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CHMP position statement on Creutzfeldt-Jakob disease and plasma-derived and urine-derived medicinal products

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This is the second revision of the CPMP[2] Position Statement on "Creutzfeldt-Jakob disease and plasma-derived and urine-derived medicinal products" (EMEA/CPMP/BWP/2879/02) published in February 2003 and revised in June 2004 and XXX 2010, which replaced the CPMP Position Statement on "New variant CJD and plasma-derived medicinal products" (CPMP/201/98) issued in February 1998.

Summary

Cumulative epidemiological evidence does not support transmission of sporadic, familial and iatrogenic Creutzfeldt-Jakob disease (CJD) by plasma-derived medicinal products. There is no change to the previous CHMP position that recall of plasma-derived medicinal products is not justified where a donor is later confirmed as having sporadic, familial or iatrogenic CJD.

Variant CJD (vCJD) is an emerging disease and the eventual number of cases of the disease is uncertain. There is a wider distribution and higher level of infectivity/abnormal prion protein in peripheral tissues than is seen with sporadic CJD. Four instances of apparent iatrogenic vCJD infection by blood transfusion in man in the UK provide strong evidence that vCJD is transmissible through blood transfusion. In 2009, the agent was detected in a haemophilia A patient who received intermediate purity FVIII prepared from pooled plasma sourced in the UK before 1998.

Residence in the UK is a recognised risk factor for vCJD and has led to the UK deciding to no longer fractionate from UK plasma. It is consistent with this decision to exclude donors who have spent long periods in the UK during the risk period from donating blood/plasma for fractionation. It is recommended that donors who have spent a cumulative period of 1 year or more in the UK between the beginning of 1980 and the end of 1996 are excluded from donating blood/plasma for fractionation.

There is no recommendation to recall batches if information that would have excluded a donor based on his/her stay in the UK becomes available post-donation, since this is a very conservative precautionary measure.

Available data indicate that the manufacturing processes for plasma-derived medicinal products would reduce vCJD infectivity if it were present in human plasma. Manufacturers are required to estimate the potential of their specific manufacturing processes to reduce infectivity using a step-wise approach. It is recommended that manufacturers consult the relevant competent authorities at each of the milestones in this estimation. CHMP and its Biotechnology Working Party (BWP) will keep progress with these recommendations and the actions to be taken under review.

In support of this recommendation, CHMP and BWP, with the involvement of external experts, have developed guidance on how to investigate manufacturing processes with regard to vCJD risk and CHMP and BWP are available to discuss issues that might arise.

The rationale for this position is that if, in the future, further cases of vCJD occur in countries collecting blood and plasma for the manufacture of plasma-derived medicinal products, a process previously shown to be able to reduce TSE infectivity will provide reassurance on the safety of past products, and could help to justify continuing fractionation.

Low levels of infectious TSE agents have been detected in the urine of scrapie-infected rodents and in the urine of deer with Chronic Wasting Disease. However, there is no epidemiological evidence of CJD or vCJD transmission by urine derived medicinal products. A general review of manufacturing

[2] In May 2004 there was a change in the name of the EMEA's scientific committee for human medicines from CPMP to CHMP.

processes for urine-derived medicinal products indicates that it is feasible to apply donor selection criteria when a product is derived from a relatively small and well-defined donor population. In addition, it indicates that manufacturing processes have at least one step that might be theoretically capable of reducing TSE infectivity if it were present in the starting material. It is noted that urine-derived medicinal products are not sourced from urine collected in the UK.

On the basis of this review and other considerations, the use of exclusion criteria for selection for a urine donor panel is encouraged, as a precautionary measure, where feasible. The same exclusion criteria should be applied with respect to CJD and vCJD as used for blood/plasma donors providing starting material for the manufacture of plasma-derived medicinal products but, unlike blood/plasma donors, these criteria would not be checked at each donation. Manufacturers of urine-derived medicinal products are recommended to evaluate the capacity of the manufacturing process to reduce/eliminate TSE agents by following a similar approach to that for plasma-derived medicinal products.

1. Introduction

Creutzfeldt-Jakob disease (CJD) is a rare neurodegenerative disease belonging to the group of human Transmissible Spongiform Encephalopathies (TSEs) or prion diseases. Mortality rate of TSEs ranges approximately from 1.5 to 2 persons per million population per year. TSEs can occur sporadically (sporadic CJD (sCJD) and sporadic fatal insomnia), be associated with mutations of the prion protein gene (genetic TSEs (gTSE)), or result from medical exposure to infectious material (iatrogenic CJD (iCJD)). In 1996, a variant form of CJD (vCJD) was identified.[1] There is strong evidence that vCJD is caused by the agent responsible for bovine spongiform encephalopathy (BSE) in cattle.[2,3,4] The most likely hypothesis is that vCJD has occurred through exposure to BSE contaminated food.

Human TSEs, including in particular vCJD, were addressed in expert meetings/workshops at the EMEA in January 1998, January 1999, December 1999, May 2000, and December 2000. A CPMP Position Statement on variant CJD and plasma-derived medicinal products was issued in February 1998[5f] and the outcome of the subsequent meetings was published on the EMEA website.[5] An EMEA Expert Workshop on Human TSEs and Medicinal Products was held on 19-21 June 2002. This provided the scientific basis for a new CPMP Position Statement issued in 2003.[5b] A further EMEA Expert Workshop was held in January 2004 to review the current state of knowledge of vCJD, in the light of the recent report of a possible human transmission by blood transfusion.[6] In addition, the Workshop discussed the CPMP Discussion document on the investigation of manufacturing processes with respect to vCJD.[5a] In October 2005, a follow-up workshop was held to discuss the number of vCJD cases reported in France and other European countries and the potential effect of additional donor exclusion measures. Urine-derived medicinal products were specifically discussed at an EMEA expert workshop in July 2007[5g] after publication of experiments indicating transmission of prions via urine using a hamster model.

