgery – and therefore potentially contaminated instruments – may increase the possibility of transmission of sCJD. An age-dependent model is one hypothesis for the pattern of cases seen in vCJD, which predominantly affects individuals exposed to BSE in early life.

A recent systematic review by López *et al.* highlights many of the issues concerning surgical risk factors for sporadic CJD (226). This review found some evidence that cardiac and vascular surgery might be associated with sCJD risk, but also postulated that this could represent a prodrome of vasculopathy prior to clinical disease onset, rather than the cardiac surgery necessarily representing a means of transmission. The authors explain that the published case control papers are of low quality of evidence, and are generally at risk of being affected by bias – including recall bias – or of being impacted upon by poor matching of controls. For example, it is typical to obtain the history of surgery from a family member in patients suspected to have sCJD, but most control histories relate to the individual him or herself answering the questionnaire.

Other than case control studies, the other type of publication covering potential sCJD surgical transmission is that of case reports. Most case reports are unable to prove causality in any observed pair of CJD cases with a neurosurgical connection, and the

best option is to track individual instruments, and if possible, perform animal transmission experiments with any implicated instruments, such as was reported by Gibbs *et al.* with depth EEG electrode transmission of CJD (227). López *et al.* also note that relatively few incidents have been published relating to re-use of neurosurgical instruments after earlier usage in a patient subsequently identified to have CJD, but that several such incidents have been reported by colleagues at international prion conferences without having been published (221).

The López *et al.* paper features a comprehensive details concerning different surgical types implicated as potential sources of surgical transmission for CJD, including forest plots (figures 4 and 5 in their paper) covering the different surgical types indicated as potential risk factors for CJD transmission in 13 different case control papers (226). I have not reproduced their work here to avoid plagiarism or copyright issues. Overall, the epidemiological results are inconsistent, but trend towards identifying some association between (different) surgical procedures and sCJD; however, the quality of evidence across these studies of low, and there is high susceptibility to bias in each.

Aims:

I aimed to look for any evidence that some UK sCJD definite or probable patients referred to the NCJDRSU between 2010 and 2015 (inclusive) may have been exposed to potentially contaminated surgical instruments which had been used on another patient within the cohort under study. Additionally, I looked for any suggestion that any UK sCJD definite or probable patient referred to the NCJDRSU since 1990 may have come into contact with potentially contaminated surgical instruments used in a high risk surgical procedure which were used on another sCJD patient between 1990 and 2015 inclusive.

Methods

UK CJD Surveillance and NCJDRSU data collection:

The process of UK CJD Surveillance and the initial steps of data collection are covered in Chapter 3 on tissue and organ transplant associated CJD. In summary, data is collected via a family questionnaire, completed by the NCJDRSU clinician at the time of meeting the patient and their family. As one of the NCJDRSU clinicians I saw many of the patients included in this cohort, and completed family questionnaires for all the patients I visited. After obtaining permission from the family (or patient, when possible), the GP records are accessed after the death of the patient, these are reviewed and each surgical procedure entered onto a GP questionnaire. Procedures from both data sources are entered into the NCJDRSU clinical surveillance database by administrative staff.

Part of the data entry procedure, both when completing the questionnaires and on entry of the data into the clinical surveillance database, includes allocating a code of operation category: neurosurgery, eye, ear, abdominal, orthopaedic, gynaecological, tonsillectomy, carpal tunnel, spine/disc, appendicectomy, transplant, cardiology/cardiovascular, stitches, nose/throat, varicose vein, dermatology/minor lumps, vasectomy/testicular, breast, plastics, dental surgery, urological, and other (including endocrine, salivary gland surgery, lymph node excision not covered by other categories, bone marrow aspiration, nail surgery, and thoracic surgery). A copy of the guide for procedure classification used at this stage is included in Appendix 1. In considering the instruments likely to be used in each surgical procedure, these categories are not necessarily mutually exclusive; some categories may potentially be totally subsumed within another (i.e. appendicectomy can be incorporated into abdominal surgery, vasectomy/testicular is a form of urological surgery, and tonsillectomy into the nose and throat category), for these groups, the smaller

category was recoded into the parent category. Details about which categories were moved within others can be found in table 4.3 of the results section.

There is also the potential for overlap between categories for certain procedures, either through the usage of the same sets of instruments within more than one category (such as some neurosurgical spinal procedures using the same instruments as certain spine and disc surgeries, since operating near the spinal cord requires access past the same bony structures operated on within the spine/disc category). Some of these type of associations are possible because more than one group of surgeons may perform similar surgery (for example carpal tunnel surgery may be performed by either orthopaedics or plastics, or both depending on local policies and individual skills/training); instrument sets may be shared between such procedures and other surgical procedures performed by the same group of surgeons, and we lack sufficient detail to know which surgical specialty performed each particular procedure in each hospital across the UK. There is the potential for these sorts of overlaps to occur between the following pairs of surgical categories listed in Table 4.1.

Table 4.1: Surgical categories in data set with potential category overlaps

Surgery category	**Potential overlap with**
Neurosurgery	Spine/Disc, Carpal tunnel
Orthopaedics	Spine/Disc, Carpal tunnel
Spine/Disc	Neurosurgery, Orthopaedics
Plastics	Carpal tunnel
Abdominal	Urology, Transplant
Cardiothoracics	Transplant
Urology	Transplant
Ophthalmology	Transplant

The potential for such overlaps between categories means that, rather than separating all procedures by the category, and then looking for "surgical time-place associations" (see below) only within each category, it is necessary to look beyond the category, considering potential overlaps as listed above, for example between carpal tunnel and neurosurgery, and possibly others not listed above but arising due to variation in local practices. The number of possible inter-category overlaps were potentially very high, and resulting analyses numerous and complex. Including them was considered beyond the scope of this thesis, but will be considered in future analyses.

An additional issue is that the categories are entered into the NCJDRSU database as a result of human interpretation of the procedure name, and some operations may be coded into one category where with only a minor change to the operation name they could be coded within another, this is particularly problematic with multistage operations (such as combined coronary artery bypass grafting and aortic valve replacement, often performed as a single procedure, where the latter aspect could potentially be coded as a transplant procedure, instead of cardiothoracic surgery).

Data extraction:

The process of creation of the 2010-2015 sCJD cohort data set is covered in the methods section of Chapter 3. The same data set was used for both chapters. In summary, a list of all surgical procedures undergone by every patient in the cohort as reported by either the GP record data or the family questionnaire was extracted to a Microsoft Excel spreadsheet from the NCJDRSU database. The extracted data included the name of the procedure, the code of operation category described above, the year of the procedure, the name of the hospital, and town where the procedure

occurred. The recording of year of procedure only, rather than an exact date, has limitations for subsequent utility of this data, discussed below.

"Surgical time-place association":

For this study, I examined the operative histories for all patients classified as either probable or definite sCJD cases (by WHO diagnostic criteria) referred to the NCJDRSU between January 2010 to December 2015 inclusive to look for potential links between the surgeries performed.

The potential for infectivity of surgical instruments will be dependent on the degree of exposure to prions of the instrument, the sterilisation procedures the instruments undergo, and the number of times they undergo usage and sterilisation cycles between patients. While data on instrument sets is tracked in theatre records, this information is not usually entered in the medical records of those individuals undergoing surgery. Unfortunately, we are unable to collect this data concerning the usage of individual instruments; therefore, we are unable to show any direct link between sCJD patients in terms of the specific instruments used in their operations, and the number and nature of any sterilisations those instruments underwent between those operations. As such, it was necessary to develop a surrogate measure of interconnection between cases. The implications of this surrogate measure are analysed in the discussion section below.

I defined a "surgical time-place association" as an occasion when two different patients underwent an operation within the same surgical category, or overlapping surgical categories (as listed above) within one year of each other, at the same hospital. The period of +/- 1 year was selected to accommodate the limitations of the data accuracy, since the NCJDRSU database recorded operative dates only by year;

for example, it was conceivable that a pair of patients might have undergone surgery on 31st December of year X, and then the other patient on 1st January of year X+1, with only one day separating them – consideration of surgeries based on only the same calendar year would therefore fail to recognise such a pairing as an association. Prion infectivity after adherence to surgical instruments is unlikely to be limited by time to a single year of storage, but without the ability to track the number of usages and the sterilisation techniques deployed on each instrument, a +/- 1 year range was decided to be the most reasonable approach in attempting to use this data – a wider range became too unwieldy to assess for surgical time-place associations. However, in the known cases of neurosurgical instrument and EEG electrode transmission of CJD, patient exposures to instruments were separated by weeks or months, meaning this date range may be reasonable (91, 94).

Errors in data extraction and efforts to improve completeness:

For the purposes of the following paragraphs relating to the errors in the data extraction process, "we" represents the NCJDRSU. On two occasions, I prematurely processed the data looking for surgical time-place associations with incomplete datasets, due to failing to try to ensure the data set was as complete as possible. On the first occasion, an error in the parameters entered into the NCJDRSU database when extracting to the Excel spreadsheet resulted in approximately one third of cases having no surgical data extracted; once identified, this was relatively easy to rectify. The second premature analysis was performed prior to later efforts made to ensure completion of the dataset – I was missing data for those patients for whom we had not yet obtained the GP records, or for those patients for whom we had only recently received those records, but had not yet completed a GP questionnaire (the step immediately prior to data entry to the NCJDRSU database). This constitutes a form of data lag, and affected approximately 30 GP questionnaires (unfortunately, I did not keep exact records of this step contemporaneously).

After rechecking the list of cases within the 2010-2015 cohort, and crosschecking against those for which I already had data, I again attempted to obtain the remaining GP notes, arranging for an administrative colleague to contact the relevant practices and central storage sites or local health boards once more. For the cases where we had the GP notes, but had not completed the questionnaire, I completed the questionnaire and entered the data into the Excel spreadsheet (before passing the now-completed questionnaire for data entry into the NCJDRSU database). All data obtained by 1st December 2016 was included in the final analysis. To ensure there had been no patients omitted in the list generated by the extraction process from the NCJDRSU database to the Excel spreadsheet, for each CJD patient for whom the extracted data set listed no operations in either one, or both of the family or GP questionnaire, I rechecked the paper copies of both questionnaires, and the scanned GP notes, to ensure that this patient had indeed undergone no surgical procedures. A sample of 10% of the CJD patient identification numbers for which data existed within the spreadsheet were randomly selected and rechecked by reference back to their GP notes, GP questionnaire and family questionnaires to ensure there were no missing procedures – no such omissions were identified among the sample.

Data cleaning:

All surgical procedures listed throughout the extracted data were manually checked to ensure they were appropriately coded, with corrections made when spurious entries were located – this most commonly occurred for family questionnaire entries, where the visiting NCJDRSU clinician occasionally recorded a procedure in the incorrect category, failing to follow the procedure classification document, or in multistage operations featuring aspects which might be coded into different categories.

One issue with the extracted data set was the variability of the reported hospital name. This occurred not only through typographic errors in data entry, but, for the GP data, in the cases of hospitals where the trust and hospital may not share the same name, and (potentially for both data sources, but more so for the family data) for hospitals that have changed name at some point in their operating period. For example, Kent and Canterbury Hospital (in Canterbury, Kent) could be entered as its correct name, or Canterbury Hospital, or East Kent Hospitals University NHS Foundation Trust, or minor combinations of this final iteration; another example is Hope Hospital, which was renamed to Salford Royal Hospital in 2008, while its trust was called Salford Royal Hospitals NHS Trust until it was renamed to Salford Royal NHS Foundation Trust in 2006, resulting in 4 variations on the name. Other hospitals changed site and often name, while moving staff and equipment between the sites, such as Frenchay Hospital, in Bristol, which closed in 2014 and transferred patients, staff and services to Southmead Hospital. Finally, some trusts have moved certain departments between their hospitals as building developments have been completed, such as the movement of urology from St Bartholomew's Hospital, Smithfield, London, to The Royal London Hospital, Whitechapel, London after the opening of their new building in 2012.

The dataset extracted included a variable of the reported town for each procedure, but this field was variably entered; for example, Salford Royal had town entries listing either Salford or Manchester, and for the operations when the trust name was used, but for a trust spread across multiple sites, the town field typically (but not reliably) listed the main site of the trust, rather than the correct one (for the example of East Kent, the main site was Canterbury, but the other hospitals in the trust, Queen Elizabeth Queen Mother and William Harvey Hospital, are situated in Margate and Ashford respectively). To overcome the issues around naming, for any procedure where the trust was listed rather than a hospital, I returned to the original data and repopulated this field if possible; for each operative entry, I ensured that a

standardised, consistent town entry for each hospital site was entered, overwriting the ambiguous or incorrect ones for every procedure listed, if possible. Entries where the hospital and town could not be accurately identified were removed before assessing for time-place associations. It was not possible to track the movement of each department within each multi-site hospital trust, but many such movements remained within the same town, and it is hoped this would largely overcome this potential pitfall.

The use of the town variable was necessary due to overlap of identically named hospitals in more than one location in the UK; for example, Lister Hospital could refer either to Chelsea or Stevenage, Royal Victoria Hospital might indicate any of Belfast, Blackpool, Folkestone or Newcastle, or St Mary's Hospital could be in Colchester, Harborough, London, Luton, Manchester, Newport or Portsmouth.

