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INTRODUCTION

Implications of emerging pathogens in the management of haemophilia

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In the early 1980s, more than 50% of persons with haemophilia became infected with human immunodeficiency virus (HIV). This high rate of infectivity was in large part because of the initially limited reaction to the possibility and implications of HIV transmission through blood and blood therapies. Significant morbidity and mortality were the result of this tragically inadequate response. While the risks of HIV transmission are now well documented, clinicians must be aware of the potential for new pathogens.

This supplement is a synthesis of the proceedings of an interdisciplinary symposium convened in August 2005 at Sydney, Australia. The symposium, part of the XXth Congress of the International Society on Thrombosis and Haemostasis, was designed to provide a unique perspective on the state of emerging pathogens and to review proactive measures to prevent their transmission in patients with haemophilia.

New, reemerging or drug-resistant pathogens continue to arise worldwide, and their presence presents an ongoing threat to the blood supply. Recent years have produced outbreaks of severe acute respiratory syndrome (SARS), West Nile virus (WNV) and avian influenzas. In his article, Dr Michael Tapper puts forth the basic definition of an emerging pathogen agreed upon by the medical and scientific communities. Using the examples of WNV, SARS and avian flu, he outlines the factors that contribute to the emergence of these pathogens, including microbial adaptation, global travel and human demographics.

Non-viral disease-causing agents, such as the prions that cause variant Creutzfeldt–Jacob disease (vCJD), also pose a real and significant threat to blood and blood-derived therapies, as exemplified by the recent experience in the UK. Dr James Ironside's article reviews the clinical and management characteristics of vCJD with an emphasis on the risks of disease transmission. He describes the three classes of prion diseases: idiopathic (also known as sporadic), inherited and acquired (the classification for variant CJD). In addition, Dr Ironside reviews the epidemiological considerations, including the theoretical role of genetics in vCJD susceptibility, the potential risks of transmission through blood and blood products and the trends in and extent of its spread to date.

In his article, Dr Gerry Dolan shares the clinician's perspective on the indirect clinical and psychological impact that vCJD may potentially have on patients with haemophilia in the UK, primarily as a result of new recommendations and policies to safeguard patients from transfusion-transmitted infections. Often developed in concert with governmental and non-governmental agencies, these policies describe how to inform and manage patients who may have been exposed to vCJD through the use of UK-based plasma concentrates, and therefore may pose a risk for human-to-human transmission. Unfortunately, these policies could have unintended consequences for the haemophilia population: potential stigmatisation and interference with necessary medical and surgical care. The emergence of vCJD argues for the utmost caution in the treatment of persons with haemophilia, both medically and sociopolitically.

By their very nature, unknown emerging pathogens will always cause uncertainty within the medical and scientific communities, but as evidenced by the HIV crisis of the 1980s, hesitation in the face of theoretical risks can result in increased morbidity and mortality. My article describes the importance of both anticipating the potential risks which emerging pathogens may pose to patients with haemophilia, and discussing these risks, as well as the relative

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merits of available therapies with patients. Practitioners must understand the real and theoretical risks to their patients with haemophilia and be able to address those fears and anxieties appropriately. I review the basic information to know when discussing emerging pathogens with a haemophilia patient. While it is likely that emerging pathogens will always be with us, they represent an opportunity for an open and frank discussion between practitioner and patient regarding optimal therapy for this disease and other bleeding disorders.

A synthesis of a question and answer session about emerging pathogens, patient management, vCJD and the impact of vCJD within the haemophilia community follows these four articles. The contents of this supplement contain the most current information on identified and potential infectious disease risks, their mechanisms for emergence, and their associated threat to the safety of the blood supply worldwide. A special emphasis is placed on the impact of emerging infectious diseases on clinical practice in haemophilia. A thorough grasp of this information enables clinicians to proactively review therapeutic regimens with their haemophilia patients in order to minimize anxiety with regard to emerging infectious disease, counsel them on the current safety of plasma-derived therapies and recommend a pharmacological course of therapy that will result in the most optimal outcomes possible.

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Emerging viral diseases and infectious disease risks

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Summary. New pathogens and antimicrobial-resistant forms of older pathogens continue to emerge, some with the potential for rapid, global spread and high morbidity and mortality. Pathogens can emerge either through introduction into a new population or when the interaction with the vector changes; emergence is also influenced by microbiological adaptation and change, global travel patterns, domestic and wild animal contact and other variants in human ecology and behaviour. Quick, decisive action to detect and control novel pathogens, and thereby contain outbreaks and prevent further transmission, is frequently hampered by incomplete or inadequate data about a new or re-emerging pathogen. Three examples of pathogens that are current causes for human health concern are avian influenza, West Nile virus (WNV) and the severe acute respiratory syndrome (SARS) coronavirus. Pathogens directly or indirectly transmitted by aerosolized droplets, such as avian influenza and SARS, pose considerable containment challenges. Rapid screening tests for other newly described pathogens such as WNV require time for development and may be <100% reliable. The importance of vigilance in the detection and control of newly recognized infectious threats cannot be overstressed. The presence of infectious agents in the blood supply could again have a significant impact on the safe use of both blood and blood-derived products in the care of patients with haemophilia, as did the human immunodeficiency virus in the 1980s. Emerging pathogens will continue to be a reality requiring the collaborative efforts of public health and individual healthcare providers worldwide to contain outbreaks and prevent transmission.

Keywords: avian influenza, haemophilia, human immunodeficiency virus, pathogens, severe acute respiratory syndrome, West Nile virus

Introduction

The emergence of new infectious pathogens and the recurrence of older pathogens in unique settings have become common topics in the medical literature and lay media, indicating an increasing concern among healthcare providers and the general public alike. The presence of infectious agents in the blood supply, for example, has had – and could again have – a profound influence on the safe use of both blood and blood-derived products in the care of patients with haemophilia. This article provides an overview of emerging infectious diseases in general and discusses some examples of viral pathogens that are currently cause for concern, including West Nile virus (WNV), severe acute respiratory syndrome (SARS) and avian influenza. It also lays the foundation for discussions about the implications of emerging infectious diseases for the safety of the blood supply and for the care of patients who depend on the safety of the blood supply, such as those with haemophilia.

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Infectious disease outbreaks of the last decade

In the last decade there have been a number of major global infectious disease outbreaks that have had the potential to be major health threats. Many of these rapidly spreading viruses, including SARS and avian influenza, appear to have originated as zoonoses in Asia [1]. These viruses have also demonstrated an extraordinary capacity to move quickly (and often surreptitiously) between animal and human populations and across continents.

Definition of an emerging infectious disease

Defining an emerging infectious disease is not necessarily straightforward. Morbidity and mortality from

emerging infectious diseases are understood to be a continual threat, yet the exact nature of that threat is not well defined. One widely accepted definition was proposed in 1992 by the Institute of Medicine (IOM) in the USA, which defined an emerging infectious disease as a new, re-emerging, or drug-resistant infection whose incidence in humans has increased within the past two decades or whose incidence has threatened to increase within the near future [2]. Based on this definition, a spectrum of potential infectious diseases becomes apparent.

Potential infectious disease threats

A continuum exists in types of pathogens that emerge and infect new populations. The continuum includes infectious diseases such as SARS that appear to be newly introduced to humans from animals as well as bioengineered organisms that produce disease in unforeseen ways, such as the transmission of anthrax by contaminated mail in the USA in 2001. Outbreaks of disease once thought to be well controlled may be associated with a breakdown in core public health measures such as treatment of established infection (e.g. tuberculosis) or routine childhood immunizations (poliomyelitis) The continuum of potential disease threats also includes new antimicrobial-resistant forms of established pathogens, such as methicillin-resistant *Staphylococcus aureus*. In addition, scientists continue to recognize previously unidentified infectious origins of some chronic diseases, such as Lyme borreliosis [3].

Factors contributing to emerging infections

In 1992 the IOM identified numerous factors that contribute to emerging infectious diseases, all of which may impact the safety of the blood supply [2]. These factors include:

1 human demographics and behaviour;
2 technology and industry;
3 economic development and land use;
4 international travel and commerce;
5 microbiological adaptation and change;
6 breakdown of core public health measures.

In 2003, the IOM published an update to the 1992 report in which additional contributing factors were identified [3]:

1 human susceptibility to infection;
2 climate and weather;
3 changing ecosystems;
4 poverty and social inequality;
5 war and famine;
6 lack of political will;
7 intent to harm.

Many of these factors are interdependent. International travel and commerce and human demographics and behaviour, for example, are closely related and have undergone considerable change in the last century. Over the last 150 years as the global population has increased dramatically, the length of time required to circumnavigate the globe has decreased dramatically (Fig. 1) [4]. International travel and commerce have affected the size and mobility of human populations, bringing some environments, humans and other animal species into contact with each other for the first time. These changing human demographics may enable an infectious agent to become adapted to and disseminated within a new host population, often resulting in an expansion of the agent's geographic range [5]. The combination of these factors has accelerated the global spread of infectious agents.

