

A Report on TSE and Transfusion Safety

by

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Executive Summary

TBD

Background

Transmissible spongiform encephalopathies (TSEs)/prion diseases include a number of related disorders of humans and animals that are characterized by long incubation period, central nervous system (CNS) degeneration, and are associated with accumulation of abnormal prion protein (PrP^{TSE}) that changed its conformation from normal cellular prion protein (PrP^{C}). Prion associated diseases of animals include scrapie in sheep and goats, chronic wasting disease (CWD) in deer and elk, and bovine spongiform encephalopathy (BSE) in cattle. In humans the disease manifests itself as Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker disease (GSS), fatal familial insomnia (FFI), kuru, and variant CJD (vCJD).

PrP^{C} is a highly conserved phylogenetically cell surface sialoglycoprotein attached to the cell membrane through the glycosylphosphatidylinositol anchor. It is sensitive to protease treatment and soluble in detergents. In mammals the PrP^{C} is encoded by a single-copy chromosomal gene (*PRNP* in humans and *Prnp* in mice) and is expressed during early embryogenesis and is found in most tissues in the adult with the highest levels in the CNS. The

normal function of PrP^C is yet to be defined but most recent data indicate that the protein is involved in cell adhesion and/or signaling processes.

The exact mechanisms responsible for the PrP^C conversion to its abnormal counterpart PrP^{TSE} are not clearly understood but it is known that, due to unknown reasons, PrP^C molecules undergo conformational modifications from mainly α -helical to β -sheet structure; and as a result, the PrP^{TSE} aggregates and becomes resistant to protease digestion. Extensive accumulation and aggregation of PrP^{TSE} in the cytoplasm may be associated with neuronal cell death. According to prion hypothesis, the PrP^{TSE} is the only component of infectious agent, and prion propagation occurs through the self-replication of PrP^{TSE} which occurs with high fidelity by the recruitment of endogenous PrP^C in the presence of as yet unidentified factor. There is no doubt that PrP^C is essential for spread of infection since mice without functioning *Prnp* gene (PrP^{0/0}) are resistant to TSE infection.

The target organ of accumulation of TSE agents is the CNS but they also accumulate at a lower level in lymphoreticular tissues including spleen, lymph nodes, and other secondary lymphoid tissues. Since the lymphoid tissue maintains an immediate contact with blood, the spread via the bloodstream probably occurs. It has been a concern for decades that blood of an infected individual contains low level of infectivity and that blood and blood components can be a source of transmission of TSE infection. All epidemiologic evidence to date suggests that transmission of non-variant CJD through blood transfusion probably does not occur, although it remains an open question which deserves further research and continued surveillances. During the last decade it has been proved that blood from a proportion of animals with both natural and experimental induced TSE could transmit the infection. Recent epidemiological evidence from the UK indicates that, almost undoubtedly, transfusions of non-leukocyte reduced red cells

(RBCs) from vCJD patients during preclinical phase have transmitted the infection at least in three instances.

This report summarizes current available knowledge on TSE infection that is related to the safety of blood transfusion, and makes recommendations for future works aimed to reduce the possible risk of TSE transmission by blood and blood products.

Current TSE Epidemiology

Approximately 85% of all human TSE cases belong to sporadic CJD which has a relatively even worldwide incidence of 1 to 1.5 cases per million per year with equal frequency in males and females. However, there is a significant increase in incidence among people that are methionine homozygous (M/M) at *PRNP* codon 129. Approximately 10% of human prion diseases have a genetic cause due to mutations in *PRNP* gene and phenotypically they manifest as CJD or GSS or FFI. The remaining cases constitute the subgroup of acquired diseases which include iatrogenic CJD, kuru, and vCJD.

The infectious agent responsible for vCJD has been found to be identical to the BSE agent. It is accepted that infection in humans occurred through consumption of BSE-contaminated meat products. BSE was first described in 1986 in the UK and later in a number of European countries as well as in Japan, Canada and the US. By end of 2005, nearly 190,000 cases of BSE had been diagnosed in 26 countries, with a vast majority (97%) from the UK. The vCJD was first identified in 1996 in the UK and subsequently endogenous cases were reported in France, Ireland, Italy, Netherlands, Portugal, Spain and Saudi Arabia and cases associated with previous residents in the UK occurred in the US, Canada and Japan. As of July 2006, a total 192 primary cases of vCJD have been reported in 11 countries with the vast majority (168 or 87.5%)

in the UK. Twenty cases were reported in France. Recently, three iatrogenic transmissions of vCJD associated with transfusion of non-leukoreduced RBCs (2 clinical and 1 sub-clinical) were reported in the UK. In a UK retrospective study in which examination for PrP^{TSE} had been undertaken in 16,700 surgically removed tonsillectomy and appendectomy specimens, most of which were from patients 10 to 30 years old at the time of operations. Three appendectomy samples showed accumulation of PrP^{TSE}, giving an estimated prevalence of 3 per 12,674 or 237 per million (95% CI: 49-692 per million) in the UK. Continuing studies by the Imperial College lead to changes in the prediction of incidence that has had fallen from the initial half million cases at upper-limit of primary outbreak to around 7,000 by 2002 and then to 540 by 2003.