<u>"Surgical time-place association" method:</u>

In order to identify the potential overlap of procedures across the differing surgical categories (as discussed above) it was necessary first to sort the data by operation category (for later convenience when reviewing the final output), then operation year, then the standardised operation town. This produced a list of surgical procedures performed within each town, and within each town further sorted by year of the operation. If there were multiple surgical procedures in the same year and town, then they would be sorted by operation category. Reversing the order of the last two sorts, so that the data was sorted by town first and then year would have produced a list of surgery by year, but without the town clumping persisting (although within a given year, the town category would be alphabetised); it would not have been possible to analyse the data to look for time-place associations in this order. With the data sorted, I then worked systematically to remove extraneous data – *i.e.* any which contained no procedures involving surgical time place associations. The first step was to separate

the data (using blank rows in Excel for easy visualisation) into blocks by town, with further separations inserted any time there was a gap of a year or more with no procedures. A considerable number of operations could be removed from the list for having no neighbours within the same or adjacent year, in the same town, or when runs of consecutive procedures involved only a single patient, rather than at least a pair of patients.

This process left clusters of operations occurring within one year of at least one other operation, involving more than one patient and in the same town. These clusters were further broken down and re-sorted by the hospital name, and occurred in sufficiently small groupings to make those occasions where multiple alternative names (both correct, incorrect and historical) of the same hospital had been used readily identifiable to avoid missing an association. Further sifting to remove extraneous pairs could occur on the basis of the surgical category, with care required in any procedure (as listed above in the methods section) where there was the potential for overlap of surgical instruments between categories – in these circumstances, clinical knowledge was required to contemplate whether there may be shared instruments.

After producing the list of surgical time-place associations (TPAs), these were cumulated into categories more in keeping with the clinical practice of different surgical teams, rather than the categories used in data entry; for example, bringing "ear" and "nose and throat" and "tonsils" into an ear, nose and throat (ENT) category, reflecting UK surgical specialties. One issue was the inclusion of vascular surgical procedures within the cardiac or cardiothoracic surgery category – the associations identified involving these procedures were considered individually, moving non-cardiac and non-cardiothoracic vascular surgery into the adapted varicose veins and vascular surgery category. The details of these reclassifications are listed in table 4.3.

Results presentation:

The process of using both family and GP data to identify potential TPAs was intended to minimise occasions where incomplete data might mask such an overlap between patients, accepting a degree of duplication. It became apparent on beginning to collect the results together that there were substantial differences in the numbers of reported procedures between the two sources, and that there are likely to be inaccuracies in the family reported data. Using only these two sources of data on surgical procedures means there is no "gold standard" to use to try to assess the accuracy of each data source. My first attempt to present this data therefore separated the TPAs into three parallel results sets, with tables and graphs for each. After feedback, I have reorganised the results section to include the complete data on TPAs in Table 4.5, and using figures to plot the percentages of TPAs and surgical procedures by specialty for both data sources together (Figure 4.2), and when using only the GP data set (Figure 4.3), as this is considered more likely to be accurate. The family only data set has been moved to Appendix 5.

Removal of non-clinically plausible surgical time-place associations:

One further level of scrutiny of the surgical time-place associations was to remove those that were deemed to be clinically implausible. A pair of procedures would not be regarded as clinically plausible if they would not use the same instrument set. For example, within the abdominal category were numerous endoscopic procedures (oesophago-gastroduodenoscopy, colonoscopy or sigmoidoscopy), as well as non-endoscopic surgical procedures (either laparoscopic or open surgical procedures, such as cholecystectomy or appendicectomy). A TPA would be plausible if it occurred between two endoscopic procedures, or between two non-endoscopic procedures, but implausible if it contained one endoscopic procedure and one invasive surgical procedure.

Similar such pairs of implausible surgery could occur in the cardiac and cardiothoracic surgery categories, between endovascular/angiographic procedures (*e.g.* coronary angiogram and angioplasty) and open cardiothoracic surgery (*e.g.* coronary artery bypass grafting); in orthopaedics between arthroscopic and open surgery; in gynaecology between colposcopic/hysteroscopic procedures and more open gynaecological surgery (*e.g.* hysterectomy, oophorectomy); and in urology between cystoscopic/ureteroscopic procedures (*e.g.* transurethral resection of the prostate) and open urological surgeries (*e.g.* nephrectomy). In TPAs where at least one procedure was listed containing both open and endoscopic (*etc.*) procedures, naturally, this was regarded as a plausible TPA paired with either sort of procedure as the other half of the pair. Any TPA containing an entirely non-invasive procedure (such as stereotactic radiosurgery) was also discounted as clinically implausible.

Two further aspects of the TPAs were considered when looking at plausibility for transmission of CJD. Any TPA containing at least one procedure which used only single use instruments or equipment were deemed implausible, such as insertion of a nasogastric tube, ascitic drain, *etc*. Any procedure which may use either single use or reusable instruments was presumed to use reusable. In TPAs where the two procedures occurred on adjacent years, and the later procedure was performed on an individual already symptomatic of CJD, this TPA would be discounted on grounds of temporal implausibility; this occurred only infrequently, but included procedures such as percutaneous endoscopic gastrostomy (PEG) or radiologically inserted gastrostomy (RIG) tube insertion on a dysphagic CJD patient, if another patient had already undergone an endoscopic procedure in earlier years. The limited accuracy of only recording year of procedure, and relatively small number of procedures performed on symptomatic CJD patients mean this accounted for few exclusions on clinical implausibility. Any TPAs where where both procedures were performed after

symptom onset in each patient were also discounted on grounds of temporal implausibility.

High risk surgical procedures:

As an additional element to this study I extracted from the list of surgical procedures all operations which were likely to include contact with high risk materials, as per the ACDP tissue classification (89). These predominantly relate to neurosurgical procedures around the brain, cranial nerves, cranial ganglia, spinal cord and ophthalmological procedures involving the posterior segment of the eye (predominantly retina). This extraction was performed manually on reviewing all surgical procedures, using the neurosurgical and ophthalmological categories as an indication, before removal of procedures which were unlikely to contact such tissues (*e.g.* ophthalmological procedures contacting only anterior eye tissues); I looked through the remaining categories for any procedures classified in other categories which also included high risk tissue exposure.

I compared this to a list of high risk procedures undergone by all earlier definite or probable sporadic CJD patients reviewed by the NCJDRSU since the creation of the Unit in 1990 until January 2010 (the start of the cohort for this study). When planning to perform this additional aspect of analysis, it was believed that a list of such high risk tissue procedures already existed as part of ongoing work at the NCJDRSU. This was not the case, so identification of these procedures required extracting all surgical procedures for earlier cases from the NCJDRSU database, and then review of the operation details for consideration of whether they may involve high risk surgery, just as for the 2010-2015 cohort. For historically older cases reviewed by the NCJDRSU, the questionnaires used to collect information from patient families were different, and we did not request GP notes for patients prior to 2000; there were no additional efforts made to increase data completeness as per the 2010-2015 cohort, as

too much time had passed. Not all these surgical procedures were coded by category in the same manner as the later cohort, meaning it would have been insufficient simply to review procedures coded as neurosurgical or ophthalmological.

With the two lists generated, I looked for surgical time-place associations among high risk tissue surgical procedures among all patients reviewed by the NCJDRSU since 1990.

Results

Data availability:

The dataset used was the same as in Chapter 3 concerning tissue and transplant. I have repeated a few key numbers of cases and procedures in the results here for completeness. 579 cases of definite or probable sporadic CJD were reported to the NCJDRSU between January 2010 and December 2015 and were included in this study. Both family questionnaire and GP record data were collected for 488 patients (84.3%), family questionnaire data only for 49 patients (8.46%), GP record data only for 3 patients (0.518%) and no data for 39 patients (6.74%). One, or both, data sources were available for 540 (93.3%) of the cases. (See Figure 3.5 in Chapter 3, which illustrates the data sources.) A total of 4437 surgical procedures were reported from both data sets (median 7, range 0-56), 1326 from the family questionnaire (median 2, range 0-12), and 3111 from the GP records (median 5, range 0-54). A summary of these figures follows in Table 4.2.

Table 4.2: Procedure and patient number by data source.

Data Source	Either or both GP /family data	GP data only	Family data only
No. of patients	540	491	537
No. of procedures	4437	3111	1326
• **Mean (2 d.p.)**	8.22	6.34	2.47
• **Median**	7	5	2
• **Mode**	5	5	2
• **Range**	0 - 56	0 - 54	0 - 12

The individual who underwent 54 GP listed procedures (explaining the wide range in Table 4.2) is of some interest, and I still remember meeting her family (unfortunately, she died before we were able to meet her) – her family reported only two procedures, but they were recorded as "numerous endoscopic procedures" and "numerous gynaecological procedures" – there had been so many that they had lost track, due to her extensive past medical history.

Coding errors and adjustments:

Three errors in coding were identified: a lymph node surgical procedure being misclassified as breast surgery, circumcision misclassified as urology (the procedure classification document in Appendix 1 indicates it should be put into the stitches group), and a flexible laryngoscopy being miscoded as abdominal, rather than nose and throat.

Incomplete data:

The 2 family reported events of surgery type "not known" (listed in Table 4.5) were removed from the data set before further analysis (each family reported that they knew their relative had had some sort of procedure around a particular time at a particular hospital, but without the details of the surgery; both families were also quite vague about the year of the procedure).

Similarly, there were 46 surgical procedures in the combined data set without a value for year of the procedure (1.0% 1 d.p.), 18 (0.6% 1 d.p.) for the GP data and 28 (2.1% 1 d.p.) for the family questionnaire data; these operations therefore could not be used for linkage.

For 443 procedures, it was not possible to identify the hospital where the procedure had occurred (10.0% 1 d.p.), 356 (11.4% 1 d.p.) for the GP data and 87 (6.6% 1 d.p.) for the family data. This included procedures where the hospital was not known or there was insufficient data to identify the hospital (e.g. "a British military hospital"), procedures performed at GP surgeries or at the patient's home, and procedures were there was an unresolvable ambiguity: for example, "Nuffield Hospital" might be recorded, which is a chain of private hospitals spread across many sites. I attempted to resolve such ambiguities by review of the GP record, and returning to the original entry in the family questionnaire, to see whether additional information was available – it was, rarely – but for the majority such efforts proved fruitless.

These areas of incompleteness were not mutually exclusive, as for several data omissions other details were also absent (therefore the total number of incomplete data listed in these paragraphs is greater than the number of operative procedures removed from the original set, before subsequent analysis). The total remaining operative entries after these exclusions, therefore, was 3977 (89.6% of the original 4437) using both data sources. 1225 of the 1326 family questionnaire procedures (92.4%) were usable, and 2752 of the 3111 GP record procedures (88.5%). Where possible, incomplete data was still used for indicating the total number of procedures in each operative category, as the majority still included the name of the surgical procedure and therefore its category.

<u>Category summation and corrections:</u>

24 events were identified within the cardiology/cardiothoracics category which were better classified as vascular surgery (procedures including carotid endarterectomy, femoral bypass surgery and abdominal aortic aneurysm repairs).

Table 4.3 (overleaf) shows the number of procedures from each surgical category, and the amalgamation process for surgical procedures which needed to be moved from their original reported surgical category. The mathematical symbols of + and - are used to indicate the addition or removal of particular categories (and therefore surgical procedures) from each row. The exact values are not of great interest, but it seems sensible to show the division of surgical procedures among both the NCJDRSU procedure classification in usage (see Appendix 1), as well as the final category sorting used in this work when the results are presented below, particularly if some aspect of this work was to be repeated in the future. For any category where I did not move or cumulate procedures, the figures can be seen in Table 4.5 without any additional processing (the totals from Table 4.3 are also included in Table 4.5).

Category overlaps:

The category overlaps identified earlier which could form time-place associations with more than one other category proved to be an unfounded concern, as (within the dataset studied) none of the time-place association events included carpal tunnel surgery, even on the later reanalysis following the late suggestion of a potential overlap between neurosurgery and carpal tunnel (a category which might then link to either plastics, orthopaedics, or neurosurgery). Nevertheless, it was important to be aware of the potential when sorting for time-place associations, as without such consideration, if such overlaps had occurred, they would have been overlooked. With such overlaps excluded, the carpal tunnel and spinal categories were incorporated into orthopaedics when cumulating the total number of procedures.