Route of transmission of emerging infectious disease

Emergence of an infectious disease can occur either through its introduction into a new population or when the interaction with the vector of a disease changes. The latter scenario is the likely manner in which viruses such as WNV and Lyme borreliosis have spread [5]. The WNV strain found in the USA, for example, is believed to have spread from the Middle East and be a variant of the virus first isolated in 1937 in the West Nile District of Uganda in Africa. It is uncertain how WNV spread to the USA. It has been hypothesized that the strain in the USA was

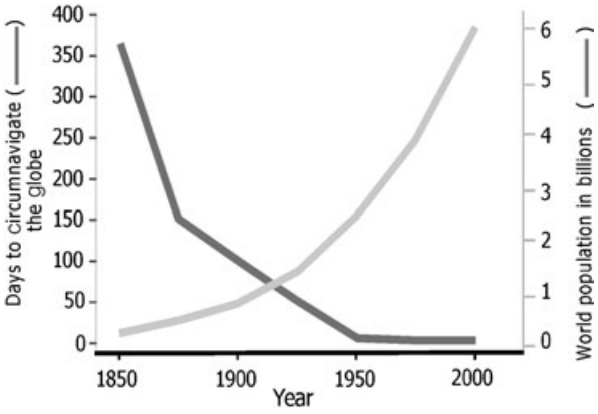

Fig. 1. Speed of global travel in relation to world population growth [4].

transported in an infected bird illegally imported from the Middle East or Central Europe where the disease had previously been endemic. Mosquito transmission subsequently resulted in transmission to birds, horses and humans in the USA. After its initial appearance in New York City in 1999, WNV spread to the lower 48 states in the US in <2 years [6].

Recent infectious disease concerns

New, emerging infectious diseases and disease agents continue to be discovered and described. While incomplete, the list in Table 1 provides an indication of the variety and quantity of pathogens that confront public health officials and present potential threats to human health [3].

West Nile virus

In 1999, the first cases of WNV infection were recognized in New York City. Over the next several years, the virus spread throughout the northeastern part of the country and subsequently spread west to the Mississippi River and south into Florida. By 2002, cases were being reported across most of the Midwest, and by 2005 every state in the continental USA had reported cases of WNV in humans, birds, mammals or mosquitoes [7].

Since 2002, following reports of transfusion-associated WNV infections, the US blood supply has been screened for the virus. As of 15, November 2005, 382 presumptively viremic blood donors had been identified and reported to the US Centers for Disease Control and Prevention (CDC). These donors were generally asymptomatic for WNV infection at the time of blood donation but tested seropositive when pooled samples were screened using nucleic amplification technology (NAT). Some of these individuals subsequently developed clinical symptoms [8].

Table 1. Partial list of emerging infectious diseases and disease-causing agents*.

HIV/AIDS
Tuberculosis
Dengue
Malaria (resistant *Plasmodium falciparum*)
Severe acute respiratory syndrome
Cholera
Meningococcal meningitis
Cryptosporidiosis
Filoviruses (Ebola/Marburg)
Legionella pneumophila
Lyme disease
Poliomyelitis
Toxin producing streptococci and *Staphylococcus aureus*
Human Herpesvirus-8
Parvovirus B19
Hepatitis C
Arenaviruses (Lassa)
Cyclospora cayetanensis
Hantavirus (Sin Nombre)
New variant CJD (BSE)
Bunyaviruses (Rift Valley)
Rotavirus
Escherichia coli 0157:H7
Bartonella henselae (cat scratch disease)
Community acquired MRSA
Avian influenza (H5N1)
West Nile virus
Salmonella enteritidis

AIDS, acquired immunodeficiency syndrome; BSE, bovine spongiform encephalopathy; CJD, Creutzfeldt–Jakob disease; HIV, human immunodeficiency virus; MRSA, methicillin-resistant *Staphylococcus aureus*.
*Data adapted from Smolinski *et al.* [3].

Severe acute respiratory syndrome

At the outset of the SARS epidemic in Asia, a number of small mammals commonly maintained in open food markets in Canton were found to be infected with the SARS coronavirus. More recent data have suggested that certain species of bats native to China may be the definitive host of the virus in nature [9].

Severe acute respiratory syndrome was first recognized in Hanoi, Vietnam in February 2003, although it is now believed to have originated in the Guangdong Province in southeast China in November 2002 [10]. In late February 2003, the first case of SARS in Hong Kong was reported in a physician from the Guangdong Province, who travelled to Hong Kong for a wedding. While staying overnight in a local hotel, it appears he transmitted the virus to 12 people on his floor. Subsequent generations of infection from the physician (who died in a Hong Kong hospital 2 days after arriving at the hotel), his relatives and others staying in the hotel involved more than 95 healthcare workers and 100 close contacts in the city of Hong Kong [11].

The global spread was rapid. Other infected hotel guests subsequently travelled to Vietnam, where 37 healthcare workers and 21 close contacts became infected, and to Singapore, where 34 healthcare workers and 37 close contacts were infected [11]. Another returned to Canada, where a cluster of infections commenced in a local hospital, involving family members, healthcare workers and other patients. Ultimately, over 200 people in Canada were infected, approximately one-third of whom died [12].

Avian influenza

Avian influenza is a major potential threat to the populations of the world and may be the source of the next flu pandemic [13]. There were three major flu pandemics in the last century: the so-called 'Spanish flu' in 1918–1919, potentially responsible for up to 50 million deaths worldwide; the Asian flu in 1957–1958, responsible for approximately 70 000 deaths in the USA; and the Hong Kong flu in 1968–1969, responsible for 34 000 deaths nationwide. Many epidemiologists believe that the human population is overdue for a pandemic [14]. Figure 2 illustrates a timeline of the emergence of several strains of the influenza virus.

Since 1918 there have been a number of shifts in the influenza virus's haemagglutinin and neuraminidase components, its key antigens. Fifteen types of haemagglutinin (H1–H15) and nine types of neuraminidase (N1–N9) have been recognized. Combinations involving subtypes H1–H3 and N1–N2 have been responsible for both seasonal and epidemic outbreaks in humans. The definitive hosts of influenza in nature are non-domesticated birds, particularly ducks that carry H1–H15 type viruses. Direct bird-to-human (and to date, rare instances of human-to-human) transmission of avian influenza has been reported [15] with increasing frequency in the last two and a half years.

Mechanism of influenza antigenic shift

Influenza viruses undergo constant subtle evolution and mutation of their principal proteins, a process referred to as antigenic drift. In addition to this naturally occurring and random process, influenza strains from different host species can periodically recombine. Swine may serve as hosts for both human and duck influenza strains and hence can function as ideal mixing vessels for major antigenic recombination and the emergence of novel influenza strains. When such shifts or recombinations occur and result in a virus with the capacity to maintain ongoing transmission between humans, a major pandemic may occur [16].

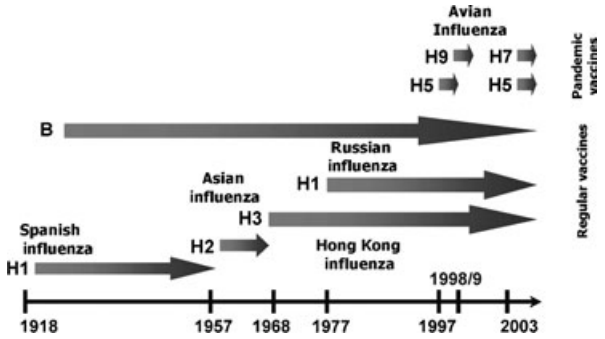

Fig. 2. Timeline of emergence of influenza viruses in humans. (Figure courtesy of the Centers for Disease Control and Prevention.)

In 1997 in Hong Kong, the first evidence emerged that avian viruses could directly infect humans without going through this interim mixing step [15,16]. In 1997, there was an outbreak of influenza associated with an avian (H5N1) strain in humans that was preceded by an outbreak of the same strain in poultry [17]. With six deaths among 18 hospitalizations, H5N1 exhibited unusual lethality and was considered by some public health officials and epidemiologists as a pandemic warning call.

By December 2003, confirmed cases of avian influenza among humans were reported in Vietnam and Thailand, and since January 2004, human cases have been reported in Vietnam, Thailand, Cambodia, Indonesia and the People's Republic of China. The total number of cases as of 17, November 2005 was 130, with 67 deaths [18]. Sustained outbreaks among domestic poultry flocks in Asia preceded these human cases.

While the major outbreaks of avian influenza have occurred among domestic poultry flocks, evidence of avian influenza viral infection in migrating birds throughout Asia (and more recently in Europe) has also been demonstrated. It has been suggested that migratory birds may be responsible for the widespread introduction of avian influenza into other bird populations, both domestic and wild [19].

Conclusion

New pathogens continue to emerge, some with the potential for rapid, global spread and high morbidity and mortality. Laboratory tests for viral detection can be developed once a virus is identified, but their development takes time and their reliability may be <100%.

Pathogens spread by aerosolized droplets, such as avian influenza and SARS, pose considerable containment challenges, although neither pathogen appears to clearly impact the safety of the blood supply. In the case of SARS, patients can be screened, but the exact mode of human-to-human transmission remains uncertain. In contrast, reasonably (although not universally) effective screening exists for some newly described blood-borne pathogens such as WNV. Nonetheless, the hard-learned lesson from the human immunodeficiency virus (HIV) experience in the 1980s is that the importance of vigilance in the

detection and elimination of newly recognized threats to blood safety cannot be overstressed. For these reasons, emerging pathogens will continue to be a reality requiring the best efforts of both public health officials and individual healthcare providers worldwide to identify emerging pathogens in a timely fashion, contain outbreaks and prevent transmission.

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Variant Creutzfeldt–Jakob disease: risk of transmission by blood transfusion and blood therapies

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Summary. In the last decade, a new variant of the human prion disease Creutzfeldt–Jakob disease (now known as variant CJD or vCJD) was identified and causally linked to dietary exposure to bovine spongiform encephalopathy (BSE) during the 1980s and early 1990s. Preliminary studies in animal models suggest that prions can be transmitted by blood. Based on two recent reports of iatrogenic vCJD transmission by blood transfusion in humans, a Department of Health-sponsored risk assessment warned that recipients of plasma therapies are now at risk of contracting vCJD from potentially infected donors. It is believed that all the population may be susceptible to vCJD infection, although clinical cases have so far occurred only in methionine homozygotes at codon 129 in the human prion protein gene. A non-invasive blood-based diagnostic assay is urgently needed. Because the incubation period may be upwards of 40 years and there is no reliable screening test, it is currently unknown how many people may be in an asymptomatic phase of vCJD infection in the UK. However, there remains a distinct possibility that some infected patients may never develop clinical symptoms but will remain asymptomatic carriers who can potentially transmit the disease to other individuals. Therefore, screening of infectious individuals will be a critical component for individuals who rely on blood transfusions and/or blood therapies. In the absence of screening tests or effective therapies to treat this disease, a formidable worldwide public health challenge lies ahead to prevent new infections, accurately assess infection rates and treat infected patients.

