



## EARLY PHASE OF vCJD INFECTION IN RECIPIENTS OF BLOOD TRANSFUSIONS

### Issue

1. The Committee on Microbiological Safety of Blood, Tissue and Organs has requested advice from SEAC on whether a scientific distinction can be drawn between historic and recent blood transfusion recipients in terms of the relative load of the vCJD agent that may be present in the bone, tissues or organs of the blood transfusion recipient. In the context of this question, a recent blood transfusion recipient is defined as having received a blood transfusion within a 24-48 hour period prior to bone, tissue or organ donation.

### Background

2. As a pre-symptomatic diagnostic test for vCJD is currently not available, blood, bone, tissue or organ donors with a sub- or pre-clinical vCJD infection cannot be identified prior to donation.
3. Two cases of vCJD infection in recipients of blood from blood donors that subsequently developed vCJD suggest that the disease may be transmitted between individuals by blood transfusion prior to development of clinical disease in the donor<sup>1,2</sup>. Experimental evidence of BSE transmission via blood transfusion from infected asymptomatic donor sheep to BSE-free recipient sheep supports the possible transfer of infection by this route<sup>3,4</sup>.
4. There have been no definite or suspected cases of vCJD transmission between humans via bone, tissue or organ

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<sup>1</sup> Llewelyn *et al.* (2004) Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. *Lancet* 363, 417-421.

<sup>2</sup> SEAC statement on the second presumed case of blood transfusion- associated infection with vCJD (2004).

<sup>3</sup> Houston *et al.* (2000) Transmission of BSE by blood transfusion in sheep. *Lancet* 356, 999-1000.

<sup>4</sup> Hunter *et al.* (2002) Transmission of prion disease by blood transfusion. *J. Gen. Virol.* 83, 2897-2905.

transplantation. However, epidemiological evidence of iatrogenic transmission of sCJD<sup>5</sup> suggests that there is a potential risk of vCJD transmission via some surgical procedures.

5. There are no data on the tissue distribution of vCJD infectivity in the first few days following transfusion with infected blood. Therefore, this paper predominantly focuses on summarising a previous assessment of vCJD infectivity in blood and limited data from clinical cases of vCJD and TSE studies in animals. Most prion disease pathogenesis studies have examined infectivity levels much later in the incubation period (e.g. from a number of weeks post-inoculation). However, very limited data from a few old studies that examined tissue infectivity levels early in the incubation period were identified from a literature search and are provided.

### **Previous SEAC consideration of vCJD transmission via tissue transplantation**

6. In 2004, SEAC considered the possible risks of transmission of vCJD via tissue transplantation. A number of factors were identified that may influence the level of infection risk:
  - type of tissue/organ and infectious load (possible influence on infectious dose),
  - mass tissue/organ transplanted (possible influence on infectious dose)
  - site of transplant (possible influence on transmission efficiency)
  - pooling of tissues (possible dilution of dose)
7. SEAC noted that, because of the scientific uncertainties about the infectious load in tissues and the prevalence of asymptomatic infection in the population, a quantitative risk assessment was not possible. However, the scientific uncertainty about infectious dose could be addressed in part by assuming that a linear dose-response relationship for infection exists with no threshold for a minimum infectious dose. Thus, the chance of infection would be proportional to the dose of infectious agent received. Under these circumstances a dose of 2 ID<sub>50</sub>s or above could be considered as highly likely to cause infection. Given estimates for the mass of tissues transplanted, it was considered that if a donor were infective, a dose above the threshold could typically result,

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<sup>5</sup> e.g. Will. (2003) Acquired prion disease: iatrogenic CJD, variant CJD, kuru. *Br. Med. Bull.* 66, 255-265.

even though the level of infectivity per gram of tissue might be relatively low.

### **vCJD infectivity in blood**

8. The infectious load and distribution of vCJD infectivity in blood constituents is unclear. One scenario is provided by a risk assessment of exposure to vCJD infectivity in blood and blood products by DNV Consulting<sup>6</sup>, which estimated the infectivity in various blood components (see Annex 1). The literature on blood-borne infectivity was reviewed as part of this assessment. The review is also provided (see Annex 1). The risk assessment was accepted by SEAC in 2002. The estimations of vCJD infectivity in blood components produced are summarised in Table 1.

**Table 1: Infectivity levels per unit whole blood<sup>a</sup>**

<b>Blood fraction</b>	<b>Infectivity intravenous ID<sub>50</sub>/450mL contaminated whole blood unit</b>
Whole blood	900
Plasma	480
Buffy coat	201
Red blood cells	219
Red blood cells (leucodepleted)	2

<sup>a</sup> Taken from DNV Consulting. (2003) Risk assessment of exposure to vCJD infectivity in blood and blood products.