Blood and blood components for transfusion are outside the scope of this Position Statement. Recommendations on the suitability of blood and plasma donors and the screening of donated blood in the European Community were described in Council Recommendation 98/463/EC.[7c] European legislation on human blood and blood components entered into force on 8 February 2003[7a] Under this legislation, a Commission Directive on certain technical requirements for blood and blood components, including eligibility criteria for donors, entered into force in April 2004.[7b] In addition, Council of Europe Recommendation No. R (95) 15 contains a technical appendix on the use, preparation and quality assurance of blood components and details the current requirements for donors.[79]

In December 2003, following the announcement of a possible case of vCJD transmission by blood transfusion, Commissioner Byrne made a statement highlighting EU activities in the area of vCJD and announcing a meeting of the Working Group of the Blood Regulatory Committee to consider the latest information available from the UK.[7d] The meeting took place in January 2004 and a summary statement was produced.[7e]

The Scientific Steering Committee (SSC) and the Scientific Committee on Medicinal Products and Medical Devices (SCMPMD) of the European Commission have published a number of opinions relating to TSEs, which are of relevance to blood and blood components for transfusion, as well as to plasma-derived medicinal products.[8] WHO Guidelines on TSEs are also of relevance to both blood components for transfusion and plasma-derived medicinal products.[9] The Council of Europe has made recommendations for blood and blood components for transfusion.[10]

2. Human TSEs current status

2.1. Sporadic, genetic and iatrogenic forms of human TSEs

There is no evidence that sporadic, genetic or iatrogenic forms of human TSEs have been transmitted from person to person through exposure to plasma products or urinary derived medicinal products. Systematic surveillance for CJD of all types has been undertaken in a number of countries, including a collaborative study in the EU since 1993,[11,12] and no case of sporadic, genetic or iatrogenic CJD has been causally linked to prior treatment with plasma products. Cases of sporadic CJD with a history of drug treatment for infertility have not been identified but there is uncertainty about the validity of this observation. (See the report of the 2007 EMA expert meeting for further details.[5g]) Although there is evidence that plasma products have not been implicated in transmission of sporadic, genetic or iatrogenic CJD, the strength of the evidence excluding transmission by urinary derived medicinal products is less secure.

2.2. Variant CJD

The official UK figures for vCJD at the beginning of April 2010 were a total of 172 definite or probable vCJD cases.[13] (One case diagnosed in Hong Kong was classified as a UK case and is included in the UK figures.) Outside of the UK, there have been 25 cases in France[15], 5 in Spain, 4 in the Republic of Ireland, 3 in the Netherlands, 3 in the USA, 2 in Portugal and Italy and single cases in Canada, Saudi Arabia and Japan. 2 of the Irish cases, 2 of the US cases, 1 French case and the Canadian case had spent more than 6 months in the UK during the period 1980-1996 and were probably infected while in the UK. [14] The third US case has been reported as most likely infected when living in Saudi Arabia. The possibility of cases occurring in other countries cannot be excluded.

Two cases of vCJD identified in Spain occurred in the same family. No family links have been reported in any other vCJD cases to date.

All definite and probable cases, which have been genotyped so far, are Met-Met homozygotes at codon 129 of the prion protein (PrP) gene.[16] In 2009 a possible case of variant CJD was reported in the UK with a heterozygous codon 129 genotype. [17]

Analysis of the UK figures for the quarterly incidence of deaths indicates that vCJD incidence in the UK is currently in decline. However, interpretation requires caution as there may be a long tail or more than one peak to the epidemic.[18]

A UK study screening specimens from surgically removed appendices and tonsils for accumulation of prion protein in the lymphoreticular system has been carried out in order to try and obtain some estimation of the number of people that might be incubating vCJD in the UK.[19] Three positive appendix specimens have been found as a result of the screening of 12,674 appendix and tonsil specimens. However, the pattern of lymphoreticular accumulation in two of these samples was dissimilar from that seen in known cases of vCJD, raising the possibility that they may be false positives. With respect to this possibility, the authors comment that although it is uncertain whether immunohistochemical accumulation of prion protein in the lymphoreticular system is specific for vCJD, it has not been described in any other disease, including other forms of human prion disease or a range of inflammatory and infective conditions. Subsequent genetic analysis of residual tissue samples from these 2 cases found that both were valine homozygotes at codon 129 in the prion protein gene[20] This finding might account for the immunohistochemical features in these cases; all patients who have developed vCJD and have undergone a comparable genetic analysis have been methionine homozygotes at codon 129 in the prion protein gene.

Statistical analysis on this finding of 3 positive specimens gives the following estimations of numbers who may be incubating vCJD:

237 infections per million population (95% confidence interval (CI): 49-692 per million)

Assuming that this estimate relates to those aged 10-30 years[3], 3,808 individuals (CI 785-11 128) aged 10-30 years may be incubating vCJD in the UK.

These estimations are higher than predictions from modelling of the clinical data (upper 95% confidence interval of 540 future cases).[21] It is not known whether those incubating vCJD will eventually develop clinical disease. However, estimates of numbers possibly incubating are important with respect to any potential for secondary transmission (e.g. by blood donation, surgical instruments) while individuals are in the incubation phase. It should be noted that plasma-derived medicinal products have not been manufactured from donations collected in the UK since 1998.