Keywords: blood transfusion, factor replacement, haemophilia, prion, transmission, variant Creutzfeldt–Jakob disease

Introduction

Variant Creutzfeldt–Jakob disease (vCJD) is a recently identified member of the transmissible spongiform encephalopathies (TSE) or prion diseases [1,2]. These disorders are fatal neurodegenerative conditions occurring in humans and other mammals, the best known examples in non-human species being bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep and chronic wasting disease in deer and elk [3]. Prion diseases are transmissible under both experimental and natural conditions. For many years, the nature of the transmissible agent was the subject of intense debate, and in 1982 the prion hypothesis was proposed by Prusiner [4]. This postulated that the transmissible agent was composed entirely of a modified host protein (prion protein) that was partially resistant to proteolytic degradation, without a nucleic acid component.

The normal form of the prion protein (PrP^{C}) is expressed in many cells and tissues in the body, but is present at highest levels in neurones within the central nervous system [3]. The precise function of PrP^{C} is uncertain, but it has a short half life and is readily degraded by proteolytic enzymes [5]. An abnormal isoform of PrP (PrP^{Sc}) accumulates in the central nervous system in prion diseases. PrP^{Sc} has an identical amino acid sequence to PrP^{C}, but a different conformation, with an increased beta-sheet content that is associated with infectivity and neurotoxicity [3]. This abnormal conformation also confers a relative resistance to degradation by proteolytic enzymes. The precise cellular mechanisms that result in this conformational change, and their locations, have not yet been fully determined.

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The BSE epidemic in the UK

In 1987, a novel progressive neurological condition in cattle was reported in the UK [6]. The new disease was named bovine spongiform encephalopathy (BSE, or 'mad cow' disease) because of its similarity to other prion diseases by pathology and immunohistochemistry. By the early 1990s thousands of cattle were diagnosed with BSE and millions were incinerated to prevent the disease from spreading [7,8]. However, BSE has still not been fully eradicated in the UK. The BSE epidemic in the UK has been attributed to TSE-infected feeds made of meat and bone meal prepared from rendered sheep offal [9]. With the prohibition of specific feeding practices and specified offals, however, the number of reported cases declined to fewer than 500 by 2003 in UK (Fig. 1) [7,8].

Since the UK continued to export cattle offals after 1986, the BSE agent spread to over 20 European countries, as well as to Japan, Russia, Canada, Israel and the USA. Thus, the exportation of contaminated animal feed from the UK to many other countries across the world resulted not only in the spread of BSE but potentially widespread human exposure to BSE-positive animals through the consumption of BSE-contaminated meat products [10]. Public health concerns about the safety of meat products around the world since the BSE epidemic two decades ago have not diminished. On 24, June 2005, the US Department of Agriculture confirmed BSE in a cow that had conflicting screening test results the previous year. Fortunately, no part of the animal had entered the human or animal food supply; however, this case heightened the awareness of the need for better testing in this country and ongoing surveillance [8,11].

Table 1. Classification of human prion diseases [12].

Class	Diseases
Idiopathic	Sporadic Creutzfeldt–Jakob disease
	Sporadic fatal insomnia
Familial	Familial Creutzfeldt–Jakob disease
	Gerstmann–Sträussler–Scheinker syndrome
	Fatal familial insomnia
Acquired	
Human origin	Kuru, iatrogenic Creutzfeldt–Jakob disease
Bovine origin	Variant Creutzfeldt–Jakob disease

Classification of human prion diseases

Human prion diseases are categorized into three distinct groups that reflect their different origin and range: idiopathic, inherited and acquired [2] (Table 1). The commonest of the idiopathic disorders is sporadic CJD (sCJD). Sporadic CJD is distributed worldwide and is the most common of all human prion diseases, accounting for around 85% of all cases [13]. It is associated with a highly aggressive clinical course with a mean duration of illness of approximately 4.5 months. Sporadic CJD occurs most frequently in middle-aged or elderly individuals and appears to be triggered by a somatic mutation of the prion gene, or by a spontaneous conformational change of the host prion protein from its normal cellular form (PrP^{C}) to its abnormal and pathogenic form (PrP^{Sc}) [3,14].

Inherited (familial) forms of prion diseases comprise up to 15% of all cases and are strongly linked to a series of pathogenic mutations and insertions in the prion protein gene [15,16]. The clinical course of these TSEs is characterized by a slow degeneration of the central nervous system, resulting in dementia, ataxia, motor difficulties and death. The inherited human prion diseases comprise three main groups of

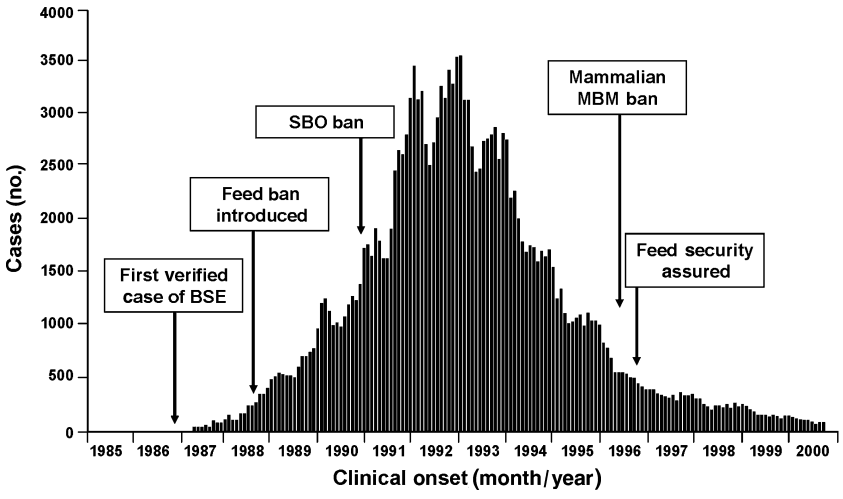

Fig. 1. Bovine spongiform encephalopathy epidemic in the UK [7].

disorders, each with a characteristic clinical and pathological phenotype: familial CJD, the Gerstmann–Sträussler–Scheinker syndrome and fatal familial insomnia [16]. All occur as autosomal dominant disorders [15].

The third group of human prion diseases, the acquired disorders, comprise <1% of all cases and are characterized by exposure to infectivity in brain or nervous system tissue either through human-to-human contact via contaminated neurosurgical instruments, tissue grafts or extracts (iatrogenic CJD) [17], or via the consumption of contaminated bovine meat products (vCJD). Experimental transmission studies have shown that the transmissible agent in vCJD has identical properties to the BSE agent, confirming the link between these 2 disorders [18,19].

Variant CJD was first described in the UK in 1996, but has now been identified in 10 other countries. Variant CJD tends to affect young adults, with a mean age of approximately 29 years (age range 12–74 years at disease onset) [1]. Interestingly, this corresponds with the general age group at which people become blood donors. The duration of the clinical illness is longer (mean duration of 13 months) than that of sCJD, and is characterized by psychiatric features and sensory symptoms at onset, followed by ataxia, myoclonus and other movement disorders; rapidly progressive dementia is very uncommon in this disease [1]. Thus, sCJD and vCJD are distinct disorders that are characterized by different geographical distributions, durations of illness, ages of onset and clinical course, and, most importantly, the causal association of vCJD with BSE.

Transmission of prion diseases by blood

While the transmission of prion infectivity through blood in rodent models of scrapie is well established, recent reports have also found evidence of infectivity in the blood of a rodent model of vCJD and in sheep experimentally infected with BSE [20,21]. These findings have raised questions over the potential transmission of vCJD by blood or blood components. Therefore, concern over safeguarding the blood supply has been gradually mounting given the potentially large number of asymptomatic carriers of vCJD who may unknowingly donate blood. This threat to the blood supply poses a unique challenge to public health officials and raises concerns for patients – especially individuals with haemophilia and other bleeding disorders – who routinely rely on the blood supply and blood therapies. Retrospective studies of haemophilia patients who died from other diseases, including HIV, have not identified any cases of sCJD that were missed or misdiagnosed, either in the UK or in the USA [22,23]. However, although epidemiological studies of sCJD have found no convincing evidence of its transmission by blood [24], the different pathogenesis of vCJD does not allow reassurance to be taken from these studies focusing on sCJD.

Genetic susceptibility to vCJD

Progress in the understanding of human prion diseases was accelerated following the identification of the PrP gene on the short arm of chromosome 20. The identification of pathogenic mutations and insertions in the PrP gene provided evidence to support the prion hypothesis, as familial prion disorders are both genetic and transmissible. Furthermore, it is now recognized that a polymorphism at codon 129 in the human PrP gene may influence susceptibility to prion disease.

Three genetic subgroups have been identified at codon 129 of the PrP gene: methionine homozygous (M/M), valine homozygous (V/V) and heterozygous (M/V). All clinical cases of vCJD have so far occurred in individuals with the methionine homozygous genotype [25,26]. This finding is important because only around 40% of the total human population are methionine homozygotes; approximately 10% are valine homozygotes and 50% are heterozygotes [27,28,29] (Table 2). However, among sCJD cases, only 65% are methionine homozygotes. Thus the methionine homozygous genotype is more susceptible to developing both sporadic and vCJD.