Table 4.3: Number of procedures by category where category correction required, including subcategory numbers, and totals after category correction

Surgery category	Number of procedures	Number from family questionnaire	Number from GP records
Ear + Nose and throat + Tonsils/adenoids	51 + 135 + 56	22 + 24 + 43	29 + 111 + 13
Total	242	89	153
Abdominal (GI) + Appendicectomy	957 + 83	194 + 56	763 + 27
Total	1040	250	790
Orthopaedic + Carpal tunnel + Spinal	550 + 40 +24	248 + 12 + 8	302 + 28 +16
Total	614	268	346
Cardiology/cardiothoracic - Vascular	176 -24	65 – 9	111 -15
Total	152	56	96
Dermatology/minor lumps & bumps + Plastics + Stitches	494 +21 + 147	97 + 9 + 65	397 + 12 + 82
Total	662	171	491
Varicose veins + Vascular	51 + 24	16 + 9	35 + 15
Total	75	25	50
Vasectomy/testicular + Urological	106 + 223	33 + 51	73 + 172
Total	329	84	245

Figure 4.1: Flow diagram of data sources for identification of TPAs

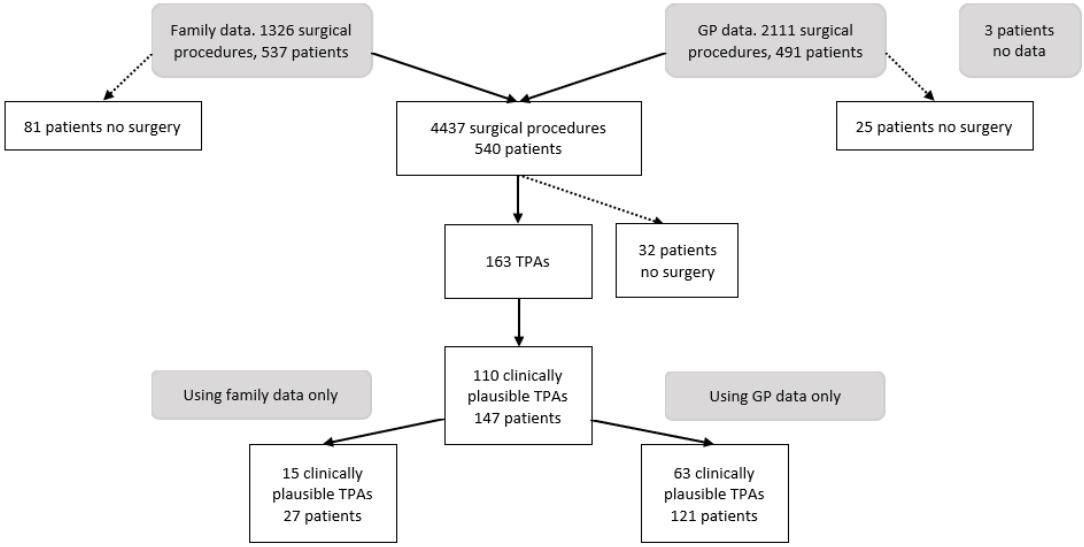


Figure 4.1, above, illustrates the numbers of procedures and patients and their data sources, and the total number of TPAs. The numbers of patients with no surgery do not sum as some of those with no family reported surgical history have data from the GP questionnaire, and *vice versa*.

Procedures and TPAs from both data sources:

163 potential TPAs were identified from the 4437 procedures, reducing to 110 after assessment of clinical plausibility (see below). 147 (27.2%) of the 540 patients for whom data was available were included within one (or more) of these clinically plausible TPAs.

Procedures and TPAs from GP questionnaire only:

3111 procedures were identified, and from this data, 63 clinically plausible TPAs were identified, involving 121 unique patients, 24.6% (1 d.p.) of the 491 patients in the cohort for whom GP record data was available.

Procedures and TPAs from family questionnaire only:

1326 procedures were identified, with 15 TPAs found within this group; these included 27 unique patients, 5.0% (1 d.p.) of the 537 patients for whom family questionnaire data was available.

Exclusion of clinically implausible TPAs

53 TPAs from the combined data set were removed on grounds of clinical implausibility. These 53 exclusions are summarised in Table 4.4, overleaf.

Table 4.4: Exclusion of TPAs on grounds of clinical implausibility.

Surgery Category	No. TPAs excluded	Reasoning
Neurosurgery	2	All procedures performed after symptom onset, at least 3 of 4 using single use items (patients seen by me)
Ophthalmology	4	Each TPA pair includes at least one purely laser (non-contact) surgical procedure
ENT	1	Single use instruments only for nasogastric tube insertions
Abdominal	14	2 TPAs excluded as all 4 patients already symptomatic of CJD 12 TPAs excluded for pairs including 1 endoscopic and 1 open procedure
Orthopaedics	14	1 TPA temporally implausible (second procedure after symptom onset) 13 TPA pairs with 1 arthroscopic and 1 open procedure
Gynaecology	10	Each pair includes 1 purely colposcopic/ hysteroscopic and 1 open procedure
Cardiology /Cardiothoracics	7	1 TPA temporally implausible (second procedure after symptom onset) 6 TPA pairs with 1 angiographic and 1 open procedure
Dermatology	1	Laser skin procedure (non-contact)

TPAs by surgical specialty:

Table 4.5 (overleaf): Number of surgical procedures by category and time-place associations. I have presented the results for time-place associations using data from both family and GP questionnaire sources, then using data only from the family questionnaire, and also using data only from the family questionnaire. The rationale for this is explained in the discussion section. I have also attempted to represent the number of TPAs per surgical procedure in each category graphically, and have included Figure 4.2 showing TPAs using the combined data source, and Figure 4.3 showing TPAs using the GP data only. The equivalent chart using family data is included in Appendix 4 for completeness, but is of limited value to subsequent interpretation in the discussion.

The majority of TPAs occurred in the abdominal and gynaecological surgery categories: 55 and 26 (respectively) of the 110 associations from the combined data (see Figure 4.2); and 35 and 17 (respectively) of the 63 TPAs when using the GP records only (see Figure 4.3).

Table 4.5 Procedures and TPAs by category

Surgery category	Number of procedures (% of total 1 d.p.)	Number from family questionnaire (% of total 1 d.p.)	Number from GP records (% of total 1 d.p.)	Time-place associations (TPA)	TPA after exclusion (clinical improbability)	Plausible TPA from family questionnaire data only	Plausible TPA from GP record data only
Neurosurgery	27(0.6)	14 (1.1)	13 (0.4)	2	0	0	0
Ophthalmology	245 (5.5)	84 (6.3)	161 (5.2)	5	1	1	0
Ear + Nose and throat + Tonsils/adenoids	242 (5.5)	89 (6.7)	153 (4.9)	2	1	0	1
Abdominal (GI) + Appendicectomy	1040 (23.4)	250 (18.9)	790 (25.4)	69	55	5	35
Orthopaedic + Carpal tunnel + Spinal	614 (13.8)	268 (20.2)	346 (11.1)	22	8	3	2
Gynaecology	761 (17.2)	193 (14.6)	568 (18.3)	36	26	1	17
Transplant	15 (0.3)	2 (0.2)	13 (0.4)	0	0	0	0
Cardiology /cardiothoracic – Vascular	152 (3.4)	56 (4.1)	96 (3.1)	9	2	2	0
Dermatology/ minor lumps & bumps + Plastics + Stitches	662 (14.9)	171 (12.9)	491 (15.8)	13	12	2	7
Varicose veins + Vascular	75 (1.7)	25 (1.9)	50 (1.6)	0	0	0	0
Vasectomy/ testicular + Urological	329 (7.4)	84 (6.3)	245 (7.9)	5	5	1	1
Breast	96 (2.2)	34 (2.6)	62 (2.0)	0	0	0	0
Maxillofacial	40 (0.9)	13 (1.0)	27 (0.9)	0	0	0	0
Other	137 (3.1)	41 (3.1)	96 (3.1)	0	0	0	0
Surgery type not known	2 (0.0)	2 (0.2)	0 (0.0)	0	0	0	0
Total	4437	1326	3111	163	110	15	63

Figure 4.2: Percentages of procedures and TPA by surgery type from both data sources, sorted by frequency of surgical procedure. Percentages based off 4435 procedures (2 unknown procedures excluded) and 110 plausible TPAs.

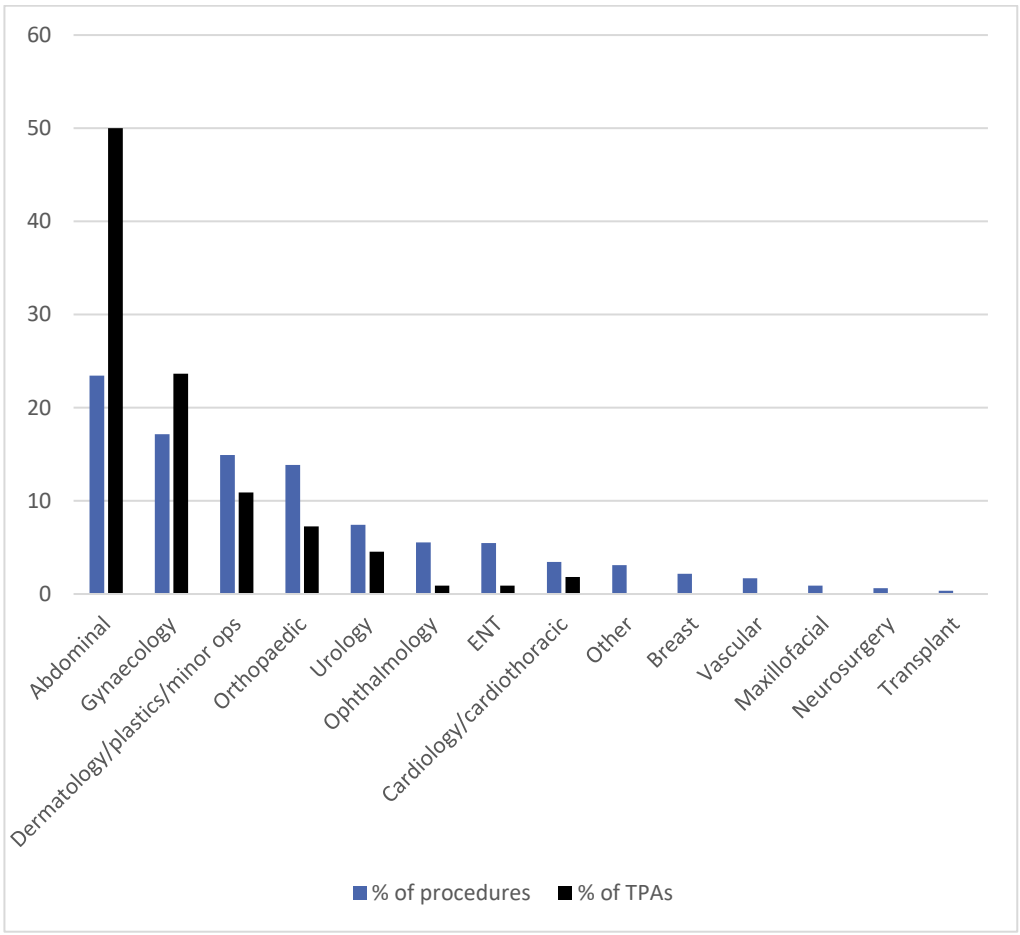
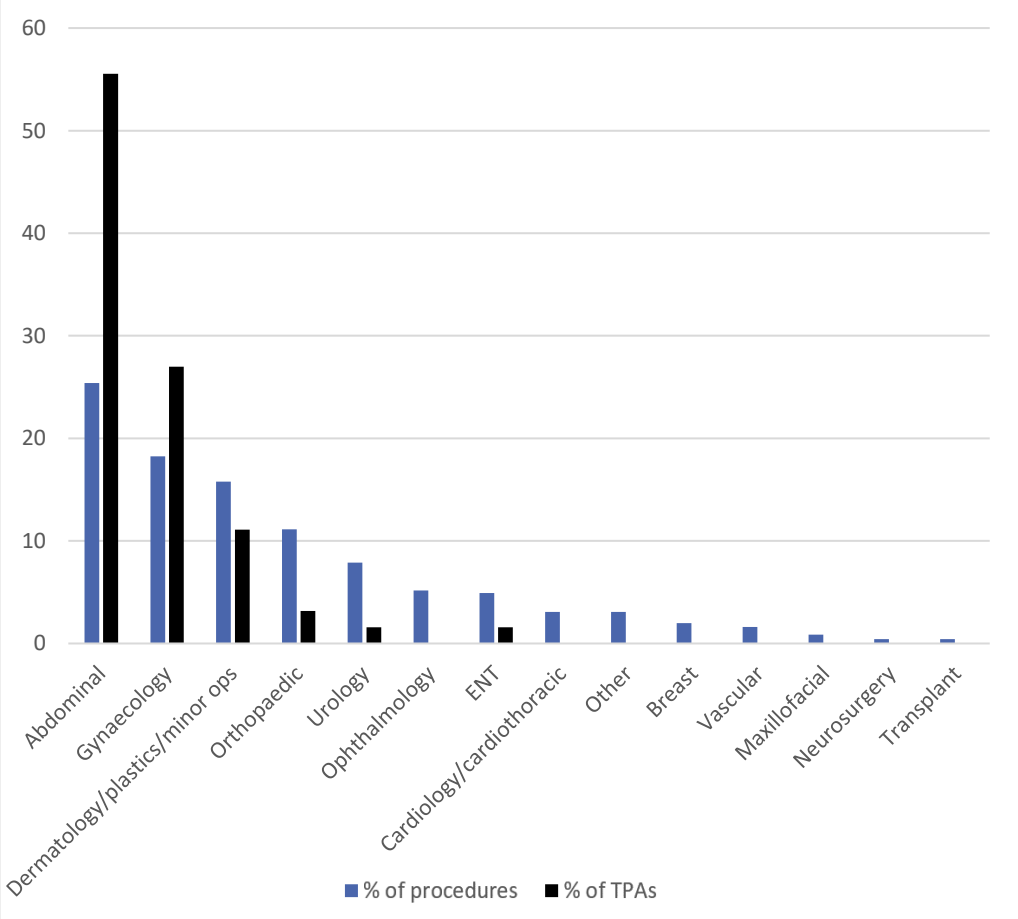


Figure 4.3: Percentages of procedures and TPA by surgery type from both data sources, sorted by frequency of surgical procedure. Percentages based off 3111 procedures and 63 plausible TPAs.