### **Distribution and accumulation of vCJD infectivity**

9. The accumulation and tissue distribution of vCJD infectivity in humans over the course of the incubation period is unclear. However, successive risk assessments reviewed by SEAC have assumed infectivity to be widely distributed around the body in the pre-clinical phase of the disease. Concentrations of infectivity increase during the incubation period, with the highest accumulation of infectivity in the central nervous system and the back of the eye but also with significant accumulation in lymphoid tissues, which are considered to carry infectivity throughout the incubation period.

### **Incubation period following possible blood associated infection**

10. The incubation period of vCJD following infection with vCJD is unclear. In the single case of possible transmission of vCJD via blood transfusion that resulted in clinical disease, the incubation period (if it is assumed the infection was acquired from the

<sup>6</sup> DNV Consulting. (2003) Risk assessment of exposure to vCJD infectivity in blood and blood products. [http://www.dnv.com/binaries/vCJD\\_Update\\_Report\\_tcm4-74414.pdf](http://www.dnv.com/binaries/vCJD_Update_Report_tcm4-74414.pdf)

transfused blood) was about 6.5 years (about 3.5 years to clinical disease)<sup>7</sup>.

11. Infection in the asymptomatic case of possible transmission of vCJD via blood transfusion was found at about 5 years following transfusion. In this case, there was no evidence of a spongiform encephalopathy in an examination of brain material. Although abnormal PrP was found in the spleen and in a cervical lymph node, it was not found in multiple regions of the central nervous system, tonsils, appendix, large intestine, skeletal muscle or thymus (see Annex 2)<sup>8</sup>.

### **Early phase of the incubation period following blood transfusion**

12. The data from the two clinical cases of possible blood transfusion associated vCJD infection suggest the incubation period following infection via blood transfusion is relatively long. However, there are no data on the dissemination and tissue distribution of vCJD infectivity in the early phase of the incubation period immediately following infection.
13. At a superficial level, a number of discrete steps could be envisaged in the very early phase of the incubation period following transfusion with infected blood:
  - (i) immediately following transfusion, dispersal of the infectious agent in the body via the cardiovascular system.
  - (ii) clearance and/or infiltration and/or retention of the infectious agent within tissues/organs.
  - (iii) replication of the infectious agent retained within tissues/organs.
14. There are few data on the dispersal of prion infectivity immediately following inoculation. As part of study by Millson *et al.* (1979)<sup>9</sup>, the scrapie infectivity associated with a number of tissues (lung, liver, salivary gland, spleen and blood), 30 minutes after intravenous inoculation, was examined using infectivity bioassays (see Annex 3). Infectivity could be detected in all the tissues analysed. Infectivity was judged to be highest in the lung and liver on the basis of the relatively lower incubation times

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<sup>7</sup> Llewelyn *et al.* (2004) Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. *Lancet* 363, 417-421.

<sup>8</sup> SEAC statement on the second presumed case of blood transfusion-associated infection with vCJD (2004).

<sup>9</sup> Millson, Kimberlin, Manning and Collis (1979) Early distribution of radioactive liposomes and scrapie infectivity in mouse tissues following administration by different routes. *Vet Microbiol.* 4, 89-99.

when preparations of these organs were analysed in infectivity bioassays. Infectivity was also examined in the liver and blood over a range of time points. Infectivity could be detected at all the time points tested (5 minutes, 0.5, 2, 7 and 16 hours and later time points). The authors suggest that scrapie infectivity is dispersed relatively rapidly following intravenous inoculation. However, it is not possible to determine from an analysis of the experimental data whether the infectivity associated with the organs tested is related solely to the blood fraction of the organs or, in part, to infectivity that has infiltrated into the organs.

15. No studies have specifically examined the clearance of infection following inoculation. Limited data on tissue infectivity levels following parenteral inoculation of scrapie in mice are available from a study by Bruce (1985)<sup>10</sup> (see Annex 4). In the study, scrapie infectivity levels in the brain and spleen of mice following intracerebral or intraperitoneal inoculation were examined using infectivity bioassays at 7, 20 and 35 days and later time points post inoculation. A reduction in the level of infectivity was found in the spleen and brain at 20 days post inoculation relative to the infectivity level at 7 days post inoculation. Infectivity levels subsequently rose at 35 days and later time points. The author suggested that the drop in infectivity levels between the 7 and 35 days post inoculation was due to clearance of the inoculum. This finding suggests that clearance of scrapie infectivity occurs over a period of weeks in mice following intracerebral or intraperitoneal inoculation.
16. Clearance of infectivity is likely to depend on a number of factors such as the infectious dose, the nature of the inoculum, the route of administration and the processing of the infectious dose in the host. For example, Dickinson & Outram (1979)<sup>11</sup> suggest that infectivity in the spleen and liver can decrease rapidly over the first few days following inoculation. However, the duration of the zero-phase of the incubation period (when infectivity levels are undetectable following inoculation and prior to measurable replication) may be influenced by factors such as dose and host (see Annex 4).
17. No data are available that would allow the rate of infiltration of a prion disease agent into tissues to be determined. The rate of infiltration may be dependent on factors such as dose and tissue

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<sup>10</sup> Bruce. (1985) Agent replication dynamics in a long incubation period model of mouse scrapie. *J. Gen. Virol.* 66, 2517-2522.