Diagnostic assays for vCJD

One of the largest issues that confront clinicians trying to manage this disease is the absence of a diagnostic screening test for vCJD. Confirmation of a clinical diagnosis of vCJD requires neuropathological examination of the brain following autopsy, with demonstration of the characteristic type 2B isoform of PrP^{Sc} in the brain and lymphoid tissues [25].

Table 2. PRNP codon 129 genotype frequencies [29].

	Genotype		
	M/M	M/V	V/V
Normal population	37%	51%	12%
Sporadic CJD	65%	17%	18%
Variant CJD	100%	–	–

CJD, Creutzfeldt–Jakob disease; M/M, methionine homozygous; M/V, valine heterozygous; V/V, valine homozygous.

Therefore, diagnostic assays are urgently needed for vCJD that are blood based and do not require an invasive brain or tonsil biopsy [30].

A major challenge to the development of such a test is that prions are devoid of nucleic acid, unlike bacteria or viruses, making rapid polymerase chain reaction-based diagnostics non-viable. In addition, as prions are modified cellular proteins and not foreign, there is an absence of a measurable host immune response; hence, an enzyme-linked immunoadsorbent assay (ELISA) diagnostic test is not feasible. The best diagnostic marker for prion diseases is the presence of the disease-associated isoform of the prion protein, PrP^{Sc} [30]. This is generally detected by western blot assay in the brain and in lymphoid tissues in vCJD [31], but attempts to detect PrP^{Sc} in blood from patients with vCJD have so far been unsuccessful, probably because of limitations in the sensitivity of this assay [32]. However, a conformation-dependent immunoassay was recently described that measures both the protease-resistant and protease-sensitive forms of PrP^{Sc} [33] and appears to be far more sensitive than western blot assays. Whether this method will be applicable to blood samples remains to be seen. Another technique that has recently been developed for enhanced detection of PrP^{Sc} is the cyclical amplification method [34]. This relies on a repeated series of incubation with normal PrP and subsequent cycles of sonication, and has recently detected PrP^{Sc} in blood from a rodent model of TSE [35].

Probable pattern of tissue infectivity in vCJD

In the UK, it is presumed that most of the adult population was exposed to the BSE agent through the ingestion of contaminated meat products in the late 1980s and early 1990s. However, because the incubation period of BSE in humans is unknown (incubation periods of 40 years or longer have been documented for other human TSE) [17], and because of the lack of a reliable screening test, it is currently unknown how many people may be in an asymptomatic phase of vCJD infection in UK.

In contrast to sCJD, vCJD infectivity is more widely distributed outside the CNS, and can readily be found in the peripheral nervous system and lymphoid tissues (tonsil, spleen, lymph node and gut) [31]. The levels of infectivity in these tissues are lower than in the CNS, but they still represent possible sources of person-to-person spread of infectivity (Fig. 2) [36]. As the asymptomatic phase of infection in vCJD may last for at least several years, infected individuals may represent a potential source of secondary spread of vCJD to others via contaminated surgical instruments (such as tonsillectomy instruments) or by blood transfusion.

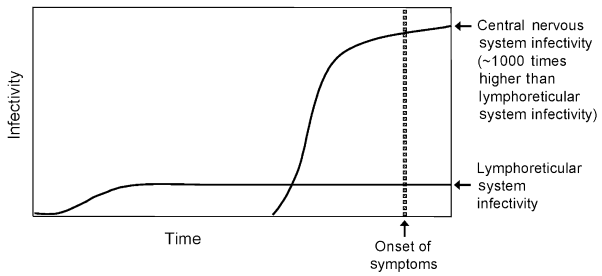

Fig. 2. Probable pattern of tissue infectivity in variant Creutzfeldt–Jakob disease [36].

Variant CJD prevalence study in UK

To estimate the number of individuals in the UK who are asymptomatic for vCJD and who could potentially contribute to the iatrogenic spread of the disease, a retrospective study of lymphoid tissues was recently performed using immunohistochemistry for prion protein in surgically removed tonsillectomy and appendectomy specimens. Researchers reported three positive samples out of 12 674 tested, or an estimated prevalence of 237 vCJD cases per million in the UK (CI 95%) [37,38].

These findings indicate a far higher prevalence than clinical cases would predict, suggesting that additional cases of vCJD are likely to emerge in the UK. Furthermore, they emphasize the importance of preventive measures already instituted by the UK Department of Health to reduce the potential spread of vCJD through blood therapies. These findings also point to the urgent need for large-scale screening of lymphoreticular tissue samples to determine with greater precision the incidence of vCJD infection in the asymptomatic UK population [38].

However, there remains a distinct possibility that some infected patients may never develop clinical symptoms but will remain asymptomatic carriers who can potentially transmit the disease to other individuals. Therefore, screening of infectious individuals will be a critical component for individuals who rely on blood transfusions and/or blood therapies.

Transmission of vCJD infectivity via blood transfusion in humans

Two cases of probable iatrogenic vCJD transmission through blood transfusion have been reported. The first case was a 69-year-old male who presented with

clinical symptoms typical of vCJD in 2002, 6.5 years after receiving one unit of non-leucodepleted packed red blood cells [39]. This patient died 1 year later. Sequencing of the prion protein gene revealed that he was methionine homozygous at codon 129 of the prion protein gene. The asymptomatic donor developed symptoms 3.5 years after donation and subsequently died.

The second case was an elderly female patient who was a known recipient of a blood transfusion from an asymptomatic donor who later developed vCJD [40]. The female patient died of an unrelated illness and without any vCJD clinical symptoms. Because of her known exposure, a medicolegal autopsy was performed. Abnormal prion protein was detected in the spleen and lymph nodes; however, PrP^{Sc} was not detected in the CNS and there were no other significant abnormalities in the CNS. Interestingly, this patient was heterozygous (M/V) at codon 129 in the prion protein gene.

Because that was the first identified case of vCJD infection occurring in the heterozygous subgroup [40], this case raises many important issues regarding the disease, including whether this genotype may have influenced either its incubation period or distribution of infectivity in this patient. These findings underscore the importance of developing effective screening tools and techniques to identify blood donors who may be asymptomatic. In addition, they highlight the need to ascertain whether all vCJD/BSE infections result in clinical disease or whether a subclinical carrier state may occur.

Epidemiological considerations

In the absence of a transfusion-transmitted infection, one statistical analysis has estimated that the probability of acquiring vCJD is approximately 1 in 15,000 to 1 in 30,000 [39]. Therefore, while dietary exposure can never entirely be ruled out, in the aforementioned cases, the infections were far more likely associated with vCJD-contaminated blood transfusions.

To examine a probable link between transfusion and vCJD infection, a review of blood transfusion policies in the UK and a risk assessment on the implications for plasma therapy recipients was commissioned by the Department of Health [41]. The commissioned research concluded that the infectivity concentrations in blood were likely to be highest in the buffy coat fraction, followed by those in plasma and whole blood (Table 3). Moreover, the report stated that levels of the infectious agent present in a full unit of blood would probably be sufficient to cause infection in recipients [41]. The Department of Health's Health Protection Agency also evaluated the risk of different plasma products in an attempt to determine which were most likely to carry the greatest degree of vCJD infectivity. Recipients of factor VIII, factor IX and antithrombin were estimated to have the highest risks: administration of even a single one-vial dose of these products was determined to be sufficient to cause transmission of the disease [42]. Intravenous immunoglobulin (IVIG) and large doses of albumin were concluded to be of medium risk, and anti-D and IVIG were determined to be of low-risk of infectivity.

Table 3. Selected infectivity of blood components [41].

	Volume (mL unit^{-1})	Infectivity (ID_{50}/unit)	Infectivity concentration (ID_{50}/unit)
Whole blood	450	900	2.0
Plasma	225	480	2.1
Filtered plasma	225	480	2.1
Red cells	212	219	1.0
Buffy coat	14	201	14.9

The risk of contracting vCJD from plasma therapies

As recipients of plasma therapies appear to possess the highest risk of contracting vCJD, it is theoretically possible that many patients with bleeding disorders in the UK have already been exposed to the agent responsible for vCJD. Patient groups and the UK Haemophilia Centre Doctors' Organisation believed that the Health Protection Agency's CJD Incidents Panel should recommend that all patients with bleeding disorders in the UK who were treated with UK-source pooled factor concentrates between 1980 and 2001 be considered at potential additional risk for public health purposes [42].

The risk of contracting vCJD has implications for the overall safety of the worldwide blood supply. To address this concern, various measures have been taken to protect the blood supply in the UK, including the sourcing of plasma from the United States (Table 4). Future efforts to minimize the risk of prion contamination of the blood supply might include improved filtration steps to more effectively remove this pathogen.

Variant CJD worldwide as of October 2005

As of October of 2005, 184 confirmed cases of vCJD have been reported worldwide. Individual countries include: UK (158), France (15), Ireland (3), Italy (1),

Table 4. Measures taken to reduce the risk of variant Creutzfeldt–Jakob disease (vCJD) transmission via blood and blood therapies in the UK.

Date	Measure
1997	Withdrawal and recall of any blood components, plasma therapies or tissues obtained from any individual who develops vCJD
1998	Importation of plasma from the USA for fractionation
1998–1999	Leucodepletion of all blood used for transfusion
2002	Importation of fresh plasma from the USA for patients born on or after 1, January 1996
2004	Blood donation is not accepted from people who have received a blood transfusion in the UK since 1980, or who are unsure of this
2005	Donors of blood to patients who have subsequently developed vCJD are advised that they may be at 'increased risk' of vCJD and should not continue to donate blood
Today	Promotion of appropriate use of blood and alternatives in NHS
The future?	Use of 'prion filters'?