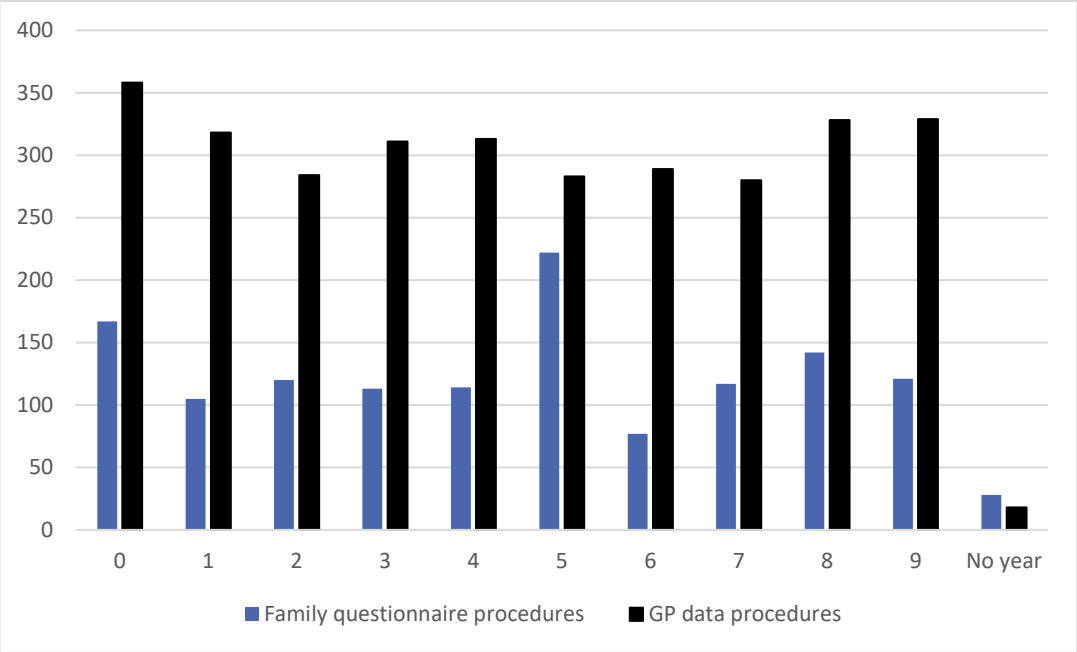


Family questionnaire vs GP record year reporting:

Plotting the number of procedures by the final digit of the year for both GP and family data in Figure 4.4, overleaf, shows that family reported surgical procedures (blue bars) were found at higher frequencies in years ending 0 and 5. The data from GP records (black bars) is more even, without such clear peaks at these final digits. This provides some evidence that the family reported year variables may be inaccurate, calling into question their utility when considering TPAs where year accuracy is critical when looking for overlaps. The numerical values can be found

in a table in Appendix 4. As a minor aside, in the NCJDRSU database, the year 9999 is input when no date is available – on first plotting this data, I failed to remove these to the "no year given" row, causing a substantial skewed in the digit distribution with an unexpected high frequency of 9, which was then corrected before creation of Figure 4.4 below.

Figure 4.4: Number of procedures by final digit of year



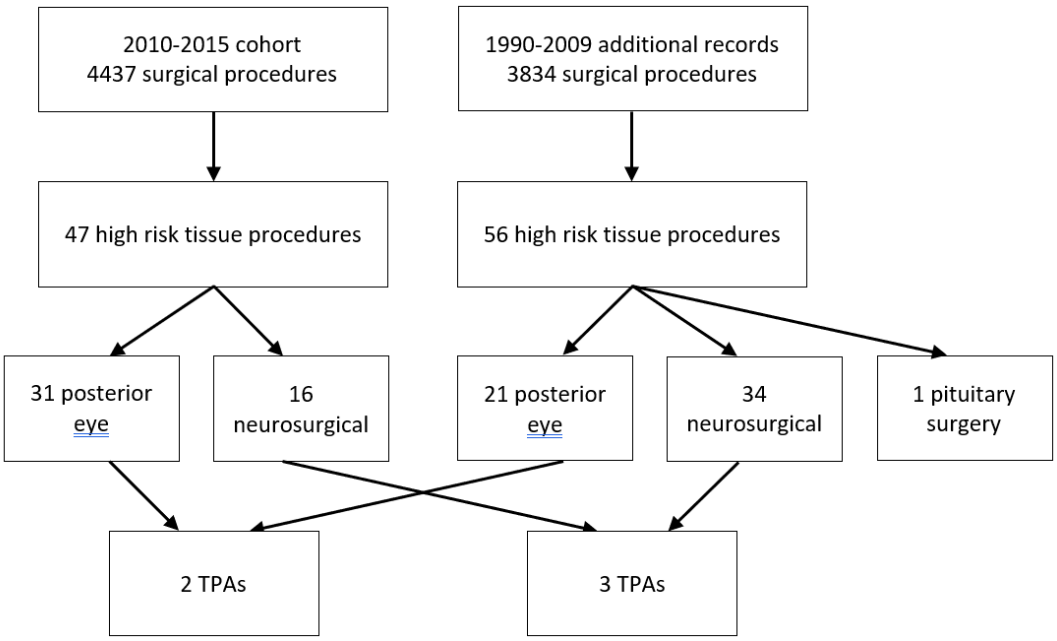
These peaks at years ending 0 and 5 are likely to have occurred due to the recording of family provided information at the time of the NCJDRSU interview. Based on observations I made while collecting around 250 such records while working in the NCJDRSU, families often report a surgical procedure happened in a particular decade, which would then be recorded by the mid-point of the decade at a year ending 5, or otherwise gravitate towards the beginning of a decade as a more memorable year. It seems this has occurred for around 150 of the 1326 (11.3%) surgical procedures from the family data. There is no way to isolate these estimate

years from the other data extracted from the NCJDRSU database with the information I have available. In some (but not all) cases, the recording clinician may document this family uncertainty, and a review of the family questionnaires to look at all 389 procedures recorded in years ending 0 or 5 might allow the selective removal of some of these estimates, but undoubtedly others will be missed as they will have simply been recorded as that year, without any comment or explanation. Since it is not possible to know which 0 and 5 year family reported procedures are accurate and which are estimates, it is very unclear whether or not the family data should indeed be used for assessment of TPAs. This is discussed further in the discussion section of this chapter.

Supplemental analysis of high risk tissue surgeries:

47 procedures involving high risk tissues were identified from the 2010-2015 cohort; 31 were ophthalmological procedures involving posterior eye structures, while 16 were neurosurgical. From the earlier group (1990-2009), a further 3834 operations were extracted from the NCJDRSU database, from a mixture of data collected from families and from GP notes for those individuals for whom these were accessed. Among these 3834 operations, 56 additional high risk tissue operations were identified, 34 were neurosurgical, one case of pituitary surgery was found (classified within the "other" category on the database, although it would be arguably more appropriate for this to be considered as a neurosurgical procedure), and 21 ophthalmological high risk surgeries were identified. There were 5 surgical time-place associations identified among this group. 2 of these associations related to retinal (ophthalmological) surgery, and the other 3 were neurosurgical. These figures are illustrated in Figure 4.5 overleaf. Details about these associations follow in the discussion section, including consideration of potential future work such as sensitivity analysis.

Figure 4.5: Flow chart of high risk surgical procedures and TPAs in this subset.



Discussion

Considerations of data quality and completeness:

Although efforts were made to ensure the best possible completeness of data for this work, these efforts met with only moderate success. We had both GP and family questionnaire data for only 488 of 579 cases (84.2%), and GP records were received for 491 of 579 patients (84.8%).

There are several reasons for this incompleteness, including families declining to meet with the NCJDRSU visiting clinician; families meeting with the clinician, but requesting not to complete (or subsequent destruction after completion of) the questionnaire; or, in cases of CJD patients dying before a visit was possible if the family then decline a posthumous visit when contacted in due course (some families do not wish to engage with the NCJDRSU, especially without any earlier awareness of our unit during the life of their loved one). The process of accessing the GP record may also have contributed to issues with completeness. Access to the GP record is usually only possible after the death of the patient, and this requires tracking the notes. The NCJDRSU will first contact the registered practice, and an issue can arise if the family gives the incorrect GP practice details. The local practice also usually holds onto the notes for only a short time after the death of the patient, after which they are forwarded to the medical records department of the local trust/health board, or a third party organisation on their behalf. If the GP practice does not hold the records, then it may take considerably longer to obtain the records, if this is possible at all, adding data lag. On occasions, the process goes awry, and since we are reliant on external bodies or individuals in helping us to access these records, we may not then be able to obtain further information. There can also be a considerable delay in sending the records through to us, which in part is

administrative, as photocopies are usually sent rather than the original notes, after invoices and payment are arranged to cover these costs, but issues relating to comprehension of the law and information governance issues can also add considerable delay. It is likely that a few of the outstanding GP records will have arrived after we closed the data acquisition phase of this work, although the majority may never be received.

For the patients for whom we had GP and/or family data, 89.6% of the reported procedures had sufficient detail to be usable for assessment of time-place associations, caused by 46 procedures not listing a year, 443 procedures listed in unknown or unidentifiable hospitals, and 2 procedures listed by the family of unknown type. At first, it might seem surprising that the family questionnaire (a verbal, recalled survey) yields a higher percentage of data with entries in each field (92.4%) – and therefore usable for TPA assessment – than the data extracted from the GP notes (88.5%). The difference is not vast, but is likely to occur in part from some creative guesswork on the part of the individual being interviewed – once they recall that their relative has had a procedure, they may be happy to make (educated) guesses about roughly when the procedure occurred and at which hospital.

Based on my experiences while collecting this data, such guesses about dates often generates ranges or estimates like "early-", "mid-" or "late-" [decade], or "some point in the 90's" (*etc*.). Guesses about hospitals usually centre around where the individual affected was living around that time – families may simply choose the local hospital. Such guesswork by families raises concern about the validity of any subsequent assessment of time-place association using the family questionnaire data. The issue is illustrated by Figure 4.4 (with the data in Appendix 4), which shows the final digit of each year in which every procedure reportedly occurred.

The family questionnaire data shows an outlying high frequency of procedures performed in a year ending 5 – a value which would be entered by the interviewer (or at the point of data entry into the NCJDRSU database) if a family recalled that a surgery had been performed in the middle of a decade. A smaller peak is identifiable for 0, another commonly selected year for such guesses. These peaks indicate that perhaps 150 family recalled procedures may be incorrectly recorded in a year ending 0 or 5.

Further concern about the validity of the family questionnaire data for this purpose is raised in light of the substantially lower number of procedures identified from recall at interview when compared with data from GP notes. Family questionnaire procedures makes up only 29.9% of the total number of procedures (4437) in the studied cohort. This occurred despite having family questionnaires for 46 more patients than the number of patients for whom GP record data is available.

The GP data is unlikely to have false procedures included in a patient's medical record, although a few procedures may be omitted by error, and on occasions the GP record may be incomplete (such as when a patient moves practice but their record fails to follow them). For the GP data set, incomplete entries (i.e. those where we lack year, hospital or procedure name details) occurred more frequently with historically older procedures, usually recorded in handwritten entries in original small page size paper notes. Clinician handwriting is notorious, and this was also a small factor. While GP surgeries have now moved to an electronic patient record, efforts to transfer the original paper notes are of varying quality, and in some cases have not occurred. Entries recorded in the GP notes about surgical procedures must have filtered through several sources, and could be reliant on the GP surgery receiving a discharge letter from the hospital, someone at the GP surgery entering this onto the record, and either the original record being available to us at the

NCJDRSU, or for the practice to have transcribed that record to the electronic one. This chain of events is reasonably complex, with the opportunity for human error. The entries in the handwritten notes not infrequently skip pertinent facts such as the name of the hospital, as such detail may have been either obvious or unnecessary at the time of the recording if a particular surgery only referred patients to a single hospital.

Human error also plays a part in the accuracy of data input at the NCJDRSU, since extraction from the data sources is not error free. We make efforts to code procedures using the standard template, but certain procedures have been miscoded, or included typographic errors. Such errors are likely to have affected both family questionnaire and GP record entries into the database equally, and while coding errors and hospital/town name typographic errors were corrected where identified (and corrections were fed back into the NCJDRSU database for any future use), any such mistake on year entry would have been overlooked if the incorrect entry was a plausible year. This sort of error is unavoidable unless all health records are perfectly digitised.

Implications of missing data:

Since the hypothesis I intended to explore with this work was that a small number of suspected sCJD patients may in fact have developed a form of iCJD due to exposure to infective prion material through surgical instruments. Since only very small numbers of cases of such transmission have been reported (91, 94), if this occurs, it is likely to be a rare event. More than 15% of GP records are missing, and within the available data, more than 10% of reported procedures lack sufficient detail to be used in analysis looking for TPAs. As such, if any such transmission had occurred within the studied cohort, this work may well have missed it.

Decisions on utility of potentially inaccurate data sources and duplicate procedures:

The concerns regarding accuracy of the family questionnaire data highlighted above draw into question its use in attempting to identify associations which are reliant on accurate time entry (albeit, only recorded to the nearest year). Ideally, an attempt to quantify the completeness and accuracy of each data source would allow clearer decisions regarding whether or not to use that data. Such quantification would require a "gold standard" third data set, after which statistical comparison could be made. One possible such standard is the Information Services Division Scotland (http://www.isdscotland.org/), who keep a surgical register for Scottish patients; if accessed, this would provide a small sample of the 579 patients in the 2010-2015 cohort with a definitive list of surgical procedures, so long as those procedures were performed in Scotland. Further investigation of these data was beyond the scope of this thesis, but is being taken forward separately as part of enhanced surveillance activities at the NCJDRSU.