A larger study of an archive of tonsil tissue from 63,007 people of all ages removed during routine tonsillectomies has been published.[22] 2,753 samples were from the 1961- 1985 birth cohort in which most cases of vCJD have arisen and 19,808 were from the 1986-1995 birth cohort that may also have been orally exposed to bovine spongiform encephalopathy. None of the samples were unequivocally reactive to two enzyme immunoassays and none of the initial reactives were positive for PrP^{TSE} by immunohistochemistry or immunoblotting. The estimated 95% confidence interval for the prevalence of PrP^{TSE} in the 1961-1995 birth cohort was 0-113 per million and in the 1961-1985 birth cohort 0-289 per million. These estimates are lower than the previous study of appendix tissue, but are still consistent with this study. Archiving of tonsil tissues continues and further studies are planned.

3. Human tissue distribution of infectivity/abnormal prion protein.

Tissue distribution has been investigated by detection of the abnormal prion protein PrP^{TSE} or by infectivity assays. Detection of PrP^{TSE} in tissues has often been associated with infectivity, however it should be noted that, in some circumstances, infectivity can be present without detection of PrP^{TSE} or PrP^{TSE} be present in absence of infectivity.[23] This may be related to limitations of assay methods for PrP^{TSE}, however, in some cases the reason for this finding is not known. It is thus recommended that

[3] The reason the age range of 10-30 years is specified is because 83% of the samples were from individuals in this age range.

any study on tissue or fluid distribution of the abnormal prion protein be confirmed with an infectivity assay.

A wider distribution and higher level of PrP^{TSE} in human peripheral tissues, including the lymphoreticular system, has been found in vCJD compared with sporadic CJD.[24,25,26] Limited data from infectivity assays of vCJD tissues are consistent with the PrP^{TSE} findings.[27] In clinical vCJD cases high titres of infectivity are found in the brain and spinal cord and lower levels in spleen and tonsil[27]. While PrP^{TSE} and infectivity are occasionally found in the spleen of sporadic CJD, the levels of PrP^{TSE} are lower than in vCJD.[8i] It is also suspected that lymphoid tissue involvement in sCJD is associated with a relatively long duration of clinical illness whereas it occurs preclinically in vCJD. PrP^{TSE} accumulations have been observed in muscles of some patients with both sporadic and variant CJD.[28]

It is likely that the distribution of PrP^{TSE} and infectivity in iCJD is more similar to sCJD than vCJD.[29] Data are lacking for gCJD.

4. Infectivity in blood and transmissibility via blood

4.1. Animal blood

Low levels of infectivity have been found in the blood of rodents experimentally infected with animal and human TSE agents.[30,31,32,33] Experiments indicate that approximately half the infectivity is in the cellular components, mainly the buffy coat, and the remainder in the plasma. Experimental studies indicate that the vCJD agent behaves in a similar way (qualitatively and quantitatively) to a genetic TSE agent[4] when adapted to RIII/Fa/Dk mice.[33] Infectivity has also been detected in buffy coat of a prosimian microcebe experimentally infected with a macaque-adapted BSE strain.[34]

The infectivity in rodent blood was transmitted by intravenous inoculation, but 5-7 fold less efficiently than by the intracerebral route.[31] In one study with mouse-adapted vCJD agent, the intravenous and intracerebral routes were found to be equally efficient for the buffy coat fraction but not for the plasma fraction.[33] However, studies in primates show that survival times were similar after intravenous or intracerebral inoculation of infected brain material.[35,36] Unpublished studies presented at scientific meetings[37,38] indicate that blood of primates experimentally infected with human TSE agent is infectious from about half way through the incubation period.

Furthermore, information from intra-species transfusion experiments indicates that experimental BSE in orally infected sheep or natural scrapie infection in sheep can be transmitted to sheep by blood transfusion.[39,40] Transmission efficiency was high for both BSE and natural scrapie, and the majority of transmissions resulted from blood collected more than half way through the incubation period[41]. The level of infectivity in sheep blood cannot be established from these experiments.

The European Union has provided funding for animal transmission projects.

4.2. Human blood

The tracing of recipients of blood transfusion from UK donors who have subsequently developed vCJD (the TMER study) has revealed four instances of secondary transmission.[42] These individuals had received transfusion of non-leucodepleted red cells from donors who were clinically healthy at the time of donation but subsequently (17–40 months later) developed variant CJD. Three of the four patients developed disease after incubation periods ranging from 6.5 to 8.5 years; the fourth died 5 years after

[4] Mouse-adapted GSS strain of human TSE (brain tissue obtained from a case of Gerstmann-Sträussler-Scheinker syndrome).

transfusion of an illness unrelated to prion disease but tested positive for PrP^{TSE} in the spleen and lymph nodes. This asymptomatic prion-infected patient was heterozygous (methionine/valine) at codon 129 of the *PRNP* gene. Taken together, these instances are strong evidence that vCJD is transmissible through blood transfusion.

Recently, another presumed case of prion infection was identified in an elderly haemophilic patient who was heterozygous at codon 129 in the prion protein gene.[43] The patient, who died of unrelated pathology, had received large quantities of UK-sourced fractionated plasma products, including some units derived from plasma pools which contained plasma from a donor who later developed variant CJD. This patient was identified through an intensive search for PrP^{TSE} positivity in all post-mortem tissues, although only 1 of 24 samples taken from the spleen tested positive. Whether someone with this limited distribution of PrP^{TSE} would be infectious is unknown, but from a public health perspective, this patient represents a warning that some plasma-derived products might contain residual prion infectivity.