USA (1), Canada (1), Saudi Arabia (1), Japan (1), the Netherlands (1), Spain (1) and Portugal (1). The individuals in the USA, Canada and Japan who contracted vCJD and one person in Ireland had all lived in the UK; therefore, these four cases are considered as UK infections.

Japan confirmed its first case of vCJD in 2005. This patient had briefly visited the UK in the late 1980s, fell ill in 2001 and died in 2004. While BSE has been identified in 15 Japanese cattle, officials contend that the patient most likely contracted the disease while in the UK [43]. Because the patient is believed to have visited the UK for less than a month, the Japanese government has changed its blood donation policy to ban donations from anyone who visited UK for a day or more between 1980 and 1996. Previously its policy had been to accept blood donors who had visited the UK for up to 1 month [44].

The fact that cases of vCJD have been reported in many different countries suggest that the disease has spread from the UK to other continents. Although the number of deaths per annum of vCJD in the UK has steadily declined from 28 in the year 2000 to only two by the middle of 2005, the onset of new cases has gradually risen to nine in 2004 from five in 2003 [45]. These data suggest that the disease may become endemic at a low level in the UK population.

Research priorities for vCJD

There are four immediate research priorities. First, to reduce the potential spread of vCJD, there is an urgent need for development of a new screening assay that is applicable to blood and is both highly specific and sensitive. Second, enhanced epidemiological surveillance of potentially infected donors should be broadened to encompass all age groups in the UK. Third, improved methods of decontamination of surgical and laboratory instruments must be developed and implemented across the country to reduce further iatrogenic infections. Finally, progress in the treatment and prophylaxis of vCJD is desperately needed.

Conclusions

In the last decade, a variant of CJD has emerged in many countries that has been causally linked to dietary exposure to BSE during the 1980s and early 1990s. Preliminary studies in animal models suggest that prions, including the BSE agent, can be transmitted by blood. Based on two recent reports of iatrogenic vCJD transmission by blood transfusion in humans, a UK DOH-sponsored risk assessment warned that recipients of plasma therapies are now at risk of contracting vCJD from potentially infected donors. In the absence of screening tests or effective therapies to treat this disease, a formidable worldwide public health challenge lies ahead to prevent new infections, accurately assess infection rates and treat infected patients.

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Clinical implications of emerging pathogens in haemophilia: the variant Creutzfeldt–Jakob disease experience

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Summary. The impact of variant Creutzfeldt–Jakob disease (vCJD) on the clinical practice of haemophilia in the UK is coloured by the haemophilia community's experience of hepatitis C virus and human immunodeficiency virus (HIV) transmission via plasma-derived therapies in the 1980s, when the delay in recognizing and acting on the potential risks cost many patients their lives and left others to manage another chronic disease. This crisis prompted organisations such as the United Kingdom Haemophilia Centre Doctors' Organisation to advocate for the introduction of haemophilia therapies that would not be susceptible to contamination with blood-borne pathogens. After the identification of vCJD in 1996, a number of public health measures were taken in response to a government-sponsored vCJD risk assessment, and following reports of transfusion-transmission of vCJD, additional guidelines have been developed to prevent person-to-person transmission, some of which may impact the quality and availability of medical and surgical care. Variant CJD has had a significant negative effect on the UK haemophilia community, shaking patient confidence in the therapies they have received over the last 21 years, affecting the quality of care and creating the risk of stigmatizing the community as it was in the 1980s. As with HIV and vCJD, emerging blood-borne infectious agents will likely affect blood and blood-derived therapies well before we become aware of its presence. As a result, only therapies with the lowest level of risk should be used for care of patients with haemophilia.

Keywords: haemophilia, pathogen, variant Creutzfeldt–Jakob disease

Introduction

This article will review the impact of variant Creutzfeldt–Jakob disease (vCJD) on the clinical practice of haemophilia in the UK, with particular attention to how haemophilia treater and patient organizations have responded to this concern. The haemophilia community's response to vCJD is best understood in the context of the significant morbidity and mortality caused by the transfusion-transmitted hepatitis C virus (HCV) and human immunodeficiency virus (HIV) infections contracted in the 1980s. Given the delayed recognition of the risk that HIV and HCV posed to patients with haemophilia, the subsequent lack of rapid response and the many missed opportunities to protect patients from contaminated plasma-derived therapies, it is understandable that many patients with haemophilia and their caregivers are now very alert to the potential implications of emerging pathogens such as vCJD. This is especially true for those patients who still rely on plasma-derived therapies and transfusions.

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UKHCDO therapeutic guidelines

The United Kingdom Haemophilia Centre Doctors' Organisation (UKHCDO) was established in 1968 by doctors treating patients with bleeding disorders who sought to improve care, conduct research into the disorders and facilitate healthcare planning. The UKHCDO and the patient organization the Haemophilia Society had, for many years, argued for the introduction of recombinant therapies. This view was reflected in the UKHCDO haemophilia treatment guidelines, published in 1997, which stated that recombinant factor concentrates were the treatment of choice for patients with haemophilia [1]. The guidelines further stated that recombinant factor concentrates were the safest with respect to reducing the risk of transfusion-transmitted infection. At the

time the UKHCDO guidelines were released, the general consensus among haemophilia treaters was that the plasma therapies used in the UK had a relatively low risk for transmission of hepatitis or HIV, but because they could transmit other infectious agents, such as parvovirus B19 and hepatitis A, [2,3] they might in theory be the route of infection for new or altered agents.

The UKHCDO guidelines were accepted by most treaters but not by the majority of healthcare commissioners. In particular, the future risk of infection by emerging pathogens through plasma therapy was not accepted. Approximately 6 months later, the potential threat of vCJD to the haemophilia community emerged.

Shortly after vCJD was first described in the UK in 1996, concerns were raised that it could be transmitted through blood transfusion and blood therapies [4]. As a result, the UKHCDO convened a meeting with experts on prion diseases, including members of the National CJD Surveillance Unit and the Spongiform Encephalopathy Advisory Committee (SEAC), both of which were formed in 1990. The National CJD Surveillance Unit is sponsored by the Department of Health (DOH) and the Scottish Executive Health Department; SEAC is sponsored jointly by the Department for Environment, Food and Rural Affairs, the DOH and the Food Standards Agency (FSA). The purpose of the meeting was to determine, by means of a thorough review of all available evidence, if there were any measures available to effectively reduce the risk to patients with haemophilia of contracting vCJD and other prion-based diseases.

At the time, in 1997, vCJD had only been identified in Great Britain. Limited research indicated that this was a new disease with a long incubation period [5]. Relatively little epidemiological data were available, but evidence from some animal studies indicated that there existed the possibility of transfusion-transmitted vCJD infections. Further, it was surmised that many vCJD-infected, yet asymptomatic, individuals were continuing to donate blood that would be used in the processing of factor VIII and factor IX therapies. At that time, many patients with haemophilia in the UK were treated with UK-sourced plasma factor concentrates.

Based on the 1997 meeting of the UKHCDO, SEAC and the National CJD Surveillance Unit, several recommendations emerged [4]:

1 Healthcare providers should reduce the risk of vCJD transmission by using plasma factor concentrates sourced in other countries.

2 Recombinant factor concentrates should remain the treatment of choice for patients with haemophilia.

3 Plasma-derived concentrates processed with non-European plasma, preferably from the US, should be provided for those patients for whom recombinant factor concentrates were not made available.

As a consequence of these recommendations, the two main UK fractionators of plasma, Bio Products Laboratory and the Scottish National Blood Transfusion Service, were obligated to stop processing factor concentrate therapies. In the meantime, the UK imported plasma from the US for processing factor VIII and factor IX. This ban on utilization of UK-derived plasma resulted in long delays in resuming the processing of factors and interrupted the supply of other niche therapies such as factor VII and factor XI.

Patients and providers respond

Prior to 1997, many patients with haemophilia and their physicians held the view that UK-sourced plasma therapies were safer than any alternative and there had been a relatively slow uptake of recombinant therapies. With the introduction of these policies recommending the use of non-UK-sourced plasma, however, patient confidence was undermined and the pressure increased on government and healthcare commissioners to make recombinant therapies more widely available.

Against a background of increasing concern about the possible risk of vCJD, England's Department of Health agreed that recombinant therapies should be made available to all children with haemophilia [6]. In other health departments, in Scotland, Wales and Northern Ireland, they took the recommendations one step further and introduced recombinant therapies for all patients. But in England, the most populous country in the UK, adults continued to be prescribed and use plasma therapies, although derived from plasma imported from the USA.

Variant CJD: a potential new threat to factor concentrate safety

In 2000, Bio Products Laboratory notified the UKHCDO about the identification of batches of factor concentrates that had been prepared in 1996 and 1997 and used before 1998. It was determined that these concentrates were prepared from plasma pools that included plasma from a donor who had

subsequently developed vCJD. Since then there have been further notifications of batches of factor concentrates prepared from plasma from donors who were later diagnosed with vCJD. Table 1 enumerates all the batches of therapies distributed and subsequently identified as being potentially infected with vCJD, as of September 2004 [7]. These therapies were produced by either Bio Products Laboratory or Protein Fractionation Centre and, in most circumstances, many patients were treated with these therapies before notification had been given.

At the time there was no clear evidence that vCJD could be transmitted by blood products. There was no test to identify potentially asymptomatic but infected donors, and there was no treatment to offer patients for reassurance or for further assessment. Because vCJD has a long incubation period, clinical examination was of little or no use. With these facts in mind, healthcare providers and policy makers were faced with the decision of what, or even if, to tell their patients.