Given the suspicion of inaccuracy of the family data, I presented the results of procedures and time-place associations in the results section above in triplicate in Table 4.4, and graphically for the combined data set of 4437 procedures which has generated 110 plausible TPAs, a number likely to include at least some spurious TPAs generated by inaccurate family recollection of procedure details (Figure 4.2). The GP record only data set of 3111 procedures is suspected to be more accurate, and the 63 TPAs identified are more likely to have actually occurred (Figure 4.3).

It is interesting to note on comparing the family and GP sources that the number of procedures in each category is consistently lower, other than for neurosurgery. This probably represents a recall bias of neurosurgery among patient families, and also

highlights that a large amount is forgotten; as a clinician, it is surprising what patients may not recall about their own past medical history, and relying on a family member adds an additional factor (this was one source of bias in several of the epidemiology studies discussed by López *et al.* (226)). The neurosurgical figures were very close in number between both GP (13 reported procedures) and family (14 reported procedures) sources, but even with this close number, attempts to identify pairs of the identical procedures reported by both sources was unreliable, mostly due to variability on year of procedure between the two sources, preventing any attempt to remove duplicate entries from the extracted procedure list when using the combined dataset in looking for TPAs. I had initially considered attempting to remove duplicate entries from the entire data set for each surgical category, to try to prevent a single procedure from flagging as two TPAs if the details were incorrect from one data source, but with the decision largely to discount the family questionnaire data, this is less relevant.

Surgical time-place associations within 2010-2015 cohort:

The two neurosurgical associations identified (in the mixed TPA analysis) were both brain biopsies performed in large, regional neurosciences centres; for each of the four procedures involved in these two associations, the biopsy was performed in an already symptomatic patient where CJD was considered high in the differential diagnosis. Since the biopsies were performed after symptom onset in each case, none of these patients could theoretically have contracted CJD by instrument contamination from each other (they each already had the disease) meaning these biopsies are not clinically plausible for transmission of CJD. Furthermore, with CJD considered in the differential, the operating neurosurgeon should have used single use surgical instruments, or quarantined the instruments pending their subsequent destruction after neuropathological confirmation of the diagnosis, as per ACDP guidelines (228), although occasions where this has not happened have occurred

(229). I reviewed 3 of the 4 patients involved in these 2 implausible TPAs (Table 4.4); in each of those three cases, the brain biopsy was performed with a high clinical suspicion of CJD, in large regional neurosciences centres. During my visits to the patients, I discussed with the local Neurology team, including ensuring that appropriate health protection measures had been taken during the biopsy. Such procedures involving high risk tissues in known sCJD patients are followed up by the local health protection team following the NCJDRSU visit to ensure appropriate protocols are followed to prevent onward transmission (230).

None of the ophthalmological associations (either plausible or implausible) involved invasive surgery of the posterior eye, which might be regarded as high risk in terms of tissue infectivity in a patient with CJD. 4 of the 5 were clinically implausible (Table 4.4), mostly involving non-invasive procedures such as laser macular surgery "paired" with more invasive techniques requiring penetrating surgery – such "pairings" will not have shared instruments and were discounted as clinically implausible.

For the two associations involving ear, nose and throat procedures, one was a pair of naso-gastric tube insertions, both performed after symptom onset (for dysphagia approaching the end of the natural disease course of sCJD), and would have used single use items only – this TPA was discounted on grounds of clinical implausibility (Table 4.4); the other two operations were both tonsillectomies and are clinically plausible. However, tonsillar tissue does not include olfactory epithelium – the only ENT tissue considered a medium risk tissue in sCJD (89).

All other (non-discussed) associations involved low risk tissues. The distribution among the different surgical categories broadly followed the frequency of that

category occurring in the procedure list, as can be seen in the charts. Those categories where TPAs were not identified were all categories with a smaller proportion of the total number of procedures (whether considering the mixed, GP-only, or family-only data).

As would be expected, limiting the data to look at GP (or family) data only (rather than using the mixed data set) not only reduces the number of procedures, but also substantially reduces the number of identified associations. While there are too few data points to extrapolate a clear trend, it is likely that the number of TPAs would not follow a linear relationship with the number of procedures. Indeed, such a relationship is indicated in Figures 4.2 and 4.3, as well as Appendix 4, where those categories with more surgical procedures have a substantially higher proportion of TPAs. Increasing the number of procedures increases the complexity of the system, and therefore the number of potential interconnections between individuals. For example, although the family questionnaire comprises 29.9% of the total number of procedures (1326 of 4437), when used in isolation to look for TPAs, it generates only 13.6% of clinically plausible TPAs (15 of 110). The GP data comprises 70.1% of the total number of procedures (3111 of 4437), and generates 57.3% of the plausible TPAs (63 of 110). The remainder require a connection between one GP record procedure and one family questionnaire procedure. This concept is similar to the "birthday paradox", where the probability of at least two individuals among a random sample sharing a single birthday reaches 100% when 367 people are sampled (to allow for leap year 29th February birthdays), 99.9% when only 70 individuals are sampled, and 50% for 23 people. Whether or not any TPAs genuinely reflect surgical transmission of sCJD, there are likely to be chance associations where two patients underwent similar surgical procedures within the same hospital within the same or adjacent years, and these encounters become more likely as the sample size increases, whether from using data from multiple sources, or by increasing the size of the studied cohort.

Prior to completing the analysis for TPAs, and on considering some of the surgical histories I obtained visiting individual patients, I anticipated that I might see many TPAs generated by particular individuals who underwent very frequent surgical procedures. This was not supported by the observed results. From the mixed data set, the 110 TPAs involved 147 individuals, the 63 associations from the GP record set involve 121 individuals, and the 15 TPAs in the family questionnaire set involve 27 individuals. Also of interest, the individual who underwent 52 GP data reported surgical procedures did not feature in any of the identified TPAs. Of course, if CJD was transferred through surgery, each such association could only transmit CJD in one direction (an individual could not both be the donor and recipient of PrP^{Sc} in the same procedure), so the maximum possible number of individuals involved in TPAs who could have developed sCJD as a consequence of iatrogenic exposure to instruments contaminated by another patient in this cohort must be no more than half of these figures.

The majority of associations identified were seen in abdominal (55 of 110 (50%); or 35 of 63 (55.6% 1d.p.) using GP data only) and gynaecological surgery categories (26 of 110 (23.6% 1 d.p.); or 17 of 63 (27.0% 1 d.p.) using GP data only). On reviewing these associations, a large proportion of the abdominal TPAs involve endoscopic abdominal procedures: 41 of the 55 (74.5% 1 d.p.) abdominal TPAs involved only such procedures, or 29 of 35 (82.9% 1 d.p.) using the GP data only. By their nature, these are common interventions, performed as either diagnostic or therapeutic procedures. If an abnormality is identified then frequently these lead to serial surveillance procedures, or successive treatment procedures of the same intervention after a fixed time window. Among the gynaecological TPAs, a smaller proportion (5 of 26 (19.2% 1 d.p.) TPAs from both data sources, or 5 of 17 (29.4% 1 d.p.) when using only GP data) consisted solely of hysteroscopic procedure pairs.

Neither the gut lumen nor female reproductive tract is regarded as a high risk tissue for sCJD (89), and no prior epidemiological study has identified endoscopic procedures as risk factors for the development of CJD (226). Ward *et al.* did find an association between gynaecological surgery and sCJD (231), but the authors comment these findings may be affected by bias, and the rarity of CJD diagnoses; subsequently, this finding has not been replicated. It may be the case that the work in this chapter has simply identified surgeries likely to happen repetitively. Nevertheless, endoscopes are difficult to decontaminate (232, 233), something which is acknowledged by the British Society of Gastroenterology who recommend that endoscopes used on variant CJD patients should be destroyed, or quarantined only to be used again on that individual (234), and their potential as an infective route of transmission of CJD should not be discounted.

<u>High risk tissue space-time associations from 1990 to 2015:</u>

The additional 3834 (see Figure 4.5) procedures identified from the 20-year window of 1990 to 2009 inclusive seems unexpectedly low, compared to the number of procedures – 4437 – obtained from the 6 year period of the studied cohort. This has occurred for several reasons. The primary factor is that GP records were not collected by the NCJDRSU prior to 2000; given the observation from the 2010-2015 cohort that the GP record contributed approximately three times as many surgical procedures compared to the family data (there is no obvious reason this should be substantially different in the 1990-2000 cohort), this is likely to be the most significant contributor. Even after 2000, earlier efforts to chase outstanding GP records were not as comprehensive as the attempt to ensure data completeness for this study. Another, smaller factor is that referral numbers to the NCJDRSU were lower in the first few years of the Unit, and increased rapidly throughout the UK vCJD epidemic; full details can be found in the NCJDRSU annual report (235), but I have also included the graph of referrals (not all of which were patients with CJD)

to the NCJDRSU by year in Appendix 6 to show the trends. The number of confirmed or definite cases of CJD seen by the NCJDRSU have increased steadily until 2003, before dipping and later climbing again, reflecting the peak of the vCJD epidemic. In more recent years, the increasing number of referrals may reflect a greater investigation of dementia, more ready access to MRI and other diagnostic tests, and greater awareness of CJD following vCJD; the gradual growth of the population will also have contributed marginally, with more individuals under surveillance.

Among the 3 neurosurgical associations listed in Figure 4.5, 2 were the clinically implausible associations of brain biopsies performed after symptom onset which were discussed above, and only involved patients within the 2010-2015 cohort. The 1 remaining neurosurgical association included 3 patients, 2 from the 1990-2009 group, and 1 from the studied 2010-2015 cohort; 2 of the 3 patients underwent only brain biopsy, after symptom onset, and as a diagnostic procedure, one in 2006, and the other in 2007; since they were both symptomatic, they cannot have conveyed CJD to each other. It is likely that appropriate instrument quarantine or destruction was followed, but we cannot be completely certain that this was the case, as this has happened in the past (229). These two patients were referred to the NCJDRSU, so CJD must have been suspected at the time of the biopsy. Patients seen by the NCJDRSU are referred to the local public health office at the time of review so that issues such as these can be followed up. The 3rd patient (from the 2010-2015 cohort) underwent neurosurgical removal of a meningioma in 2008, within a one year range of the second patient's brain biopsy. If appropriate infection control procedures were followed (228), overlap of instruments with those used for the second patient's brain biopsy would then be unlikely; furthermore, this individual became symptomatic more than 5 years after the neurosurgical procedure, considerably longer than the reported incubation periods for neurosurgical transmitted iCJD (73). Nevertheless, I have highlighted these patients to my supervisors (since I have left

the NCJDRSU), in case any additional efforts to clarify instrument usage can be made. None of the neurosurgical TPAs include Middlesbrough General Hospital, the hospital implicated in the past CJD brain biopsy incident mentioned above (229) (in any case, those potential recipients have been informed they are at risk of developing CJD, and I understand are under enhanced surveillance by Public Health England).

The 2 eye surgery associations both occurred across adjacent years (i.e. first surgery in year X, the second in year X+1). 1 of the 2 occurred in a clinically implausible order on the basis of time – i.e. the second procedure was performed upon a patient who became symptomatic of sCJD within a year of the procedure, and more than 10 years before the first patient in the TPA pair (who underwent the earlier procedure and would therefore be the potential donor). This association is highly unlikely to be of clinical relevance, as it would be expected that a donor expressing PrP^{Sc} to contaminate surgical instruments must themselves be further along pathogenesis of CJD than a recipient of a minute dosage of PrP^{Sc} adherent to said instruments. If this was to represent iatrogenic transmission, at the point of contact with the instruments, the recipient would only then be beginning the cascade of PrP^{C} transformation leading to onset of clinical symptoms. It seems highly unlikely that the recipient could manifest the disease and die 10 years earlier than the donor. No such reversal of order of presentation has been identified in other, known cases of iatrogenic transmission of CJD (2, 73, 91, 94).

The other ophthalmological association occurred in a temporally plausible order, with the first surgery occurring 7 years prior to symptom onset in the patient who became symptomatic first, with the second patient becoming symptomatic 15 years after the surgery. This is the only identified high risk tissue space-time association which could be of relevance in potential iatrogenic transmission of sCJD from this

work, but it is impossible to know whether this TPA represents any more than a chance encounter, and this long after the event, it is highly unlikely that it will be possible to acquire any potentially contaminated instruments for analysis, if any instruments were indeed shared between the two procedures. The only known ophthalmological transmission of CJD has occurred in the context of corneal transplantation, and is discussed in Chapter 3; the incubation period from corneal transplant associated CJD is believed to be between 1-30 years – see Table 3.1 (179). Nevertheless, I have highlighted the individuals involved in this TPA to colleagues in the NCJDRSU to try to see what records may still remain, and whether this can be taken any further.