All clinical vCJD cases, including 2 secondary cases, have occurred in M/M homozygous individuals at *PRNP* codon 129. Approximately 40% of the population in the UK carries this genotype. However, the identification of the third secondary case with subclinical evidence of infection, which was methionine/valine (M/V) heterozygous (50% of the UK population is M/V heterozygous) has raised a possibility that exposed individuals of other genotypes may develop clinical symptoms much later or remain in some asymptomatic carrier state.

Blood Infectivity

Blood has been shown to transmit infection in naturally occurring scrapie or induced BSE in sheep and in vCJD in humans even thus the levels of infectivity are not determined. Low levels of infectivity, comparing to the brain, have been experimentally detected in buffy coat and plasma in rodent models of TSEs both preclinically and at the end stage of the disease. The source of infectivity in blood is not clearly understood, but likely the lymphoid tissues are targeted first and then the infectivity spreads and reaches the brain through the hematogenous as

well as neuronal routes. It is generally believed that the infectivity in blood is present in higher amounts in plasma component than in buffy coat with minimal or no infectivity in red cell and platelet fractions.

Current models on the risk and distribution of infectivity in blood take an assumption that blood from an infected person may contain 2 intravenous ID₅₀/mL. This assumption is based on the data from experimental animal models which showed that the overall levels of infectivity in blood are on the order of 10 intracerebral median infective doses and that the intravenous transmission is five-fold less efficient than intracerebral. It has been assumed that transmission would certainly occur if the blood component contained ≥ 2 ID₅₀/ml in total. Some assume that the infectivity is distributed in blood as: 24% red cells, 22% buffy coat, and 54% plasma, and that leukocyte reduction would reduce infectivity from red cells and platelets by 2 log but there would be no reduction in freshly frozen plasma. Based upon these assumptions, four strategies were adopted: 1) To reduce exposure to donor leukocytes; 2) to reduce exposure to donor plasma; 3) to minimize the number of donors contributing to a therapeutic dose of product; and 4) to promote an evidence-based approach to prescribing donor-derived blood components and to encourage the use of alternatives.

In relation to blood safety issue it is important to understand the TSE infectivity propagation and distribution during progression of disease which has long incubation period which in some instances may extend to more than a decade. A few scenarios can be predicted: 1) The level of infectivity is low during the incubation period and does not begin to rise until clinical disease develops; 2) the infectivity linearly increases during the whole incubation period, and 3) an early increase in infectivity is followed by a plateau. According to data from animal

models the infectivity starts to be present in blood at around a third of the incubation period and increases linearly toward the clinical stage of the disease.

TSE and Transfusion

Transmissibility of prion diseases through blood has been established in several experimental animal models: BSE, GSS, and vCJD in mice; sporadic CJD in guinea pigs; scrapie in hamsters, BSE in sheep; and GSS in chimpanzees. However, epidemiologic evidences indicate that infection of human sporadic CJD cannot be readily transmitted through blood and blood components. An ongoing study by the American Red Cross together with the US Centers for Disease Control and Prevention has enrolled a total of 368 known recipients of blood components from persons who later died of sporadic CJD, and of which, 125 have been followed for at least 5 years after transfusion. None have died of sporadic CJD. In addition, up to date, no case of CJD has been reported among more than 20,000 hemophilia patients who have been repeatedly treated with coagulation factors during the past 10 years.

In the UK, of patients who died of vCJD, 22 were blood donors prior to their appearance of clinical symptoms. From transfusion records, a total of 64 recipients were identified and 26 of whom are still alive. Of these recipients, two have developed vCJD and one had evidence of subclinical infection at postmortem examination. The first case of vCJD transmission through transfusion was reported in December 2003. A patient with *PRNP* codon 129 M/M homozygotes developed vCJD 7 years after having received non-leukocyte reduced RBCs donated by a 24 years old donor 3 years prior to illness. In the second transmission case patient died from non-vCJD related disorder 5 years after receiving the transfusion from a young adult who became ill after 1.5 years following donation of the implicated blood. The recipient had

asymptomatic evidences of vCJD infection and was identified only by the postmortem discovery of PrP^{TSE} in lymph node and spleen, and has been tested as M/V heterozygous at codon 129.