Response to possible risk of transfusion-transmitted vCJD

In 2004, the decision was made to inform all patients about the possible risk of transfusion transmitted vCJD, irrespective of whether they had received concentrates or not from the implicated batches. Patients were given three choices: they could come into their healthcare providers' offices and discuss the information in person; they could choose to be fully informed by letter; or they could refuse to be informed in any way. Many patients chose the third option. Patients who chose to be educated about the potential risks were given information disclosing that they might be infected with vCJD. Given that the majority of patients were not able to have access to recombinant therapies, this situation caused considerable concern.

For the UKHCDO, responding to the potential infection of haemophilia patients created a huge administrative burden. There was an urgent need to review all records, to contact all patients possibly infected and to give each of them the option to review all information then known about vCJD. Added to the administrative burden were government-mandated timelines as to when the patients needed to be informed.

The threat of vCJD among members of the haemophilia community increased the political pressure for more widespread use of recombinant coagulation factor concentrates in the UK. And as a result, as of April 2005, all patients with haemophilia A and B have been offered recombinant factor concentrates.

Table 1. Batches of 'implicated' UK plasma therapies [7].

Factor VIII	16*
Factor IX	8*
Antithrombin	1
Immunoglobulin G	11
Albumin 4.5%	28
Albumin 20%	21
Factor VIII with albumin excipient	76
Intramuscular immunoglobulin	12

*Indicates widely distributed throughout the UK.

Risk of vCJD from implicated plasma-derived concentrates

One of the questions that remain unanswered today is what risk do the recipients of plasma concentrates exposed to vCJD pose to others? This issue came to the forefront in December 2003 when the Health Secretary informed the UK Parliament of the first death probably related to transfusion-transmitted vCJD. This case was later confirmed as being related to vCJD [8,9].

The Department of Health established the CJD Incidents Panel, an expert committee sub group of the Advisory Committee on Dangerous Pathogens Working Group on Transmissible Spongiform Encephalopathies, in 2000 in order to help the medical community handle cases such as this. The mandate of this committee is to review the available literature, establish a formal risk assessment of infectivity of blood and blood therapies and formulate guidelines for response by the medical community. The CJD Incidents Panel advises hospitals, trusts and public health teams throughout the UK on how to manage incidents involving possible transmission of CJD between patients.

Based on a risk assessment commissioned by the DOH in 2003, the CJD Incidents Panel attempted to identify patients who had received at least one dose of a plasma therapy, which the committee judged to increase the risk of vCJD exposure by more than 1% over background. Therapies that were considered the highest risk were factor VIII, factor IX and antithrombin. The administration of just one vial, or 500 units, was considered enough to put patients in a high-risk category. Medium risk therapies included intravenous immunoglobulin G and albumin 4.5% administered in large doses. Low-risk therapies were defined as albumin 20%, intramuscular immunoglobulin and factor VIII with excipient albumin administered in extremely large doses [10].

In refining the risk assessment, the question emerged: which of the 'at risk' patients need be treated with precaution: those with known exposure to contaminated or potentially contaminated batches of plasma concentrates, or any patient treated with plasma-derived concentrate in the period from 1980 to 2001? Because the possibility existed that, over time, additional donors might be identified as having vCJD, it was decided to treat all haemophilia patients who had used therapies from UK-derived plasma in this 21-year-period with measures designed to reduce the risk of human-to-human transmission [11].

Measures to prevent human-to-human vCJD transmission

Following the 2001 release of a DOH-sponsored summary of the risks of vCJD transmission via surgical implements [12], the Advisory Committee on Dangerous Pathogens and the Spongiform Encephalopathy Advisory Committee published a set of guidelines in 2003 for the precautionary management of potentially-infected patients, both healthy and deceased, in order to minimise the risks of transmission to other patients and healthcare staff [13]. These guidelines were a significantly expanded version of recommendations that were released in 1998 but kept under review until a number of uncertainties were better understood, including the routes of infection, threshold infectious dose, potential for inactivating the agent and the quantity of people who might be incubating the disease.

The detailed guidelines recommend measures for laboratory containment and control, infection control of CJD and related disorders in a healthcare setting, decontamination and waste disposal and quarantining of surgical instruments, among others. For example, when patients who used UK-sourced plasma-based therapies in the years 1980–2001 undergo any surgery involving high-risk tissues, such as the central nervous system or the lymphatic system, the surgical instruments used must be subsequently destroyed [14].

Some general precautions included using single-use instruments wherever possible; performing all procedures in a controlled environment, such as an operating theatre; performing the procedure after all others; involving the minimum number of healthcare personnel; and using liquid-repellent operating gowns over plastic aprons, as well as goggles or full-face visors [15].

More controversially, the guidelines stipulated that if these patients have an endoscopic procedure of the gastrointestinal tract or the olfactory mucosa, the instruments used in those procedures also must be quarantined, i.e. not used again or destroyed [15]. The quarantine or destruction of surgical instruments has, of course, financial consequences: the quarantine of an endoscope is estimated to cost approximately £30 000 per instrument per year. Endoscopy services are in high demand, and quarantining an endoscope, or destroying it after every use, is not a reasonable or cost-effective policy for any healthcare institution. In the risk-assessment guidelines, it was suggested that capsule wireless endoscopes be used instead, but expertise in capsule endoscopy is limited, so the issue has yet to be fully resolved.

Potential stigmatization

One of the negative outcomes of the distribution of the guidelines of the CJD Incidents Panel was that persons with haemophilia became identified as presenting a risk of infection to others. In some medical centres, reluctance to performing invasive procedures became an issue in all but serious cases.

Despite assertions that these precautions should not compromise care for patients with haemophilia, the potential exists that these patients will be stigmatized again, as they were early in the HIV crisis, and that their normal medical and surgical care may be interrupted.

Scope of the problem

Cases of vCJD have also been reported outside the UK. In France, for example, 14 cases of vCJD have been reported, with three identified in persons who donated blood over a 10-year-period. Again, most of the donations have been used to make factor VIII, von Willebrand factor, and other plasma therapies. In response, the French have recalled all plasma-derived therapies, where possible, and all patients have been informed.

To further complicate matters, it is known that the French fractionators have exported concentrates to other countries, such as Belgium. And in the UK, Bio Products Laboratory also exported factor concentrate to other countries. At this point in time, there are no clear guidelines on how to manage potential risk in these situations.

Another concern involves haemophilia patients who visited the UK: unknown numbers of visitors were treated with UK-sourced factor concentrates during the crucial 21-year-period. Because records on the treatment of visitors to the UK are not readily

available, it is very difficult to identify or advise those patients.

Conclusion

The phenomenon of emerging vCJD is yet another warning against the complacent assumption that plasma-derived therapies can be made completely safe. Variant CJD has had a significant negative effect on the haemophilia community in the UK, shaking patient confidence in the therapies they have received over the last 21 years, affecting the quality of current and future medical and surgical care and creating the risk of stigmatizing the community as it was in the 1980s, at the beginning of the HIV crisis.

Our awareness of vCJD is not even a decade old. Much about the disease is still unknown, including the best means for preclinical detection and effective inactivation. But given its long incubation period, it's possible that the impact of vCJD on patients with haemophilia may be significant.

As described elsewhere in this supplement, the barriers to the emergence of pathogenic agents, both air- and blood-borne, continue to diminish. And as with HIV and vCJD, the next emerging blood-borne infectious agent will likely affect blood and blood-derived therapies well before we become aware of its presence. It is because of these reasons that only the therapies with the lowest level of risk should be used for care of patients with haemophilia.

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The physician's role in selecting a factor replacement therapy

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Summary. Over the past 20 years, transmissions of human immunodeficiency virus (HIV), hepatitis B virus or hepatitis C virus have been virtually eliminated from plasma-derived or recombinant therapy in the USA, a record that can be largely attributed to the use of effective screening and inactivation technologies for known pathogens. The next significant threat will likely come from the emergence of a new, blood-borne infectious disease, perhaps one transmitted by a non-lipid-enveloped virus or prion, for which current inactivation methods are ineffective. Following the HIV crisis of the 1980s, government, patient advocacy groups, medical and scientific communities and the manufacturers of clotting therapies can learn from the past and approach potential threats from emerging pathogens in a proactive and productive manner. For clinicians, this includes actively engaging patients in a dialogue about all the factors that may influence their choice of clotting factor therapies, including emerging pathogens, patient convenience, consistency and reliability of supply, relative cost/benefit ratios, reimbursement issues (where applicable), patient preference and brand loyalty. It is our obligation as healthcare providers to understand potential risks and help make proactive decisions with our patients, decisions that often must be made in an environment of scientific uncertainty. Threats from infectious agents that were once deemed theoretical can, and often do, ultimately become real, with serious implications for morbidity and mortality.

Keywords: haemophilia, pathogens, plasma, recombinant, therapy

Introduction

The principle of evidence-based medicine promotes the judicious and conscientious use of the current best evidence when making healthcare decisions for individual patients. While this strategy, popularized in the early 1990s, is frequently useful, it is clearly of limited benefit in the context of emerging infectious diseases. By the time concrete evidence of an emerging infectious agent is available, it is often too late to prevent infection in the most susceptible populations. Proactive decision-making processes regarding the safety of our blood supply cannot rely solely on an evidenced-based approach. Rather we can take the lessons of the past and, using our current knowledge of disease causing agents, extrapolate potential risks in order to better formulate effective healthcare policies.