The number of these high risk surgical procedures is sufficiently small to allow a sensitivity analysis using the full date of the procedure to explore what effect using a smaller time window than +/- 1 year would have on identifying TPAs. This is of relevance given the significantly shorter time interval identified in existing neurosurgical and EEG transmissions of CJD, which is more in the range of weeks to months, rather than in the same or adjacent years (91, 94). Equally possible would be using a longer time period, something that was not possible using the full 2010-2015 cohort, due an such change substantially increasing the complexity of the interconnections when using the larger data set. The NCJDRSU database (and my extracted data set) does not include the exact date of any procedure, only the year, and the level of accuracy required for such an analysis is therefore not available to me at this stage. This would be an area of potential further work, to look through the GP records (and any available hospital notes) to see whether exact procedure date could be identified for these procedures, and then proceed to such analysis.

Limitations of the surgical time-place association method:

The absence of control data dramatically affects this study. Without control data, it is impossible to know whether the observed number of associations are likely to have occurred simply by chance, and no statistical analysis of the collected data is possible. Surgical time-place association is a surrogate metric which identifies potential linkage between cases, the results cannot indicate any firm epidemiological connection. Without a control group, this surrogate is of some interest, but does not represent a robust tool; the collection of valid control data to compare would improve its utility, and would turn this into a case-control study. Such control data would require age and sex matched controls, collected regionally (perhaps in the same post code region as the individual), and then access to those individuals' GP records. This access would allow retrospective data collection, in a manner similar to the access to GP records of the sCJD cases, reducing potential bias. It would not be as easy to collect family questionnaire data which would be comparable to the studied cohort in this work, unless the family data and a new cohort of patients were collected prospectively, otherwise this would be reliant on recall significantly after the event for only the control group, and thereby introducing significant bias. When the concept of identifying linkage through surgery was first discussed, my supervisors had suggested I use historical control data collected for earlier NCJDRSU case control studies (19, 42, 231). However, the historical nature of this control data means it is not a valid comparator, and any attempt to use this as a control for the 2010-2015 cohort would be invalid; this idea was discounted. The decision was agreed to attempt this TPA analysis so that we could attempt to use the collected NCJDRSU data to look for surgical linkage, accepting that the methodological limitations would prevent any hard epidemiological evidence from being identified.

The 6-year (January 2010 to December 2015 inclusive) time window is a further limitation of the study. The greatest theoretical infectivity of a patient with CJD must occur at the point when they have the highest load of abnormally folded PrP^{Sc}; this is offset by clinical presentation and subsequent diagnosis, after which efforts would be made to avoid surgery or prevent onward transmission. The greatest risk of onward transmission would therefore occur prior to disease diagnosis (225). If infected, any individual undergoing surgery from contaminated instruments could take years before they too became symptomatic. Since it is not known what the incubation period would be for CJD transmitted by surgical instruments (other than in the reported cases of known neurosurgical transmission), we must consider the range associated with iatrogenic transmitted CJD, which lies between 19 months and 40 years (75). The 6 year inclusive date range was used to ensure the data set and time-place association process was workable, but limits the capability of this study to demonstrate clinically relevant associations for the majority of the cases – peripheral inoculation of small doses of PrP^{Sc} on contaminated surgical instruments would be unlikely to cause symptom onset in the recipient at the lower end of the range of incubation periods (the lower range were associated with cranial implantation of contaminated cadaveric dura mater grafts).

Due to the non-linear association between number of procedures and TPAs, expanding this method to cover the complete NCJDRSU patient cohort would be likely to break the (already labour intensive) time-place association method, while the quality (and variability) of the stored data prevents using an automated process with this method. As was evident in the high risk tissue assessment which included the 1990 to 2009 patients, earlier NCJDRSU records are less complete than those from the later cohort; it is also possible (depending on the incubation period) that perhaps we have not yet been collecting case histories in the NCJDRSU for long enough to identify iatrogenic transmission by surgical instruments – if this occurred around the 40 year mark in non-CNS exposures to sCJD PrP^{Sc}, we might start to see

such transmission from around 2030, if the NCJDRSU is still collecting such data at that point.

The method may be susceptible to bias from non-surgical spatiotemporal clustering. Prior studies, such as that by Linsell *et* al. (27) have indicated potential overlaps between sCJD patients, particularly when looking many years prior to onset of symptoms, and potentially hinting at an environmental trigger for the condition. If there was environmental clustering, then patients who lived in proximity would be likely to be treated at the same hospitals, potentially leading to identification of additional TPAs without any surgical connection. Not all such studies have had such positive results; see also the following: (236-238)

Potential future studies:

I discussed earlier that the observation of time-place associations was a surrogate metric, representing the possible reuse of surgical instruments. It is not known how many of these TPAs actually involved any instruments being reused across more than one patient. If it were possible for the NCJDRSU to access data concerning tracking of instruments (which should be possible, particularly since the NICE guidance in 2006 strengthening traceability of high risk instruments (239)), a better study could be performed, using details of each individual instrument, such as: when it was used, in which procedure, and on which particular CJD patient. This would allow prospective development of a database of potentially contaminated instruments. If an instrument could be connected to two sCJD patients this would be a far more tangible association and better epidemiological support of potential iatrogenic transmission. It would then be necessary to explore where that instrument had been used previously, what sterilisation the instrument had undergone between patients, and whether any other individuals had also been exposed. Such a structure would be similar to that employed in the Transfusion

Medicine Epidemiology Review (TMER) (1, 2), although the numbers of instruments could be substantially higher than the number of blood and blood products tracked by the TMER.

A very similar process is already undertaken by Public Health England (PHE) who monitor patients exposed to potentially contaminated instruments from high risk sources (230, 240), although this could potentially be extended to procedures involving medium and low risk tissues. Again, there are similarities to the TMER which both looks at the outcome of every donated blood product from a CJD patient (equivalent to the activities of PHE), and the source of every donated blood product to a CJD patient – this potential study could look for connections in cases where instruments were not suspected to have been contaminated through contact. If each instrument was allocated a unique identifier at a national level such a repeat study could be entirely automated, removing a great deal of the human error inherent in this study. Such a process might require a national NHS IT system, or at least greater collaboration of sterile services departments, infection control teams, public health bodies and the NCJDRSU.

On a local level, instrument tracking using barcodes and RFID tags does occur in certain centres, and is an attractive source of potential efficiency savings, since the tracking process can be automated (241). Much of the literature relating to this has been published by the manufacturers of the barcode or RFID systems, but such material is still illustrative (242). Similar procedures are being considered by Health Facilities Scotland, and if developed, a pilot study looking only at Scottish CJD cases could be undertaken as an intermediate future work, perhaps as a proof of concept initially. I have not attempted to take this further for the purpose of this

thesis, as the primary objective was to explore the existing NCJDRSU data set to which I had contributed while working in the NCJDRSU.

Conclusions

This study has identified occasions where sCJD patients in the 2010-2015 cohort may potentially have encountered surgical instruments which were used on another sCJD patient. These occasions appear to occur disproportionately among gastrointestinal endoscopic procedures, something not previously reported in case control studies, but there are several potential sources of bias which may have triggered this. It is suspected that this method simply highlights those procedures performed repeatedly.

The limitations of this study, in particular the absence of a control group, prohibit the formation of any strong conclusions. It does show that many sCJD cases can be linked together by past surgery overlapping in space and time, and that such overlaps are not infrequent. Although there is no evidence to suggest any of the cases studied were linked epidemiologically, I cannot rule out the possibility that this may have occurred, perhaps in association with other CJD cases who were not part of the 2010-2015 cohort.

Two potential high risk tissue connections have been identified between the 2010-2015 cohort, and those patients seen by the NCJDRUS between 1990 and 2010. One of these occurred through neurosurgery, but if appropriate ACDP guidelines were followed, this would not convey a risk of surgical transmission, and the interval between the surgical connection would be considerably longer than the incubation period reported from other neurosurgical instrument CJD transmission (although much wider ranges of incubation period are reported in other forms of iatrogenic CJD, such as dura mater grafting); as such, this connection was deemed unlikely to be plausible. Another high risk tissue connection was identified among

ophthalmolgical surgical procedures, with symptom onset in the potential recipient 7 years after the surgery; this overlap could be plausible, but its significance cannot be determined.

Having reviewed around 250 patients during my tenure in the NCJDRSU, and completed questionnaires for each of these patients (many of which are included this data set), it is of immense frustration that the collected data is of such little utility. Further efforts to obtain appropriate control data, and then subsequent statistical analysis would improve the utility of this data.

Thesis Conclusions

This thesis comprises an introduction (Chapter 1) containing a summary of the neuroepidemiology of human prion diseases, with some background information concerning relevant animal prion diseases, followed by Chapters 2, 3 and 4 reviewing NCJDRSU collected data concerning potential blood, tissue and organ, and surgical transmission of CJD respectively. The title of this thesis was agreed on discussion with my supervisors at the time of the original research proposal. The original intent of the work was to review the data which is acquired by the NCJDRSU at the time of each visit to every suspected CJD patient across the UK to look for any evidence that at least some of the cases currently classified as definite or probable sporadic CJD might represent acquired transmission of CJD. If such an acquired transmission had occurred, this could have happened through an iatrogenic route of transmission, either through blood transfusion, transfusion of other blood products, tissue or organ transplantation, or through contamination and cross usage of surgical instruments; however, other means of acquired transmission could conceivably be possible, including by diet, or through some unknown environmental transmission. In hindsight, narrowing the title of the thesis to reflect the work performed relating to iatrogenic, rather than environmental, transmission would have been helpful.

I have described the current evidence relating to potential transmission of sporadic CJD in the UK by blood transfusion. Although the first two cases of sporadic CJD have been seen in patients with clotting disorders who have received plasma products, there was no evidence to suggest that their development of sporadic CJD had any relation to their transfusion and blood product history, and it is believed that these two individuals may have developed sCJD by chance. A crude estimate of the incidence of sCJD among patients with clotting disorders suggested we may see other patients in the future. There were significant limitations in accessing data pertaining to the sources of all blood and blood products received by these individuals.

The update to the Transfusion Medicine Epidemiological review has not shown any evidence that sporadic CJD, unlike variant CJD, has been transmitted by blood transfusion. Like the observation of the two patients with clotting disorders, there are significant limitations in availability of data, with large amounts of missing data. If transmission of sCJD by blood was to occur, this could easily be missed among the missing data. The accuracy of tracking of blood products is improving, and the TMER will be repeated in the future to look once more for evidence of sCJD transmission.

The evidence of tissue or organ transplantation associated transmission in the UK in the 2010-2015 definite and probable sCJD cohort is inconclusive. Some individuals have been identified who are suspected to have received tissue grafts, including possibly Lyodura® dura mater grafts, and they could represent iatrogenic transmission of CJD through a known means of transmission. The record availability limits our ability this long after surgical procedures to identify which graft materials were used, and there are limitations associated with the means of data collection in the NCJDRSU (such as the GP record not including sufficient operative detail to know whether tissue allograft material was definitely used). A single patient who underwent 7 corneal transplants could represent corneal transplant iCJD transmission, but there was no evidence among the NCJDRSU data set of any other recipient of another organ from any of the corneal donors to add evidence of potential transmission; there is no information pertaining to the corneal donors. The NCJDRSU lacks a formal process to look into these patients in greater detail, and the development of a structure akin to the TMER might allow greater understanding about the nature of these potential transmissions. While there is no evidence to suggest tissue or organ transplant associated transmission of sporadic CJD in this cohort, there are cases of interest which are of indeterminate significance.

Once again, with considerable missing data, any such transmission of CJD by tissue or organs could easily be overlooked.

The data we collect in the NCJDRSU concerning surgical procedures allowed me to identify multiple potential connections where different sCJD patients underwent surgical procedures within the same surgical domain, within the same hospital, within the same or adjacent year. This assessment of time-place associations highlights the potential for sCJD patients to be connected to one another through surgical instruments, but we lack the ability to track individual instruments – something that would allow far greater understanding of these potential connections. The evidence I have produced is of very limited utility as we lack a suitable control data set to use for comparison. With these limitations, this study cannot lead to any firm conclusions. As instrument tracking becomes increasingly automated, there is the potential for future studies to look into this issue with greater accuracy and relevance, and with less labour intensive sorting. As in the other areas of the thesis, there is considerable missing data, and surgical transmission of sCJD could easily be missed by this method. The NCJDRSU will need to ensure we collect a contemporaneous control data set if any such further assessment is to be performed.

At the time of the original research proposal, it was intended that a fifth chapter (fourth piece of research) would be completed to look for spatiotemporal clustering of patients, as a means to look for an evidence of a potential environmental factor leading to the development of sCJD. This fifth chapter was not completed as I completed my post in the NCJDRSU and returned to clinical neurology before the data could be processed and extracted. On discussion with my supervisors, it was agreed to leave such work for a future study. Such clustering could be performed from the residential address histories routinely collected at the time of the

NCJDRSU clinician visit. Such work would require conversion of the addresses to Ordnance Survey coordinates, and analysis for clustering using SaTScan™, or similar (see https://www.satscan.org/, accessed 05.08.18), looking for clustering of patients both at specific time periods (e.g. calendar year), and for clustering of patients at time intervals prior to symptom onset (e.g. 5, 10, 20 years before onset of symptoms). This would be a follow up study to the paper by Linsell *et al.* (27).

Overall, there is no convincing evidence from this work to suggest that sporadic CJD is an acquired disease, although it is possible that a small number of iatrogenic cases, transmitted by previously established means of transmission, have been missed and classified as sporadic CJD. There is considerable missing data in each section of this work, and it is also possible that the evidence of acquired transmission could be hidden among this missing data, or that such a case might not have been identified as a case of CJD, and thereby not referred to the NCJDRSU.