The surveillance described above emphasises the importance of the TMER study for identifying the risk of blood transfusion in transmitting vCJD. Moreover, national databases of blood donors and the maintenance of traceability from donor to recipient and vice versa are essential to establish whether a vCJD case has been a blood donor (UK experience has shown that questioning of family members is unreliable for establishing whether a patient has been a blood donor). Traceability is a specific requirement in Article 14 of Directive 2002/98/EC.[7a]

Infectivity or PrP^{TSE} were not detected in blood of vCJD cases using methods capable of detecting infectivity/PrP^{TSE} in peripheral tissues such as tonsil or spleen, indicating that if infectivity is present it is at levels below the sensitivity of these methods. [27,24]

There is no epidemiological evidence that blood of sporadic CJD may transmit disease.[44,45] Prospective studies, similar to the TMER study, are in progress in the UK and USA and have not yet revealed any possible case of sporadic CJD linked to blood transfusion. However, current data are scanty to unequivocally exclude the possibility that such an event could occur in a small number of cases with a long (10 or more years) incubation period.[46]

A review of transmission studies to detect infectivity in the blood of humans with CJD (sporadic, iatrogenic and variant) shows that although experimental transmissions to animal models have occasionally been reported[47-50], other studies failed to detect infectivity.[51,27] It remains possible that PrP^{TSE} is present at low levels in the blood of clinically affected cases of sCJD. Data are lacking for gCJD but the assumption is that the tissue distribution of infectivity will be more similar to sCJD than vCJD.

For the purpose of risk assessments, it is recommended that, as a worst case assumption, a relative efficiency of the intravenous and intracerebral routes of 1:1 should be used.[52] This is because the accumulated information now available from animal studies indicates that the intravenous route can be an efficient route of transmission and in certain cases can give a transmission rate and/or an incubation period similar to the intracerebral route (see also 4.1).

5. Detection techniques

Several techniques are under development for the detection of PrP^{TSE} in blood including methods based on epitope protection[53] and PrP^{TSE} specific antibodies[54]. Approaches based on surrogate markers are also under investigation. Development and validation of all methods is on-going but there is no screening test yet. Confirmatory tests that have been proposed include Protein Mis-folding Cyclic Amplification (PMCA) [55] which is extremely sensitive, but has not yet been validated.

Several WHO reference preparations are available and further materials are under development[9b]. These reference preparations will allow calibration of assays versus infectivity bioassays, and can be used for collaborative studies to compare the performance of different assays to see whether they are sufficiently sensitive and specific to justify further evaluation for screening blood.

PrP^{TSE} detection methods for screening human blood for evidence of infection are being considered for inclusion as Annex II List A devices under the IVD Directive. There are very few samples of blood or plasma from clinically affected patients or from individuals known to have been infected at a particular time. This contrasts with other blood borne agents such as viruses. Alternative development and evaluation strategies have been proposed to assess whether a candidate assay is sufficiently promising to be given access to the available samples.[56]

6. Leucoreduction and specific prion affinity filters

Leucoreduction is used in transfusion medicine to reduce the level of white blood cells in blood and blood components. It was implemented in the UK in 1999.

The rationale for considering leucoreduction as a precautionary measure is:

- The lymphoreticular involvement in vCJD
- The detection of low levels of infectivity, in studies with rodents, in the buffy coat (associated with white blood cells).

The SCMPMD opinion on leucoreduction [8a, 8b] for blood and blood components for transfusion states that it might be a precautionary step to remove white cells as completely as possible. For plasma for fractionation the opinion states the following:

'Taken together, there is no compelling scientific evidence to date for the introduction of leucoreduction of plasma for fractionation, or other methods aiming at removal of cells and debris, as a precaution against vCJD transmission. The question should be further explored by suitable experiments.'

Results reported at the 2002 EMEA Workshop, suggested that leucoreduction does not provoke fragmentation of cells and lysis. Results of a comprehensive study involving a number of different filters and procedures indicate that leucodepletion is not detrimental in terms of the generation of microvesicles or the release of prion proteins[57].

Infectivity data from hamster studies indicate that leucoreduction alone is not totally protective against prion transmission, with between 42 to 72 percent reduction in infectivity of whole blood[58,59].

Specific affinity ligands that bind prion proteins are being evaluated for their ability to reduce TSE infectivity present in blood and plasma.

A study in hamsters showed that a leucocyte-reduction filter based on modified polyester fibres exhibited a prion clearance capability between 99.0 to 99.9 percent on the endogenous and exogenous infectivity of red cell concentrates[60].

Initial studies using leucoreduced human red blood cell concentrates spiked with hamster brain-derived scrapie infectivity indicate that some ligands immobilised on a chromatographic resin matrix are capable to removing 3 to 4 log ID_{50} per ml[59]. A further study using scrapie-infected hamster whole blood demonstrated an overall reduction of infectivity of more than 1.22 log ID[61].

The prion binding capacity of an affinity ligand chromatography step has been investigated in the processing of a plasma medicinal product using hamster brain derived spiking material[62]. This preliminary data requires further evaluation before conclusions can be drawn on possible efficacy.