<sup>11</sup> Dickinson & Outram (1979) The scrapie replication-site hypothesis and its implications for pathogenesis. *Slow Transmissible Diseases of the Nervous System: Volume 2.* 13-31.

type. A study by Kimberlin and Walker (1990)<sup>12</sup> provides some indirect data that may point to infection occurring relatively quickly following injection of a prion inoculum (see Annex 5). In the study, mice were administered with a number of substances (e.g. phytohaemagglutinin, silica, carbon, lipopolysaccharide) by intraperitoneal injection either shortly (0.5 or 2 hours) before or after intraperitoneal inoculation with scrapie. Infection efficiencies were altered when the substances were administered shortly before, but not after, scrapie inoculation. To explain these findings, the authors suggested that when the substances were administered before inoculation they acted non-specifically to influence infection efficiency. However, when administered after inoculation the substances had no effect on infection efficiency because infection had already become established. Thus, it was suggested that infection was established in less than two hours after intraperitoneal inoculation.

18. The large body of data on the pathogenesis of prion diseases suggests that the replication of the infectious agent is dependent on numerous factors such as strain of agent and host genotype. In general, replication appears to be relatively slow, being detectable over a period of several weeks<sup>13</sup>. Thus, replication may not occur to a significant extent in the first few days following infection. In addition, replication is dependent on infiltration of the infectious agent into tissues capable of replicating the infectious agent. It may also be dependent on the type and nature of the tissue. For example, a recent paper by Heikenwalder *et al.* (2005) recently discussed by the committee suggests that inflammation may promote replication in tissues not normally associated with the accumulation of prion infectivity<sup>14</sup>.

## Discussion

19. In the absence of robust and direct data it could be assumed that the vCJD agent is widely distributed within the body in the first 24-48 hours following a blood transfusion but may not necessarily have sufficient time to replicate to a significant extent in that time. Thus, the infectivity level in a particular tissue/organ shortly after transfusion of infected blood may be dependent predominantly on some or all of the following factors:

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<sup>12</sup> Kimberlin and Walker (1990) Intraperitoneal infection with scrapie is established within minutes of injection and is non-specifically enhanced by a variety of different drugs. *Arch Virol.* 112, 103-114.

<sup>13</sup> e.g. Daude (2004) Prion diseases and the spleen. *Viral Immunol.* 17, 334-349.

<sup>14</sup> Heikenwalder *et al.* (2005) Chronic Lymphocytic Inflammation Specifies the Organ Tropism of Prions. *Science.* 307, 1107-1110.

- type of tissue/organ
  - possible clearance of infectivity
  - possible infiltration and retention of the vCJD agent in the tissue/organ
  - blood content of the tissue/organ
  - infectious dose in the transfused blood
20. Compared with the original infectious dose in the transfused blood, the infectious dose in a particular tissue/organ removed for transplantation could be significantly diluted after distribution of the infectious dose in the body. The infectious dose could be further diluted if the tissue/organ is washed to remove a proportion of the infectious blood/plasma prior to transplantation. Thus, because of the possible dilution of the infectious dose, the risk of transmission of vCJD to a recipient of a tissue/organ shortly after a blood transfusion may be lower compared with the transmission risk to the tissue/organ donor that received the blood transfusion.
21. By comparison, in a historic recipient of infected blood, the factors that determine the possibility of a particular tissue/organ being infective (on transplantation) may be somewhat different. The infectivity level in a particular tissue/organ may be dependent on some or all of the following factors:
- type of tissue/organ
  - ability of that tissue/organ to replicate/accumulate infection
  - stage of incubation (i.e. the time for replication and disease progression)
  - infectivity in the blood and the blood content of the tissue/organ

## **Summary**

22. Due to the paucity of relevant data, it is not possible to quantify the possible levels of infectivity in the tissues/organs of peri-operative/peri-mortem and historic recipients of transfusions of infected blood. However, different sets of factors may influence the level of infectivity in tissues/organs in these two groups of blood recipients.

## **Advice sought from the committee**

23. The committee is asked to comment and consider whether a scientific distinction can be drawn between historic and recent

(within the 24-48 hour period prior to donation) blood recipients in terms of the relative likelihood of passing on infection through bone, tissue or organ transplantation.

24. Is the committee aware of further information relevant to the discussion on the dissemination of vCJD infectivity in the first few days following blood transfusion?
25. It is envisaged that a short statement will be produced on the basis of the committee's consideration of this issue.