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Learning from the past

Within the USA, in the years immediately preceding the human immunodeficiency virus (HIV) epidemic, medical and scientific communities, government agencies and the blood therapies industry operated in an environment of shared responsibility to ensure the safety of the blood supply. Through the exercise of regulatory authority, established standards for plasma collection, product manufacturing and licensing, blood surveillance and fundamental research efforts, the system worked effectively to supply the nation with necessary blood and blood products that checked for most human safety threats. The weakness of this system was deemed to be its inability to deal with a new threat that was characterized by substantial uncertainty. This was the subject of a report from the Institute of Medicine commissioned by the Department of Health and Human Services [1]. From the first description of cases of HIV in haemophilia patients in 1982 to the development of a serum test for HIV in 1985, policy-making was extremely difficult as much more was unknown than known. During that time, evidence of the risk of acquired immunodeficiency syndrome (AIDS) was

downplayed, even regarded as 'theoretical', and time was lost while evaluating the estimates of the substantial costs of safeguards. The high regard for the efficacy of antihaemophilic factor concentrates for treating a devastating disease like haemophilia, as well as scepticism that AIDS was transmitted through blood products, led to a lack of specific recommendations about blood product use from patient groups and physicians treating individuals with haemophilia. In turn, rather than inform patients about risks and benefits, physicians tended to decide for themselves what was best so as not to burden patients with difficult decisions about treatment options [2].

In defence of these stakeholders, initially there was little or no reliable evidence of HIV [and later hepatitis C virus (HCV) transmission] via plasma-based therapies. As the 1980s proceeded, however, research showing that these diseases were, in fact, transmitted through blood products began to increase and come to the attention of all involved with the haemophilia community.

While the transmission pathways and disease processes for HIV and HCV are now well understood, uncertainty remains about the potential for variant Creutzfeldt–Jakob disease (vCJD) and other emerging pathogens to harm patients with haemophilia. The clear message is that it is our obligation as healthcare providers to help make proactive decisions with our patients, and that often these must be made in an environment of scientific uncertainty. Threats from infectious agents that were once deemed theoretical can, and often do, ultimately become real, with serious implications for morbidity and mortality.

Safety concerns: pathogen risks and inactivation efforts

The current safety of haemophilia therapies can largely be attributed to the use of effective screening and testing technologies available for HIV, Hepatitis B virus (HBV) or HCV. No seroconversions to HIV, HBV or HCV have been reported with any of the FVIII products currently marketed in the USA [3]. The real challenge, however, is presented by the likely emergence of a new, blood-borne infectious agent. For this, we rely on inactivation measures.

For the purposes of discussing inactivation measures, infectious agents can be classified in three categories:

1 Lipid-enveloped viruses, for example, HBV, HCV and HIV
2 Non-lipid-enveloped viruses, for example, parvovirus B19 (PVB19) and hepatitis A (HAV)
3 Disease-causing prions, for example, CJD and vCJD

Lipid-enveloped viruses

Lipid-enveloped viruses have a protective fatty membrane, or envelope; if this membrane is destroyed the enclosed virus is also destroyed. There is very good evidence that the lipid-enveloped viruses are effectively inactivated with current technologies [4–6]. All licensed processors of plasma therapies and recombinant therapies made with human and animal protein additives use validated methods to both detect and eliminate HBV, HCV, HIV, and the other lipid-enveloped viruses for which screening and inactivation methods have been developed [7]. West Nile virus is another lipid-enveloped virus for which effective screening methods exist; for example, in the period January–November 15, 2005, 382 presumptively viremic blood donors were successfully detected in the USA [8].

Non-lipid-enveloped viruses

As the term would indicate, non-lipid-enveloped viruses lack a protective enclosure. The lack of an envelope makes it difficult to target these viruses for inactivation. In addition, the physical and chemical conditions created by vapour/heat, solvent/detergent and gamma irradiation technologies adequate for inactivation of non-lipid-enveloped viruses may denature the factor VIII (FVIII) protein [9]. The small size of some non-lipid-enveloped viruses, such as PVB19, requires filter sizes generally not practical for the successful large-scale filtration of commercial blood products [10].

Parvovirus B19

Human PVB19 is a widespread non-lipid-enveloped virus that causes the childhood illness fifth disease. Community-acquired exposure and seroconversion to this virus is common and occurs quite early in the lives of most people. PVB19 shares the hallmark of other non-lipid-enveloped viruses: resistance to inactivation technologies. PVB19 is of particular concern to the haemophilia community because of reports that it has been found in factor concentrates. A prospective study published in 1997 by Santagostino *et al.* [11] indicated that very high temperatures applied to lyophilized factor concentrates did not prevent the transmission of PVB19 to patients with haemophilia.

Soucie *et al.* [12] evaluated the risk of PVB19 transmission as a result of treatment with recombinant antihaemophilic factor. To compare the seroprevalence of PVB19 antibodies in 2- to 7-year-old males with haemophilia, 798 subjects were grouped by their exposure to types of factor VIII or IX concentrates: those who had received only plasma-derived therapies, only recombinant therapies both recombinant and plasma-derived therapies, or no antihaemophilic factor at all (control group). The study found that the prevalence of seropositivity was higher in both the plasma-derived only and recombinant and plasma-derived therapies groups than in the control and recombinant-only groups [12].

The results of this study illustrate the potential for non-lipid-enveloped viruses to persist in blood and blood-based therapies. In 2001, processors of plasma-derived factor therapies instituted the use of nucleic acid amplification technology (NAT) to screen plasma and adopted a voluntary industry standard for the management of PVB19. Nonetheless, Soucie *et al.* [12] recommended the development of effective virus inactivation techniques for parvovirus and other non-enveloped viruses that have yet to be identified or may emerge in the future.

Disease-causing prions

As with PVB19 and other non-lipid-enveloped viruses, current inactivation methods are relatively ineffective against prions. Prions are soluble cellular protein particles (PrP^{C}) that lack nucleic acid and do not depend on genes or other factors for transmission. As discussed elsewhere in this supplement, transmissible spongiform encephalopathies (TSEs) such as the sporadic and variant forms of CJD are characterized by the accumulation of an abnormal form of this protein particle (PrP^{Sc}) in the brain.

Because prions lack nucleic acid, standard NAT testing cannot be used to detect vCJD and other TSEs. Also, the presence of abnormal prions does not trigger a measurable host immune response, making standard enzyme-linked diagnostic tests that measure antibodies, such as those used for AIDS, useless. New, hopefully more effective screening methods are currently in development.

During the disease's incubation period, estimated to be upwards of 40 years, infected individuals appear clinically healthy. Without a screening method for vCJD, donations by infected individuals will continue to pose a threat to patients using blood-derived therapies.

Opportunities for discussions

Most patients become aware of the worldwide emergence of air- and blood-borne pathogens through articles and news broadcasts in the popular press. Without the benefit of broad-based medical knowledge for context, patients may become unduly alarmed about the risks these infectious agents pose. Haemophilia treaters have an opportunity to be proactive in addressing their patients' fears about these diseases and the particular health threats that blood-borne infectious agents might pose. To be effective, practitioners must themselves be informed about these new infectious agents and the relative safety of the available therapeutic options.

The point of these discussions is to address patients' fear and anxiety with regard to these new infectious diseases and to enhance patient trust in both haemophilia treaters and their therapeutic options. Fear is unavoidable if the potential threats of emerging pathogens are not met with an informed and appropriate response.

Discussing the risk of transmission via factor therapies

Haemophilia treaters must be cognizant of and acknowledge the risk that emerging pathogens present their especially vulnerable patients. The majority of practitioners are cautiously confident about the safety of current plasma-derived clotting factors and recombinant therapies [13,14]. Their optimism is the result of >20 years of access to blood therapies free of HIV and >10 years of access to therapies free of HCV.

Today paediatric haemophilia treaters, for example, see few if any HIV- or HCV-infected patients [15]. As a result, it is unlikely that these practitioners engage in regular conversations with their patients regarding emerging pathogens or the safety of the blood supply. Haemophilia consumer or advocacy groups have shared the responsibility for educating patients about these risks. However, in order to offer their patients with haemophilia the best possible care and to maintain trust, clinicians themselves must address their individual patients' needs and concerns with up-to-date information.

Current treatment guidelines

In recent years, patient advocacy groups and haemophilia treaters' organizations have provided information, recommendations and guidelines to help educate practitioners and others in the haemophilia community about the issue of the safety of the blood

supply. Recommendations for proactive therapeutic measures have been implemented in the past regarding clotting factor concentrates. Within the USA and the EU, most patients with haemophilia have switched from plasma-derived to recombinant therapies since the first recombinant FVIII was approved in 1992 [16,17]. The majority of Canadian haemophilia patients were converted to recombinant FVIII in 1994 [18]. This decision was made by the Canadian Blood Agency at the advice of the Association of Hemophilia Clinic Directors of Canada who considered recombinant FVIII to be the safest replacement therapy available. Further recommendations have followed regarding vulnerabilities that may remain in the recombinant therapies owing to the addition of human or animal proteins during processing and final formulation. For example, in their 1999 update of the 1995 clinical practice guidelines for patients with haemophilia and von Willebrand disease, the Association of Hemophilia Clinic Directors of Canada stated that, 'until methods for total viral removal or inactivation are available, alternative methods of cell culture and stabilization of recombinant clotting factor concentrates should be sought to avoid the need for plasma-derived human albumin that's currently in use' [19]. Similarly, in November 2003, the Medical and Scientific Advisory Council (MASAC) of the US-based National Hemophilia Foundation (NHF) published Recommendation 151, advising manufacturers that 'all efforts should be made to remove human albumin from recombinant FVIII products' [3]. The first recombinant factor replacement therapy processed without the addition of any human or animal plasma proteins and albumin was approved by the US Food and Drug Administration (FDA) earlier that year, in July 2003. The UK Department of Health began a rolling phase-out of plasma-derived factor replacement therapies [20] and promoted the use of recombinant therapies that do not contain human or animal protein additives as the first-line choice for adults with haemophilia A and B; children under the age of 16 had previously been transferred to recombinant products following a 1998 provision. These expanded measures were taken as a precaution against possible vCJD transmission through blood and blood products. These guidelines indicate the proactive measures some organizations are taking to protect their communities from blood-borne infectious agents.