The third case, also received non-leukocyte reduced red cells, and developed the disease approximately 8 years after transfusion from a donor who developed vCJD almost 2 years after the donation. This case was confirmed to be homozygous M/M carrier at codon 129. These three cases provide almost indisputable evidences that blood of vCJD infected persons can be infectious during the preclinical period. The second case also indicates that genetic susceptibility to vCJD is not limited to *PRNP* codon 129 M/M homozygous persons and that individual with M/V and even V/V genotypes might be infected as well.

So far there is no report of transmission of prion disease, including vCJD, by plasma products, although the risk of such transmission cannot be totally discounted. The major concerns are that plasma products are manufactured from large volume of plasma pools and that many patients who receive plasma products have been repeatedly treated due to chronic coagulopathies or immunologic disorders with therapeutics from the same pool which might contain some infectivity. Experimentally it has been shown that multiple exposures of animals to small doses of the TSE agent can cause the diseases in rodent model. Currently the plasma collected in the UK is not used for fractionation of plasma product. Available data indicate that the separation processes used in plasma product manufacture can significantly remove prion agents and consequently, the risk of transmission of vCJD by plasma products is believed to be low. However, most of experiments have evaluated the efficiency of removal in multiple steps rather than as a whole process. Furthermore, the highly infectious exogenous brain homogenates were used as spiking materials creating probably highly artificial conditions to that in blood for

removal of prion. Therefore, further research is needed to better define the safety of plasma products.

Donor Deferral Policies

The UK Department of Health made an announcement in March 1996 acknowledging a possible link between BSE and vCJD. In the meantime, a number of precautionary measures were taken to reduce the risk of infected meat being sold for human consumption. Other actions taken in the following few years include protection against transmission through surgical instruments, and guidelines on the handling of animal-derived materials for pharmaceutical use including vaccines. In terms of blood-borne transmission, a range of precautionary measures have been put in place since 1997 to safeguard the blood supply against the risk of vCJD transmission. These include withdrawal and recall of any blood components, plasma products or tissues obtained from any individual who later develops vCJD (1997); sourcing of plasma from outside the UK (1998) and preparation of products such as clotting factors from plasma imported from the US (1999); removal of white blood cells (leukocyte depletion) from all blood components (1999); import of fresh frozen plasma from the US for neonatal patients and for older children as appropriate (2004); not accepting donations from people who have received a blood transfusion since January 1980, including apheresis donors, and those who are unsure whether or not they have received a blood transfusion (2004); notify a selective group of patients who are considered to be at risk as a result of receiving plasma products (2004); and giving out information about better transfusion and the appropriate use of blood (2002).

In 2002, the US Food and Drug Administration (FDA) issued a revised guidance in order to reduce the possible risk of transmission of TSE by blood and blood products. In terms of

donor deferral, it recommends that donors who are diagnosed with CJD or vCJD or are suspected of having CJD or vCJD, or are at risk of CJD because they received pituitary-derived growth hormone or dura mater transplant, or have family history of CJD, should be indefinitely deferred. In addition, anyone who have resided in the UK for 3 months or longer between 1980 and 1996, and who have resided in Europe for 5 years or longer since 1980, and who received a transfusion in the UK since 1980, are indefinitely deferred. In August 2006, the FDA issued a draft guidance proposing the indefinite deferral of donors who have been transfused in France since 1980.

Technologies for Infectivity Detection and Removal

Currently, the TSEs can not be properly diagnosed until the disease enters its clinical phase. Brain tissues from suspected subjects are targeted specimens for laboratory testing, such as immunohistochemistry, enzyme immunoassays, and immunoblotting (Western blot), all of which require protease treatment. Infectivity in blood is believed to be four even six orders of magnitude lower than what is in brain and is considered to be even lower during the preclinical period. Recent studies indicated that one mL of blood containing 10 infectious doses (ID) could only contain approximately 140 to 280 molecules of PrP^{TSE}, an amount which would be very difficult to detect with currently available methods or methods at the different stages of development. Even thus a number of approaches have been applied for detection of PrP^{TSE} in blood, none of them has reached the stage of being validated.

Immunocapillary Electrophoresis (CE): The CE was the first method that claimed successful detection of PrP^{TSE} in blood of sheep with natural scrapie and of experimentally infected hamsters. However, its performance has not been confirmed with samples of human or monkey

origin. The assay is based on competitive inhibition of binding of specific antibody to the antigen (PrP^{TSE}) by a synthetic peptide analogous to the fragment of PrP molecule to which the antibody was produced. Even thus efforts continue to apply to this test for detection of PrP^{TSE} , it is necessary to conclude that the methodology applied to sample preparation and test itself is difficult to optimize and reproduce.