7. Manufacturing processes for plasma-derived medicinal products

Taking account of the available data concerning blood infectivity, it is of utmost importance to investigate the capacities of the manufacturing process (fractionation) to eliminate/inactivate the infectious material potentially present in the plasma pool used as the starting material for preparation of plasma-derived products. Initial results from animal studies, using blood from rodents infected by intracerebral inoculation, indicated that the fractionation process contributes to the removal of endogenous plasma infectivity.[30,31] Information reported at the EMEA Workshops in 2002 and 2004 suggested that endogenous infectivity might persist through the fractionation process to a greater extent than would be expected from spiking studies,

Many investigational studies have now been carried out with different strains of agent and spiking materials of different nature and purity, and using different assays to follow the partition of PrP^{TSE} and/or infectivity. In most cases, the correlation between the capacity to partition PrP^{TSE} and infectivity has been demonstrated for the spiking preparations used until now (mainly brain homogenates of various strains). It is now confirmed that biochemical assays can be useful for spiking experiments to investigate manufacturing processes in a reasonable timeframe and less costly protocols than the *in vivo* bioassay. However it is still necessary to correlate such results with those from infectivity assays in animals. Cell-based assays may also be useful if properly validated for this purpose.

Studies aimed at investigating the contribution of the various manufacturing steps to reduction of infectivity (including precipitation followed by centrifugation or depth filtration, chromatography and nanofiltration) have accumulated convergent data supporting the removal of infectivity by steps that are commonly used in the manufacture of plasma-derived medicinal products.[62-68] For coagulation factors derived from cryoprecipitate, downstream fractionation using various precipitating agents or conditions allow to discard PrP^{TSE} in the precipitates. Reduction level achieved may vary according to the specific manufacturing process and probably depends on the concentration of the precipitating agent and salts, and the pH. Chromatographic steps, classically used in the separation of coagulation factors but also in the purification of other plasma derivatives have been described to remove TSE infectivity or PrP^{TSE}. Again, the reduction factors may be variable according to the fraction eluted. However, caution is still needed in the interpretation of those data since the effectiveness of a given step is dependent on a number of variables including the process conditions and the state/nature of the agent in the spiking preparation sample and in the spiked product intermediate. Consequently, effectiveness of removal may vary from one manufacturer to another. In addition, recent studies have highlighted the fact that removal capacity may be variable according to the state of dispersion of the agent in the spiking preparation particularly for steps based on retention mechanisms.

Overall, there is a need i) to investigate the partitioning or removal capacities of the various fractionation steps used in the preparation of the plasma-derived medicinal products, ii) to investigate the partition and removal of endogenous infectivity and the extent to which this is comparable with data from spiking studies, iii) to gain better knowledge of the form of infectivity present in blood in order to confirm the relevance of the spiking material used in the validation studies.

8. Infectivity in urine

Low levels of infectivity have been detected in urine of scrapie-infected rodents by several research groups and in the urine of deer with Chronic Wasting Disease.[5g, 9c]

Gregory *et al*.[69] demonstrated that the disease could be transmitted by intracerebral inoculation of pooled urine from scrapie-sick hamsters. The infectivity titre of the urine was calculated to be around 3.8 infectious doses/ml. Titration of kidney and urinary bladders from the same animals gave 20,000-fold greater concentrations. Histologic and immunhistochemical examination of these tissues showed no indication of inflammation or other pathologic changes, except for occasional deposits of disease-associated prion protein in kidneys.

Kariv-Inbal *et al*.[70] have observed transmission of the disease after intraperitoneal (i.p.) administration of enriched urine fractions from scrapie sick hamsters. Transmission via the oral route was also investigated. The recipient hamsters remained without symptoms but secondary transmission was observed after inoculation of brain extract from an asymptomatic hamster.

Seeger *et al*.[71] have studied transmission via urine using mouse models of chronic inflammation. They have detected prionuria in scrapie infected mice with coincident chronic lymphocytic nephritis. Transmission has been shown upon intracerebral inoculation of purified proteins from pooled urine collected from scrapie sick or presymptomatic mice. In contrast, prionuria was not observed in scrapie infected mice displaying isolated glomerulonephritis without interstitial lymphofollicular foci or in scrapie infected wild type mice lacking inflammatory conditions.

Prionuria was also detected in chronic wasting disease (CWD) of deer. Experiments by Haley *et al*.[72] provided evidence that concentrated urine from deer at the terminal stage of the disease, that also showed mild to moderate nephritis histopathologically, was infectious when inoculated into transgenic mice expressing the cervid PrP gene. In addition, the urine collected from the CWD sick deer that was used for mouse inoculation, showed positive results when assayed for PrP^{TSE} by serial rounds of protein misfolding cyclic amplification (PMCA) assay. The concentration of abnormal prion protein was very low as indicated by undetectable PrP^{TSE} by traditional assays and prolonged incubation periods and incomplete TSE attack rates in the transgenic mice.

Using the highly sensitive PMCA technology Gonzalez-Romero *et al.*[73] and Murayama *et al*.[74] have detected PrP^{TSE} in urine of scrapie sick hamsters. The results by Gonzalez-Romero *et al.* suggest that the concentration of PrP^{TSE} in urine is in average 10-fold lower than in blood. Animal experiments have demonstrated that *in vitro* generated PrP^{TSE} by PMCA starting from urine produced a disease indistinguishable from the one induced by infected brain material.[73]

Epidemiological evidence in the last 25 years, during which urinary-derived medicinal products and particularly gonadotrophins have been widely used, does not suggest a risk from sporadic CJD. Since epidemiological evidence has identified the few cases of iatrogenic transmission of CJD through the use of pituitary-derived gonadotrophins, it is possible that transmission from urinary-derived gonadotrophins would have been detected if it had occurred.

9. Recommendations and proposals

9.1. Sporadic, genetic and iatrogenic CJD and plasma-derived medicinal products

Cumulative epidemiological evidence does not support transmission of sporadic, genetic and iatrogenic CJD by blood, blood components or plasma-derived medicinal products.[75, 76, 12] Nevertheless, rigorous epidemiological studies for tracing blood-related sCJD cases have not yet reached sufficient statistical power to formally exclude the possibility of blood transmission in a small number of cases. Moreover, the experimental evidence of peripheral tissue infectivity in various subtypes of sCJD is very limited but available data show presence of infectivity in spleen and lymph nodes in human TSEs other than vCJD.