Additional factor replacement considerations

The primary goal of any haemophilia practitioner is to enable one's patients to live as actively and productively as possible. Selecting the appropriate factor replacement therapy for each patient can be very complex. Safety and efficacy are first and foremost considerations in choosing a haemophilia therapy. Inhibitor risk is also an important concern. Other considerations include patient convenience, and issues related to the consistency and reliability of supply for any particular therapy [14,21]. The relative cost/benefit ratio may also be a consideration, depending on which therapy is chosen [13,22,23]. In the USA, policies regarding reimbursement for therapies can differ from state to state, which can cause confusion and consternation for patients and providers alike. Last, but not least, it is important to take patient preference and brand loyalty under consideration when choosing the appropriate factor therapy as some patients have been resistant to switching to new products [22,24].

Conclusion

Underestimating what were seen as merely theoretical risks in the late 1970s and early 1980s ultimately resulted in the tragedy of HIV and HCV infection in patients with haemophilia worldwide. With the benefit of hindsight and the commitment of a proactive approach to emerging pathogens even in the face of scientific uncertainty, patients with haemophilia should hopefully never have to endure such a crisis again.

The most fundamental lesson learned in the past 25 years is that government, patient advocacy groups, medical and scientific communities and the manufacturers of clotting therapies all have an opportunity, and perhaps even an obligation, to approach potential threats from emerging pathogens in a proactive and productive manner. For clinicians, this includes actively engaging patients in a dialogue about emerging pathogens and the relative risks they might pose via available clotting factor therapies.

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DISCUSSION SESSION

Implications of Emerging Pathogens in the Management of Haemophilia

Discussion Session

1. Is there any evidence that haemophilia patients in the UK have been infected with variant Creutzfeldt–Jakob disease (vCJD) via therapies made from contaminated blood donations? Phrased differently, are there good data to support the decision in the UK to phase out the use of recombinant factor VIII (rFVIII) therapies processed with plasma additives, and are the surgical precautions in treating haemophilia patients necessary?

DOLAN: Initial discussions surrounding these issues were definitely controversial, and we in the medical community were not sure how far we needed to go in trying to protect patients. But the recommendations and surgical measures were devised after very detailed consultation with experts who knew far more about prion disease than we did.

Certain decisions, such as ceasing use of UK plasma-derived therapies, were difficult for both patients and their providers. But the subsequent events, in particular the later evidence that there have been at least two probable cases of transfusion-transmitted variant CJD, seem to justify that early stance by not just the UK but other countries as well.

2. Do you think that the fact that vCJD has not been identified in any patient receiving plasma derivatives worldwide since 1980 suggests that the risk of vCJD is minimal or non-existent from these therapies?

IRONSIDE: First of all, let's be quite clear about why 1980 has become a benchmark. The date 1980 was chosen simply because that was thought to be the earliest date at which human exposure to bovine spongiform encephalopathy (BSE) in the UK was likely to have occurred. Overall, human exposure to BSE probably would be very low in the early 1980s and highest in the late 1980s and early 1990s. It is also important to remember that we are dealing with a primary disease transmission with an incubation period of approximately 15 years on average. So, we may have to wait a few more years before we can be certain about the absolute risk of contracting vCJD.

I would be very cautious about relaxing policies and guidelines at present because, as we all understand, there are other emerging infectious agents – identified and unidentified – that are cause for concern in addition to the vCJD-causing prion.

3. Do you know of any vCJD transmissions by plasma-derived FVIII/FIX therapies?

IRONSIDE: At present, no. There is no evidence that vCJD has occurred or infection has been transmitted by these therapies. Although, as I stated earlier, this may be due to the fact that we are dealing with an agent that has a long incubation period. The level of infectivity in plasma therapies may be lower or variable. But it is too soon to exclude that possibility.

The United Kingdom Haemophilia Centre Doctors' Organisation, along with several patient groups, is engaged in enhanced surveillance of the haemophilia population. We are looking for evidence of vCJD – even of subclinical infection – in patients who died or who have a lymphoid tissue biopsy for whatever reason.

4. What is the likely impact of the UK experience with vCJD in the United States and what might those treatment implications be?

DOLAN: Reported cases of BSE in the United States are very few. And if the number of cases remains at this low level, or even disappears altogether, then perhaps US practitioners and policy makers won't be obligated to take the more sweeping measures that we did in the UK. However, as a general concept, we must all remember that emerging pathogens can affect transfusion therapy. So, based on the UK experience, if healthcare providers have an opportunity to minimize risk to patients, then it is a prudent course of direction that should be considered seriously and likely taken.

5. Are there data that leukodepletion of blood will decrease the risk of transmitting vCJD? If not, what is the rationale?

IRONSIDE: This is a very interesting question because the UK has been using leukodepletion as one of its main strategies for risk reduction in terms of blood transfusion. The data from experimental

studies do indicate that although leukodepletion will reduce infectivity, it will not remove it entirely.

Because leukodepletion does not remove all infectivity, there have been a number of other approaches that utilize additional filters that might bind more specifically to any free prion protein in the plasma and thus, further reduce the risk.

6. Please describe the results of experiments in which blood was spiked with vCJD concentrate to determine whether prions could be removed.

IRONSIDE: Results of a spiking experiment were published using blood containing a range of prions, including both sporadic and variant CJD prions. The study looked at the effect of plasma fractionation in removing the prions. And indeed, fractionation did seem to have a positive effect.

However, there are a number of concerns about these spiking experiments because they involve inoculating brain homogenate into blood and using that as the spike. Essentially, it is infected brain tissue, which is very unphysiological. Therefore, it is unlikely to replicate the form of infectivity found in blood-endogenous infection, where it is probably free in plasma and not aggregated as it would be in brain. So, while the spiking experiments do provide some reassuring information, a number of questions persist as to just how valid the spiking method is.

7. What about the results of the study in which 11% of patients who received recombinant therapy only were seropositive for parvovirus B19 antibodies soon after start of treatment? Aren't recombinant therapies totally free of any virus transmission risk?

TAPPER: As has been stated, the non-lipid-encased viruses are obviously much more difficult to inactivate. So if you ask, do the current technologies inactivate all pathogens, the answer is clearly no, they do not.

Parvovirus is one of the classic markers for these types of viruses. In children, parvovirus is relatively benign, but older people tend to get sick from it. Parvovirus can be viewed as a marker for pathogens that are either difficult to inactivate or that simply have not been fully described as yet. There are many viruses that fall into this latter category. For example, where did severe acute respiratory syndrome come from? Where did the coronavirus come from? It is clearly a novel virus that probably made a cross-species jump. You could say very much the same thing about human immunodeficiency virus when it was first described in industrialized countries in the 1980s, but clearly, phylogenetically, it had been present in Africa for at least 50 years prior to that time.

Factors such as the vastly increased ability of populations to travel, the issues surrounding land encroachment and the disruptions of the natural barriers between humans and humans and between humans and animals are clearly going to continue. And within that context, you can anticipate that new pathogens will continue to emerge, at least some of which, like West Nile virus, will be transmissible via blood.

PIPE: The medical community is not particularly concerned with parvovirus, but we're looking at it as a marker because it is one of the non-lipid-enveloped viruses for which we can actually screen. At this point in time, the theoretical concern would involve early seroconversions among patients who have depended solely on recombinant therapies. We would need to ask: is there the potential for another infectious agent – which either has or has not emerged yet, or that we don't have a test for – to become a threat to these patients?

What it comes down to is an issue of vigilance, and I think it is encouraging to see that when testing is available, such as prion screening, we are actively looking for patients who have the protein. Another encouraging example involves West Nile virus. It was only a very short period of time from its appearance to actually having an effective screening tool; this rapid response illustrates that the scientific world can respond quickly to address these kinds of issues.

8. What is the justification of continuing to use a therapy that is processed with bovine plasma protein?

PIPE: In a single clinic, I might talk to a patient with von Willebrand disease and a patient with another rare coagulation deficiency, both of whom would rely on plasma derivatives. With these patients I discuss the continued vigilance and screening that have resulted in the safety of these therapies thus far. I think it is important to inform them that there are ongoing concerns with respect to emerging pathogens, but also that as we learn more about potentially infective agents, we establish policies that will go a long way toward preventing another crisis in which emerging pathogens contaminate blood-derived therapies.

Alternatively, I will have a conversation with a family member or patient with either haemophilia A or haemophilia B and discuss with them the availability of newer therapies that are not processed with human or animal protein additives. The conversation with the patient with von Willebrand disease is very different than the one with the haemophilia patient: one is a conversation of reassurance, and the other a conversation of striving to be proactive, to help these

patients and their caregivers consider new therapies that may reduce the risk of infection with disease-causing agents.

Our history with haemophilia patients is interesting. In 1992, we switched all of our paediatric patients on FVIII to recombinant therapies. Then, in 1998 when recombinant FIX was available, we switched all of our patients from plasma-derived FIX to recombinant. That therapy had reduced recovery time in paediatric patients, and as a result, many patients had to use up to twice the amount of factor units that they would have had they remained on plasma-derived therapies. There is also the increased cost associated with the therapy.

The decision to switch patients to recombinant therapies was not based on any evidence of a known infectious agent being transmitted by plasma derivatives. Yet if you look at the data from the US Centers for Disease Control and Prevention on the adoption of recombinant therapies in paediatric patients, and indeed for adult patients around the US, it is quite remarkable how enthusiastically patients and clinicians have embraced recombinant technology.

For some patients, unfortunately, choice is not an option. There are patients in some areas of the US who do not even have access to recombinants. So, for these patients we must rely on the 20 years of safety that we have enjoyed with plasma derivatives. This relative safety should not lull us into a mode of complacency where we ignore emerging pathogens such as vCJD.