PrP^{TSE} -Specific Antibodies: A few antibodies have been reported to react specifically with PrP^{TSE} in the absence of protease treatment. This reaction could increase the sensitivity of some assays to the point of PrP^{TSE} detection in blood, although currently it appears that only monoclonal antibody 15B3 is being actively exploited in this way.

Conformation-Dependent Immunoassay (CDI): The assay is based on the differences in binding of 3F4 antibody to PrP^{C} and PrP^{TSE} molecules. The antibody-binding site is fully accessible in the normal protein but largely inaccessible in its pathologic form which requires unfolding by denaturation. Thus, PrP^{C} gives similar signal intensity whether in its native or denatured state, whereas the signal from PrP^{TSE} increases after denaturation. The sensitivity of CDI is two orders of magnitude greater than that of the immunoblot assays for the same samples. It has been also claimed that the sensitivity of the method approaches the sensitivity of bioassay in transgenic mice.

PrP^{TSE} -Binding Ligands: Sulfated polyanionic compounds such as pentosan sulfate or dextran sulfate have been shown to bind PrP^{TSE} in vitro. Plasminogen has been also shown to bind predominantly PrP^{TSE} but not to PrP^{C} . Seprion (Microsens Biotechnologies, London, UK) is a ligand from the polyanionic family that specifically captures aggregated PrP^{TSE} without the requirement for protease treatment. It can be used in variety of animal species with appropriate species-specific anti-PrP antibodies. The test has been approved for BSE, scrapie and CWD in

the US. The testing of brain- and spleen-derived samples is underway. However, on the base of very limited data on testing of blood, it is too preliminary to conclude whether this test will be sensitive and specific as a blood screening test. A different ligand, calyx-(6)-arene (bioMérieux, Durham, NC, USA) has been used to capture aggregated oligomers of protease digested PrP^{TSE} that were precipitated by streptomycin. The detection of macromolecular aggregates is accomplished by using a labeled monoclonal antibody. The method has been applied for detection of PrP^{TSE} in BSE and CJD brain homogenates and in small numbers of plasma samples from scrapie-infected sheep, BSE-infected cattle, and humans with the sporadic, iatrogenic, and variant forms of CJD. All 200 normal human plasma samples tested negative. A third ligand-based approach by Adlyfe (Rockville, MD, USA) uses a peptide analogous to the narrow region of the PrP protein that is believed to be involved in the conformational transition from PrP^C to PrP^{TSE}. The palindromic polypeptide labeled at each end with a fluorophor is mixed with a test specimen containing PrP^{TSE} which upon the contact with a ligand causes its folding into a β -sheet conformation leading to change in fluorescence wavelength. The folded ligand induces more ligand molecules to adopt the same conformation. The kinetic of changes is examined and the ratio of relative excimer to monomer fluorescence emission provides a measurement of the presence of PrP^{TSE} in sample. The assay does not require either a protease digestion step or an antibody, and has broad reactivity across different species. The test has been successfully used to demonstrate PrP^{TSE} in blood of experimentally infected hamsters, mice and monkeys as well as in blood of sheep with natural scrapie and cattle with BSE. It also showed some promises while applied to a small number of samples from patients with sporadic CJD discriminating them from uninfected control subjects. The threshold sensitivity is claimed to be at a femtomolar level of detection.

Amplification Method: Protein misfolding cyclic amplification (PMCA) is the methods that emerged from the concept that under an optimal condition in the presence of sufficient number of PrP^C molecules, even small amount of PrP^{TSE} could initiate the conversion of the PrP^C into PrP^{TSE}. As a result, PrP^{TSE} could be amplified to detectable levels by repeated cycles of conversion and fragmentation by sonication. Theoretically, the process of amplification can go indefinitely when sufficient amount of PrP^C is available for conversion. Thus, brain homogenates from scrapie-infected hamsters could be amplified about 60-fold by 5 cycles of sonication and incubation. It is also claimed that the method can amplify not only PrP^{TSE} but the infectivity as well. When PMCA was applied to buffy coat samples from clinically affected hamsters, 16 out of 18 samples tested positive. While none of 12 samples from uninfected hamsters gave positive result. More recent study demonstrated the presence of PrP^{TSE} in samples of buffy coat as early as 20 days (1/4 way through the incubation period) following inoculation in 50% of animals while no samples tested positive at 14 days. Very surprisingly the number of positive samples started to decrease after 40 days with no sample tested positive at 70 day, but then 80% of samples tested positive 80 days following infection at the clinical stage of the disease, [Saa et al. Science 2006; 313: 92-94]. No control sample tested positive. Even thus the method shows such a promising results, it takes a few days to perform which diminishes its potential usefulness as a test for routine blood screening.