The implementation of appropriate actions in relation to CJD depends on accurate diagnosis in suspected cases. There is a potential for diagnostic confusion between sporadic and variant CJD, particularly in younger age groups.

Donor selection criteria include criteria to exclude donors who might be at higher risk of developing CJD. The following permanent deferral criteria are specified in Commission Directive 2004/33/EC: Persons who have a family history which places them at risk of developing a TSE, or persons who have received a corneal or dura mater graft, or who have been treated in the past with medicines made from human pituitary glands.[7b] Precautionary recalls of batches of plasma-derived medicinal products after post-donation reports of CJD or CJD risk factors in a donor contributed to severe shortages of certain products.[9a]

On the basis of the current epidemiological evidence, the CHMP recommendation that recall of plasma-derived medicinal products is not justified where a donor is later confirmed as having sporadic, genetic or iatrogenic CJD or CJD risk factors is maintained.

9.2. Variant CJD and plasma-derived medicinal products

Uncertainties still exist concerning the number of cases of vCJD that will occur although the number of cases is in decline in the UK and France. Variant CJD has a different distribution of infectivity in tissue outside the central nervous system to sporadic CJD.

There is now strong epidemiological evidence of human to human transmission of vCJD by blood transfusion (see Section 4.2). In addition, one vCJD infection was detected in a patient with haemophilia treated with high doses of intermediate purity factor VIII. Estimates of the relative risks of exposure through diet, surgery, endoscopy, blood transfusion and receipt of UK-sourced plasma products suggest that the most likely route of infection in the patient with haemophilia was receipt of UK plasma products. At least one batch came from a pool containing a donation from a donor who later developed vCJD.[43,77,]

The following measures are aimed at minimising the risk of transmission of the agent by plasma-derived medicinal products.

9.2.1. Exclusion Criteria

a) Consideration of Country-based exclusions

There is currently no screening test to detect donors who may be incubating the disease or in the early clinical stages. Therefore, other approaches are considered in order to try and identify donors who may present a higher risk.

UK plasma

Residence in the UK is a recognised risk factor for vCJD and has led to the UK deciding no longer to fractionate from UK plasma.

Exclusion of donors based on cumulative period of time spent in the UK

Since UK donors are excluded from donating plasma for the manufacture of plasma-derived medicinal products in the UK, it is consistent to exclude donors who have spent long periods in the UK. This is supported by the finding of vCJD cases, which have a risk factor of long periods spent in the UK, in other countries[5].

It is, therefore, recommended that donors who have spent a cumulative period of 1 year or more in the UK between the beginning of 1980 and the end of 1996 are excluded from donating blood/plasma for fractionation. Countries are highly encouraged to choose their national cumulative period limit for plasma-derived medicinal products according to a nationally calculated benefit/risk balance, which will take into account the endogenous risk of BSE exposure (and introduction in the food chain) and the risk of shortages of blood and plasma for the manufacture of medicinal products. The national limit is recommended to be of cumulative periods in the UK below or equal to 1 year.

Countries may still apply a stricter limit than 1 year for exclusion of donors for blood/plasma collected for fractionation within the country (e.g. 6 months) but will accept plasma-derived medicinal products from other countries provided that at least the one-year time limit is applied.

The rationale for this recommendation is to exclude donors who have the highest individual risk from stays in the UK and to be consistent with the UK decision to no longer fractionate from UK plasma. This is further explained in the first version of this Position Statement published in February 2003.[5b]

French plasma and plasma from other BSE-exposed European countries

France published an analysis of the risk of transmission of vCJD by blood and its derivatives sourced from French plasma in December 2000.[78g] This concluded that plasma collected in France could continue to be used for fractionation. The safety margin for plasma-derived medicinal products was considered to be sufficient. However, introduction of additional steps to further increase the safety margin of some products was recommended (e.g. nanofiltration of Factor VIII introduced in January 2001). Leucodepletion for plasma for fractionation, as for plasma for transfusion products, was also recommended in 2001 as a precautionary measure. The subsequent risk-analyses published in 2002, 2003, 2004, 2005, 2007 and 2009 re-confirmed these conclusions and acknowledged that the size of epidemic was revised to a lower estimate by more recent modeling, and the risk to collect blood from vCJD-incubating donors lower than previously estimated. [78]

Based on the limited data on human exposure to BSE-risk materials in other European countries it is still difficult to estimate the epidemiological risk in those countries which have small number of vCJD cases or have not yet reported any vCJD cases.

Donors who have spent a cumulative period of time in France and other BSE-exposed countries

Exclusion of donors who have spent a cumulative period of time in France is not recommended because of the lower risk associated with time spent in France compared with time spent in the UK (the risk in France is estimated to be 1/10 of that in UK). Since the previous version of the Position Statement, endogenous vCJD cases occurred in some other countries (see Section 2. Human TSEs current status) placing them close to or lower than France in terms of incidence and ratio of risk in

[5] Two cases in Ireland, two cases in US, one case in France and the Canadian case associated with long periods spent in the UK.

comparison to UK. Exclusion of donors who have spent time in other European countries having a risk ratio in the same order of magnitude as France is not recommended.