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Appendix 1: Operation coding document

Coding of ops & other ops – (created February 2006, update July 2010)
Reviewed January 2013 to include new ops

This document has been prepared to provide guidance when coding operations under the main categories used previously in the risk factor questionnaire. This new coding is used with the risk factor form as well as aiding in placing surgery into correct categories on the medical history form and coding of the "other operations" section.

These guidelines are intended to help with: -
1) Coding consistently particularly for those operations that could be coded under more than one category
2) Those of us not medically trained to code frequently occurring operations.

New operations will be added when they occur in the data collected and medical opinion has been consulted as to where to place them.

If two operations are performed at the same time and operation category then these are coded as 1 op, if two ops performed at the same time but different operation categories (and are not listed below) then ask someone with medical training whether these are to be coded as 1 or 2 ops. If in doubt, ask.

Note
Tonsillectomy with any combination of myringotomy\grommets\adenoid code as 1 op under tonsillectomy
Myringotomy\grommets and adenoid code as 1 op under ear
Meckel's diverticulum & appendectomy code as 1 op under appendectomy

Please also note that over time some coding decisions have been made and subsequently removed. These include not counting cryotherapy\cauterisation of warts\growths etc as an op as it was decided that in many instances this would be the treatment but the treatment was not specified in such detail therefore excluding ops where this was specifically mentioned was only excluding a small proportion of the real number. Also, angioplasty was considered to not be an op for a time.

Not currently counted as operations:

- Aspiration\fine needle aspiration at most sites except bone marrow, abdominal\ascites, liver, post-op parotid abscess drainage.
- Only most invasive "open" drains included: pilonidal abscess, uchio-rectal abscess, peri-anal abscess\haematoma.
- Aspiration of cysts in lumps/bumps/etc included if incision/curettage involved.
- Manipulation (even if under GA) and examination under GA if no mention of anything else
- Angiogram unless with catheter, epidural, hysterosalpingogram, intrauterine device (IUD) inserted or removed, knee effusion\housemaids knee\pre-patella bursa, parenthesis, removal in-growing eyelashes, syringing\probing of nasolacrimal system(e.g. eyes flushed), venogram.
- Cryotherapy is not an operation.
- Oestrogen implants under injections.

Ask if in doubt

Main categories

1 Neurology includes
Embolisation of AV malformation, Morton's neuroma, repair nerve, severed nerve, stitching of open intracranial wound, sympathectomy, temporal artery biopsy, trigeminal thermo-coagulation, vagotomy if no other op done at same time.

2 Eye includes
Capsulotomy, excision TB focus, foreign body removal (does not include removal of ingrown eyelashes), phacoemulsification, probing punctoplasty, strabismus, tarsorrhaphy, tarsotomy

3 Ear includes
Cholesteatoma, mastoid, myringotomy\grommets (if myringotomy\grommets and adenoid just code under ear), stapedectomy

4 Abdominal includes
Adhesions removed, anal biopsy/stretch, cholecystectomy, colonoscopy, debridement of wound(anal area) , endoscopy (unless other site specified), ERCP (endoscopic retrograde cholangiopancreatography), gall bladder (cholecystectomy), haemorrhoids, hernia, ileorectal or (ileotransverse) anastomosis, kidney removed (nephrectomy), laparoscopy (unless stated as gynaecological), laparotomy(if part of other procedure code elsewhere), liver biopsy, oesophageal stent, PEG, peritonitis, pharyngeal pouch stapled, pilonidal sinus, polypectomy (unless other site specified), proctoscopy, pilonidal sinus, pyloromyotomy (Ramstedt's operation), rectal abscess, renal biopsy, resection caecum, RIG, sigmoidoscopy, splenectomy, vagotomy with pyloroplasty (NB vagotomy alone is neurological)

5 Orthopaedic includes
Arthroscopy, arthrotomy, Bankart repair, (bi)lateral release, bunions, cartilage, cervical rib, debridement of leg wound, dupuytrens contracture, excision of exostosis calcaneal tuberosity, ganglion, inferior capsular shift, K-wire arthroscopy, meniscectomy, muscle, osteotomy, patellectomy, subtalar fusion/arthrodesis, surgery for clawed toes, synovectomy, tendon repair, tenotomy, thumb fusion (arthritic related treatment)
Not manipulation even under anaesthetic. Not aspiration of knee effusion, house maids knee, pre-patella bursa, repositioning\reduction fractured nose. Broken nose (including reduction of broken nose under GA) included under nose\throat since 2010.

6 Gynaecological includes
Amniocentesis, ball diathermy to cervix , Bartholin's glands, caesarean section, colposcopy, dilatation and curettage (D&C), termination of pregnancy(TOP) if surgical, episiotomy, hysteroscopy, IUCD if under GA, laser treatment of HPV lesion, loop excision (LEEP), sacrocolpoplexy, skin tag of perineum , suture of perianal or perineum laceration post childbirth, TVT (tension-free vaginal tape).

7 Tonsillectomy (for post onset ops, code tonsil biopsy as tonsillectomy=4)

8 Carpal tunnel (at one time part of orthopaedic category)

9 Discs (at one time part of orthopaedic category) includes
Cervical disc, discectomy, laminectomy, slipped disc, spinal fusion

10 Appendectomy

11 Transplant includes
Aortic valve replacement, tissue valve recorded as transplant rather than op even though most likely non-human (pig) tissue.

Other categories

20 CVS/Cardiovascular includes
Angioplasty, aortic aneurysm, arteriovenous fistula, blood clot, CABG; haematoma, heart bypass, pacemaker, revascularisation, stents, thrombosis
Not angiogram unless catheter mentioned. (Varicose vein was included here and then moved to own category 23 minor operation in February 2010)

21 Stitches includes
Circumcision, infected web space of finger, installing central line/Hickman line, phimosis, sutures only.

22 Nose & Throat includes
Adenoids but not if done at same time as tonsillectomy (just code under tonsillectomy) or myringotomy\grommets (just code under ear), antral washout, broken nose repositioning/reduction (changed from orthopaedic in 2010), cautery to inferior turbinates intranasal antrostomies, dacryocystorhinostomy, deviated septum\septoplasty
EUA ears, laryngoscopy, nasal polypectomy, nasendoscopic surgery, nasendoscopy, oesophagoscopy, panendoscopy, pharyngoscopy, polyp on voice box removed, rhinoplasty, submucous resection, tracheostomy, turbinectomy\turbinate, uvuloplasty\uvulopalatopharyngoplasty\UPPP, vocal chords

23 Varicose veins includes
Saphenofemoral\varicose vein ligation, saphenous vein, sclerotherapy, strip/injection varicose veins, Trendelenburg NB check injection question!

24 Lumps, bumps, growths & cysts (generally of skin or body surface)
includes removal of moles (naevus), abscess, cyst (including in mouth), fatty tissue, warts, lymph glands, boils, papilloma, lipoma, rodent ulcer, birthmarks, skin biopsy, xanthelasma, verruca, papule, neurofibroma, excision of subcutaneous nodule axilla (unless history of breast problems), sebaceous

This category is intended for more minor ops typically performed by dermatologists and which cannot be classified elsewhere. For example, a breast cyst should be recorded in the breast category, testicular cancer recorded under testicular/vasectomy, bladder warts under urology, mention of plastic surgery under plastic surgery etc, cyst in mouth under this category. If more severe or deeper structures (or the severity is unclear due to lack of information) record under "other" e.g. TB gland removed from neck, parotid tumour from neck, tumour removed from tongue, ulcer\growth\abscess with no information as to site. Some less minor ops should be recorded under orthopaedic category e.g. growth in knee, cyst from knee. Cryotherapy (freezing) is not an op.

25 Testicular/Vasectomy includes
Adhesions to foreskin, anything penile, epididymal cystectomy, hydrocele, orchidectomy, torted hydatid of morgagni, varicocele

26 Breast includes
Breast augmentation, excision of subcutaneous nodule axilla with history of breast problems, lumpectomy (including with lymph node removal), mastectomy due to gynaecomastia

27 Plastic Surgery includes
Blepharoplasty, metoidioplasty (sex change), removal scalp skin, revision of scar, skin graft

28 Dental includes
Maxillofacial including jaw, fractured jaw, reduction of bilateral TMJ (tempero-maxillary joint) dislocation and screw fixation

29 Urology includes
Bladder op, cystoscopy (including removal of stent using cystoscopy),
kidney stones (pyelotomy\pyelolithotomy), Marshall-Marchetti-Krantz procedure for stress incontinence, nephrostomy, prostate, pyeloplasty, retrograde pyelogram, sphincterotomy (unless another site specified),TURP, urinary tract, ureteroscopy/utereroscopy.
Kidney removed should be recorded under abdominal.

30 Other & unknown includes
Bone marrow aspiration\biopsy, bronchoscopy, excision of sinus from thigh, ingrowing toenail/removal of toenail for other reasons, lung op (pneumonectomy), muscle biopsy, parotid tumour from neck, removal fishbone from foot, removal submandibular gland, removal subpectoral abscess, salivary glands removed, skin biopsy, splinter removed, sweat glands removed, TB gland removed from neck, thyroid op (including fine needle aspiration), tongue tie, tumour removed from tongue

<u>**Appendix 2**</u>: CJD Diagnostic Criteria

1. SPORADIC CJD

2010 Rotterdam Criteria

1.1 **DEFINITE:**

Progressive neurological syndrome **AND**

Neuropathologically **or** immunocytochemically **or** biochemically confirmed

1.2 **PROBABLE:**

1.2.1 I + 2 of II and typical EEG*

OR

1.2.2 I + 2 of II and typical MRI brain scan**

OR

1.2.3 I + 2 of II and positive 14-3-3

I	Rapidly progressive cognitive impairment
II	A Myoclonus B Visual or cerebellar problems C Pyramidal or extrapyramidal features D Akinetic mutism
III	Typical EEG
IV	High signal in caudate/putamen on MRI brain scan

1.3 **POSSIBLE:**

I + 2 of II + duration < 2 years

* Generalised periodic complexes

** High signal in caudate/putamen on MRI brain scan or at least two cortical regions (temporal, parietal, occipital) either on DWI or FLAIR

2. ACCIDENTALLY TRANSMITTED TSE

2.1 **DEFINITE**

Definite CJD with a recognised iatrogenic risk factor (see box)

2.2 **PROBABLE**

2.2.1 Progressive predominant cerebellar syndrome in human pituitary hormone recipients

2.2.2 Probable CJD with recognised iatrogenic risk factor (see box)

RELEVANT EXPOSURE RISKS FOR THE CLASSIFICATION AS IATROGENIC CJD

The relevance of any exposure to disease causation must take into account the timing of the exposure in relation to disease onset

- Treatment with human pituitary growth hormone, human pituitary gonadotrophin or human dura mater graft.
- Corneal graft in which the corneal donor has been classified as definite or probable human prion disease.
- Exposure to neurosurgical instruments previously used in a case of definite or probable human prion disease.

This list is provisional as previously unrecognised mechanisms of human prion disease may occur

3. GENETIC TSE

3.1 **DEFINITE**

3.1.1 Definite TSE + definite or probable TSE in 1st degree relative

3.1.2 Definite TSE with a pathogenic PRNP mutation (see box)

3.2 **PROBABLE**

3.2.1 Progressive neuropsychiatric disorder + definite or probable TSE in 1st degree relative

3.2.2 Progressive neuropsychiatric disorder + pathogenic PRNP mutation (see box)

- **PRNP MUTATIONS ASSOCIATED WITH GSS NEUROPATHOLOGICAL PHENOTYPE**
  P102L, P105L, A117V, G131V, F198S, D202N, Q212P, Q217R, M232T, 192 bpi
- **PRNP MUTATIONS ASSOCIATED WITH CJD NEUROPATHOLOGICAL PHENOTYPE**
  D178N-129V, V180I, V180I+M232R, T183A, T188A, E196K, E200K, V203I, R208H, V210I, E211Q, M232R, 96 bpi, 120 bpi, 144 bpi, 168 bpi, 48 bpdel
- **PRNP MUTATIONS ASSOCIATED WITH FFI NEUROPATHOLOGICAL PHENOTYPE** D178N-129M
- **PRNP MUTATION ASSOCIATED WITH VASCULAR PRP AMYLOID**
  Y145s
- **PRNP MUTATIONS ASSOCIATED WITH PROVEN BUT UNCLASSIFIED PRION DISEASE**
  H187R, 216 bpi,
- **MUTATIONS ASSOCIATED WITH NEURO-PSYCHIATRIC DISORDER BUT NOT PROVEN PRION DISEASE**
  I138M, G142S, Q160S, T188K, M232R, 24 bpi, 48 bpi, 48 bpi + nucleotide substitution in other octapeptides

ADDITIONAL LIST OF MUTATIONS

• PRNP MUTATIONS WITHOUT CLINICAL AND NEUROPATHOLOGICAL DATA	T183R, P238S
• PRNP POLYMORPHISMS WITH ESTABLISHED INFLUENCE ON PHENOTYPE	M129V
• PRNP POLYMORPHISMS WITH SUGGESTED INFLUENCE ON PHENOTYPE	N171S, E219K, 24 bp deletion
• PRNP POLYMORPHISMS WITHOUT ESTABLISHED INFLUENCE ON PHENOTYPE	P68P, A117A, G124G, V161V, N173N, H177H, T183T, D202D, Q212Q, R228R, S230S

4. vCJD

4.1 **DEFINITE**
1A and neuropathological confirmation of vCJD[e].