Concluding remarks

Country-based exclusions may appear unjustified in the sense that the vast majority of donors who will be excluded will not develop the disease. There is a lack of spare plasma capacity to make up for shortfalls if countries that are major producers of plasma-derived medicinal products discontinue the use of nationally collected plasma for fractionation.

b) Other possible exclusion criteria

Commission Directive 2004/33/EC indicates that further deferral criteria for vCJD may be recommended as a precautionary measure.[7b]

Other possible exclusion criteria that could be considered include permanent exclusion of recipients of blood transfusion (general exclusion or exclusion of recipients of transfusion in UK[6]), transplant recipients, and donors who have undergone neurosurgery.

Caution is needed because of the risk of loss of donors and consequent supply problems. Since such criteria could apply to both blood and blood components, and plasma-derived medicinal products, it was appropriate to consider this further within the scope of Directive 2002/98/EC.[7a] The technical meeting of blood experts, convened by the European Commission in January 2004, considered exclusion criteria, as well as blood component preparation and processing, recipient tracing and surveillance, and optimal use of blood.[7e]

9.2.2. Leucoreduction and specific prion affinity filters

The benefit of inclusion of leucoreduction to improve the safety of plasma has not been demonstrated.

At present it is not appropriate to recommend the introduction of leucoreduction for the safety of plasma-derived products.

Efficacy of introducing recently developed affinity media / filters is still under investigation.

9.2.3. Manufacturing processes for plasma-derived medicinal products

The available data support the reduction of infectivity by steps in the manufacturing process. Manufacturers are required to estimate the potential of their specific manufacturing processes to reduce infectivity. This should follow a step-wise approach as described below and illustrated in the accompanying flow diagram. It is recommended that manufacturers consult the relevant competent authorities at each of the milestones in this estimation. A decision to undertake an infectivity assay and/or to add a further manufacturing step(s) to increase reduction capacity should only be made after a careful consideration of all benefit-risk factors for a certain product.

Firstly, manufacturers should compare their own processes to those with published data on reduction of infectivity in order to estimate the theoretical potential of their specific manufacturing processes to reduce infectivity. *(Flow diagram, step 1)*

Whereas the general information available on manufacturing processes provides useful background information, the actual effectiveness of a manufacturing process might be dependent on the specific

[6] In April 2004, the UK implemented exclusion of persons who have previously received transfusions of whole blood components since January 1980, as a precautionary approach.

process conditions. Manufacturers should consider the relevance of the published data to their specific manufacturing processes and whether the removal capacity can be expected to be comparable.

If it cannot be concluded that the removal capacity would be expected to be comparable, it is recommended that manufacturers undertake product-specific investigational studies on key steps in their manufacturing processes using biochemical assays. Priority should be given to studies on products with the lowest potential removal capacity. *(Flow diagram, step 2)*

Investigations using biochemical assays may be sufficient if a clear correlation with infectivity data has already been established for similar processes (e.g. ethanol fractionation). If such a correlation is not established (e.g. a novel step) and the step is considered critical for removal of infectivity for the specific product (e.g. it is the only step for removal), the investigations should be confirmed using an infectivity assay for the critical step(s). *(Flow diagram, step 3)*

The above steps will allow manufacturers to estimate the reduction capacity of their manufacturing processes. *(Flow diagram, step 4)*

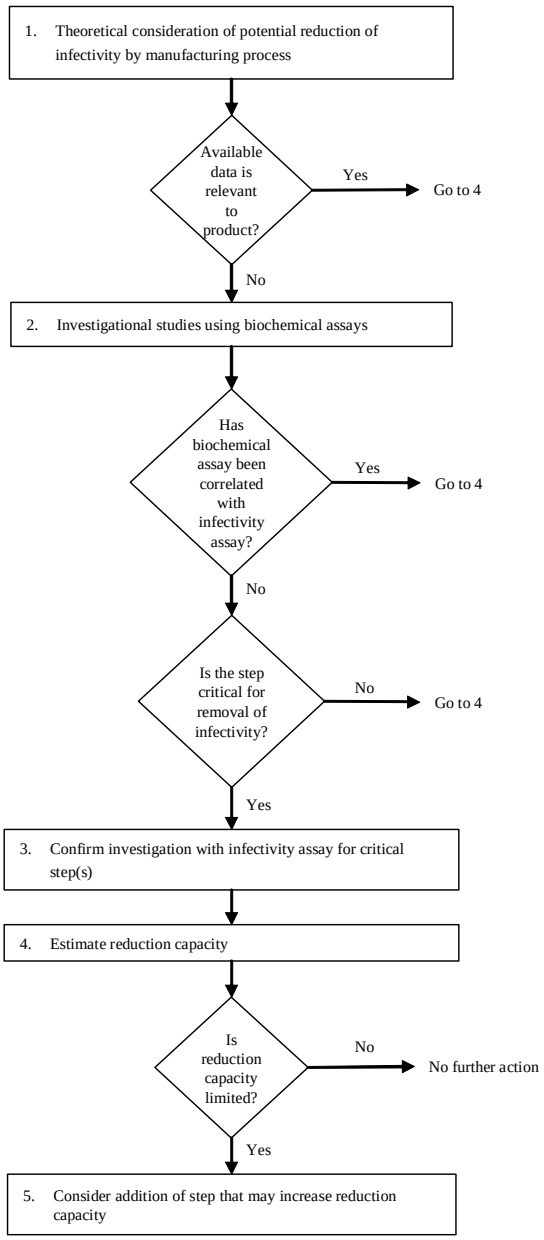
In cases where the overall reduction capacity is limited, manufacturers should consider the addition of steps that may increase the removal capacity where this is feasible without compromising the safety, quality and availability of the existing products. Discussion with the relevant competent authorities is recommended. *(Flow diagram, step 5)*

The outcome of the estimates of the theoretical potential of manufacturing processes to reduce infectivity and the results of product-specific investigational studies should be reported to the relevant competent authorities for the medicinal products concerned, as information becomes available. Applicants submitting new marketing authorisation applications for plasma-derived medicinal products will be expected to include such information in the application dossier. The outcome of the estimation of the theoretical potential to reduce infectivity should always be included in the application.

In support of these recommendations, CHMP's Biotechnology Working Party, with the involvement of external experts, has developed guidance on how to investigate manufacturing processes with regard to vCJD risk.[5a]

Figure 1: Plasma-Derived Medicinal Products: estimation of potential reduction capacity of specific manufacturing processes

Important Note: this flow diagram should be read in conjunction with the preceding text in 9.2.3. It is recommended to consult the relevant competent authorities at the milestones in this estimation. Give priority to studies on products with the lowest potential removal capacity.



9.2.4. Recall of batches where information becomes available post-donation

In view of the lack of adequate information on vCJD, it is prudent to recall batches of plasma-derived medicinal products where a donor to a plasma pool subsequently develops vCJD. Recall should also include medicinal products containing plasma-derived products as excipients. However, in both cases, consequences for essential medicinal products where alternatives are not available will need careful consideration by the competent authorities.

A case-by-case consideration would be appropriate where plasma-derived products have been used in the manufacture of other medicinal products. This consideration would include the nature of the product, the amount used, where it is used in the manufacturing process and the downstream processing.

Look-back to identify the fate of donations should be taken as far as possible. Regulatory authorities, Official Medicines Control Laboratories, surveillance centres and the supply chain should be informed of all batches of product and intermediate implicated whether or not supplies of the batch are exhausted.

There is no recommendation to recall batches if information becomes available post-donation, which would have excluded a donor based on his/her stay in the UK since this donation exclusion criteria is a very conservative precautionary measure (see 9.2.1).

9.2.5. Albumin used as an excipient or in manufacturing processes

The available data on the removal of infectivity during the fractionation process used in the manufacture of albumin indicates that the risk of transmission of infectivity by albumin would be particularly low. Nevertheless, in the case of albumin used as an excipient, recall is still recommended as a precautionary measure where a donor to a plasma pool subsequently develops vCJD. A single batch of albumin may be used to produce a number of batches of a medicinal product because of the small amounts that are typically used as an excipient. As a consequence, a recall could affect complete stocks of a product and create severe shortages. Therefore, to avoid a negative impact on supply, companies should consider the origin of plasma and select countries where the probability of having to recall batches is as limited as possible.

Development of substitutes for plasma-derived albumin used as an excipient or in manufacturing processes is encouraged although it is recognised that this can be difficult (requiring development and validation and usually non-clinical and clinical investigations) and should thus be considered as a long-term approach.

9.2.6. Substitution with alternative products

Use of alternative products to plasma-derived medicinal products could be considered, where these are available. It is felt that this choice should remain with users, taking into account the needs of the individual patient. It should be noted that plasma-derived products such as albumin may be used in the manufacture of recombinant products.

9.2.7. Optimal Use

Optimal use of plasma-derived medicinal products is encouraged, as this will maximise the benefits of the products compared with any potential risk.

9.3. Urine-derived medicinal products

The recommendations for urine-derived medicinal products are based on the following considerations:

There is no epidemiological evidence of CJD and vCJD transmission by urine-derived medicinal products.

TSE infectivity in urine has been reported in some animal models.

The review of manufacturing processes described below.

Investigational studies of infectivity reduction by the manufacturing processes should be done following the same general, stepwise approach as recommended for plasma derived medicinal products (see Section 9.2.3).[5a]

Results from different assay systems are not necessarily directly comparable (Western blot, cell based assays, bioassay). The approach recommended for plasma-derived medicinal products would be applicable (i.e. confirm reduction capacity using infectivity assays for steps critical for reduction of infectivity if a clear correlation between data from biochemical assays and infectivity assays has not been established for similar process steps). For inactivation studies, investigation of different TSE strains should be considered as they may vary in resistance.

Potential accumulation of prions on chromatographic columns or a potential batch to batch contamination due to carry-over of prions should be addressed in the studies.

Bibliographic data could be acceptable as additional supportive data to the investigational studies provided. Similarity of the compared process and materials should be established. Extrapolation of results for plasma-derived medicinal products is not justified particularly for chromatographic steps at the beginning of the manufacturing process because of the high protein content in plasma.

General review of the manufacturing processes indicates that, in each manufacturing process, there is at least one step that might be theoretically capable of reducing infectivity if it were present in the starting material. In cases where the reduction capacity is limited, manufacturers should consider the addition of steps that may increase the overall removal capacity.

For particular products, such as hormones from a relatively small well-defined donor population, some manufacturers have put in place limited exclusion criteria for the selection of a donor for inclusion in a donor panel. For other products manufactured from very large donor pools (e.g. urokinase), such measures are more difficult to apply.

Urine should be collected from countries where there is a surveillance system for both human and animal TSEs. It is noted that urine-derived medicinal products are not sourced from urine collected in the UK.

On the basis of these considerations, the use of exclusion criteria for selection for a donor panel are encouraged, as a precautionary measure, where feasible. The same exclusion criteria should be applied with respect to CJD and vCJD as used for blood/plasma donors providing starting material for the manufacture of plasma-derived medicinal products. Although these criteria would not be checked at each donation unlike blood/plasma donors, manufacturers should follow up the donor criteria at defined intervals. The exclusion of donors with known inflammation of kidney and/or chronic renal inflammatory diseases is encouraged.

Record keeping for traceability is recommended for products where it is possible to trace back to donor level.

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