4.2 **PROBABLE**
4.2.1 I and 4/5 of II and IIIA and IIIB
4.2.2 I and IV A[d]

4.3 **POSSIBLE**
I and 4/5 of II and III A

I	A	Progressive neuropsychiatric disorder
	B	Duration of illness > 6 months
	C	Routine investigations do not suggest an alternative diagnosis
	D	No history of potential iatrogenic exposure
	E	No evidence of a familial form of TSE
II	A	Early psychiatric symptoms[a]
	B	Persistent painful sensory symptoms[b]
	C	Ataxia
	D	Myoclonus or chorea or dystonia
	E	Dementia
III	A	EEG does not show the typical appearance of sporadic CJD[c] in the early stages of illness
	B	Bilateral pulvinar high signal on MRI scan
IV	A	Positive tonsil biopsy[d]

a depression, anxiety, apathy, withdrawal, delusions.
b this includes both frank pain and/or dysaesthesia.
c the typical appearance of the EEG in sporadic CJD consists of generalised triphasic periodic complexes at approximately one per second. These may occasionally be seen in the late stages of variant CJD.
d tonsil biopsy is **not** recommended routinely, nor in cases with EEG appearances typical of sporadic CJD, but may be useful in suspect cases in which the clinical features are compatible with vCJD and MRI does not show bilateral pulvinar high signal.
e spongiform change and extensive PrP deposition with florid plaques throughout the cerebrum and cerebellum.

1. SPORADIC CJD (from January 2017)

2017 Euro CJD Criteria

1.1 **DEFINITE:**

Progressive neurological syndrome **AND**

Neuropathologically **or** immunocytochemically

or biochemically confirmed

1.2 **PROBABLE:**

1.2.1 I + 2 of II and typical EEG*

OR

1.2.2 I + 2 of II and typical MRI brain scan**

OR

1.2.3 I + 2 of II and positive 14-3-3

OR

1.2.4 Progressive neurological syndrome and positive RT-QuIC in CSF or other tissues

I	Rapidly progressive cognitive impairment
II	A Myoclonus
	B Visual or cerebellar problems
	C Pyramidal or extrapyramidal features
	D Akinetic mutism
III	Typical EEG
IV	High signal in caudate/putamen on MRI brain scan

1.3 **POSSIBLE:**

I + 2 of II + duration < 2 years

* Generalised periodic complexes

** High signal in caudate/putamen on MRI brain scan or at least two cortical regions (temporal, parietal, occipital) either on DWI or FLAIR

Table 14 – Exclusions from organ and/or tissue donation based on possible TSE exposure

	LIVE TISSUE DONORS		CADAVERIC TISSUE DONORS				SOLID ORGAN DONORS
	Bone	HSC	Musculoskeletal (ligaments, tendons & cartilage)	Bone and processed bone	Ocular	Skin/ Heart Valves	
Definite, probable or possible case of human TSE, including CJD and vCJD	Exclude	Exclude	Exclude	Exclude	Exclude	Exclude	Exclude
Individual with a neurological disease of unknown aetiology	Exclude	Exclude	Exclude	Exclude	Exclude	Exclude	Exclude
Individual whose blood relatives have had familial CJD [1]	Exclude	Exclude	Exclude	Exclude	Exclude	Exclude	Exclude
Individual "presumed infected" with vCJD [2]	Exclude	Exclude	Exclude	Exclude	Exclude	Exclude	Exclude
Individual "at increased risk of CJD/vCJD" (for public health purposes) [3]	Exclude	Individual assessment required [4]	Exclude	Exclude	Exclude	Exclude	Individual assessment required [4]
History of definite [5] or probable [6] blood transfusion since 1980	Exclude	Individual assessment required [4]	Exclude Do not exclude if transfusion is within 1 week prior to death	Exclude	Do not exclude [7]	Do not exclude	Individual assessment required [4]
History of receipt of *dura mater* graft	Exclude	Individual assessment required [4]	Exclude	Exclude	Exclude	Exclude	Individual assessment required [4]
History of definite receipt of tissue since 1980	Exclude	Individual assessment required [4]	Exclude	Exclude	Exclude	Do not exclude	Individual assessment required [4]

History of receipt of pituitary derived growth hormone and/or gonadotrophin	Exclude	Individual assessment required[4]	Exclude	Exclude	Exclude	Exclude	Individual assessment required[4]
History of receipt of organ	Exclude	Exclude	Exclude	Exclude	Exclude	Exclude	Individual assessment required[4]

1 However, if a donor has had two or more blood relatives develop a prion-associated disease and, following genetic counselling, they have been
informed they are not at risk, they may be accepted for donation
2 Donors who have received blood components, tissues and/or organs from donors who have gone on to develop vCJD.
3 Donors who have been notified that they are at increased risk of vCJD (for public health purposes) due to possible exposure.
4 Level of risk or exposure should be clarified and weighed, on an individual basis, against the expected benefit of the transplant and the availability of
alternative donors. The recipient (and/or relatives) should be informed of the nature of the estimated risk of vCJD transmission.
5 Definite transfusion is defined as at least one of the following:
- Recorded in medical notes available to clinical staff at time of donation;
- Documented during interview;
- Reported by GP;

6 For tissue and organ donors, probable transfusion is defined as:
- previous major surgery; and/or
- previous major accident.

7 Ocular donors should not be excluded if they have a history of definite or probable transfusions, in view of supply issues. However it is essential that:
- information is provided to recipients;
- wherever possible donor and recipients are age matched;
- efforts are made to increase yields of ocular tissues;
- donors excluded on the basis of public health measures are not accepted as ocular donors.

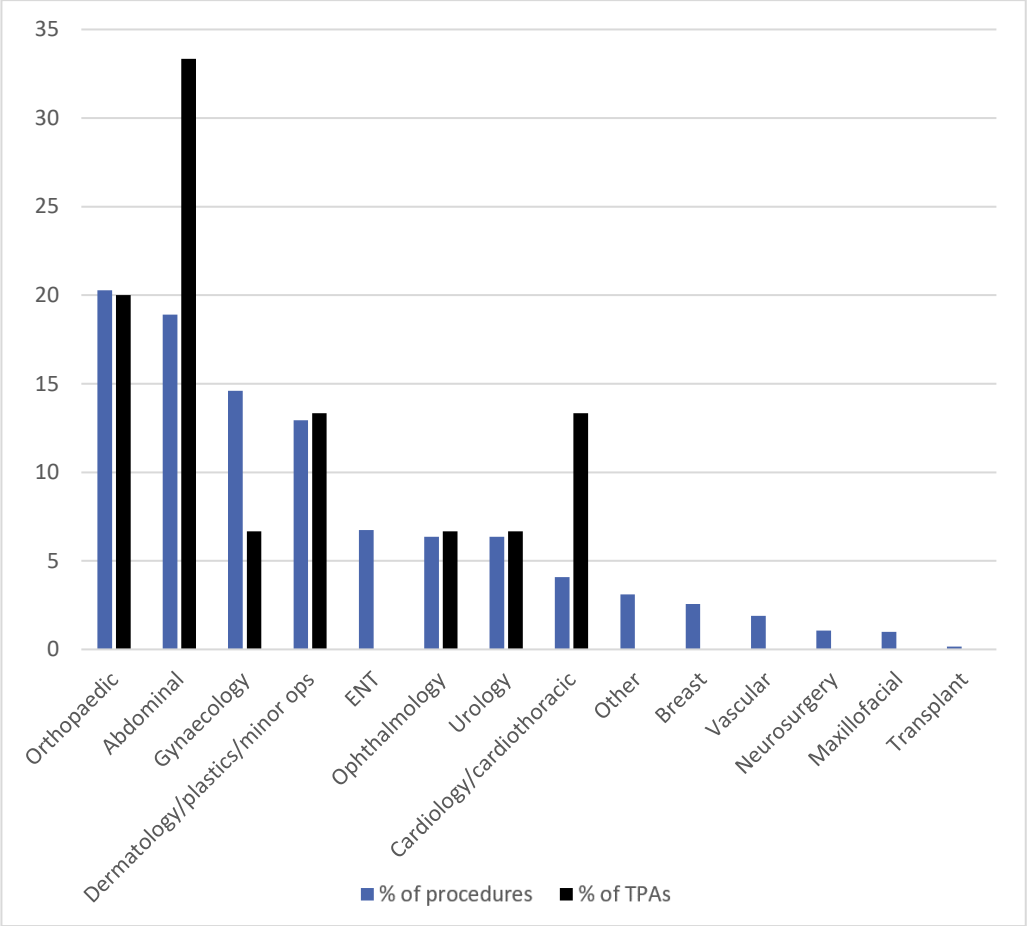
Reproduced from https://bts.org.uk/wp-content/uploads/2016/09/Guidance-on-the-microbiological-safety-of-human-organs-tissues-and-cells-used-in-transplantation.pdf

Appendix 4: Family questionnaire vs GP record year reporting

Table: Number of procedures by final digit of year. Data for Figure 4.4

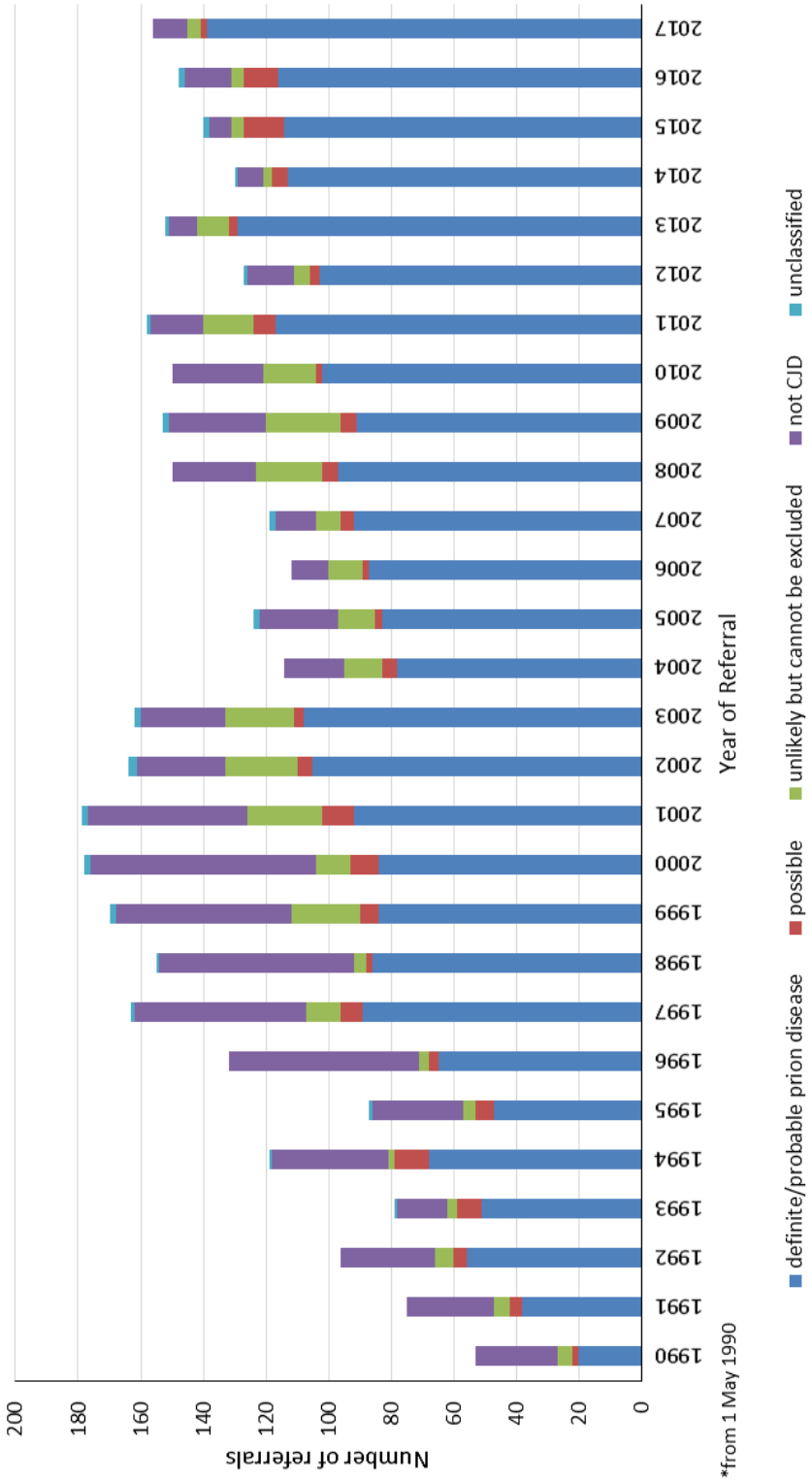
Procedure by final digit of year	Family questionnaire	GP records
0	167	358
1	105	318
2	120	284
3	113	311
4	114	313
5	222	283
6	77	289
7	117	280
8	142	328
9	121	329
No year given	28	18

Appendix 5: Percentages of surgical procedures and TPAs using family questionnaire data only



Percentages based off 1326 procedures and 15 plausible TPAs.

Appendix 5: Referrals to the NCJDRSU by year



Reproduced from NCJDRSU 26th Annual Report 2017 (235)