Other Methods: Number of other methods that has been developed for detection of PrP^{TSE} in the brain homogenate or spiked with CSF or plasma brain or spleen homogenates include intensely fluorescent targets (SIFT), immuno-PCR, flow cytometry of seeded aggregates or different variations of capturing methods (magnetic beads) with the following detection in sandwich

ELISA formats. However, there is no report that any of these methods was successfully applied to detect the PrP^{TSE} in blood.

Cell-Based Bioassays: Development of sensitive and effective cell culture based infectivity assays will be extremely important to confirm the data of the blood screening assays or to substitute expensive animal models. Few cell lines that may be useful in this regard have been developed but they are of limited use because of their restricted sensitivity to some of scrapie agents only.

A different but complementary to the detection approach will be to remove prion infectivity from blood and blood components. Two companies are developing prion-removal filtration-type technology for this purpose:

Leukotrap Affinity Prion Reduction Filter (LAPRF) (PALL Corp., East Hills, NY, US) is built up from a polyester fibers with proprietary chemically modified surface that allows to trap prions from residual plasma present in RBCs and remove any residual leukocytes that may carry the infectious molecules. The filter works as a cation/anion exchange column and also allows prion retention through nonspecific ionic interaction, and hydrogen bonding. It also removes any residual aggregates. The filter can be used on day one RBCs. It has been validated in two studies: exogenous RBC-brain spiked model and endogenous RBCs from infected hamsters. In the first study the exogenous scrapie infected brain homogenate from hamsters was spiked into units of human RBCs which then were passed through the LAPRF and the filtered material was assayed for the PrP^{TSE} by Western blotting. It has been shown that the filter removed PrP^{TSE} by approximately 3 log. In addition, similar experiment was performed with a prototype filter and pre- and post-filtration material was 10-fold serially diluted into PBS and intracerebrally inoculated into groups of healthy hamsters. Bioassay showed removal of infectivity by 3.7 log

ID₅₀/ml. Endogenous infectivity study used blood from scrapie infected hamsters that was collected at the clinical stage of the diseases, pooled and processed into RBC unit which was filtered through the LARPF. Samples of pre- and post-filtered RBCs were intracerebrally inoculated into groups of healthy hamsters. While 6 out of 43 animals that received nonfiltered blood developed clinical symptoms and were confirmed to carry PrP^{TSE} in their brains, none of 35 (p = 0.03) animals that had received filtered blood developed the disease even after long observation period of 400 days.

The MacoPharma P-Capt Filter incorporates patented PRDT (Pathogen Removal and Diagnostic Technology, Falls Church, VA, USA) ligand into established, biocompatible filter from MacoPharma (Tourcoing, France). The ligand has been shown to bind the abnormal forms of prion protein from both sporadic CJD and vCJD-infected human brain, familial GSS-infected mouse brain, and scrapie-infected hamster brain with high affinity in solution. The prototype filter removed infectivity from human RBCs spiked with scrapie-infectious brain homogenate below the level of detection by Western blotting, and reduction of 4 log ID₅₀/mL of infectivity has been documented in the *in vivo* bioassay. The filter has been tested in removal of infectivity from leuko-reduced whole blood collected from scrapie-infected hamsters at the clinical phase of the disease. Pre- and post-filtered blood was intracerebrally inoculated into a large cohort of 100 hamsters that were followed for 18 months for signs of the disease. It is claimed that the filter removed 2.8 ID/mL of blood infectivity to a level below detection of the bioassay.

Finally, it also should not be disregarded that leuko-reduction filters applied to the RBCs and even to whole blood may also reduce the infectivity below some threshold level which will be not sufficient to cause infection through transfusion. Unfortunately, at the present time there is no sufficient experimental data available which can support this assumption.

Future Prospective

Epidemiologic evidence demonstrates that blood of vCJD-infected individuals is infectious and can transmit the disease upon transfusion. The experimental evidences indicate that blood of TSE infected animals contains the infectivity during the incubation period and at the clinical phase of the disease. However, many key uncertainties remain and number of assumptions awaits confirmation. It is, therefore, important for us to continue to explore our knowledge in the following areas:

- Basic researches in prion protein chemistry, mechanisms of conformational changes and conversion, natural history of infection in humans, distribution of infectivity in blood, transmissibility via transfusion, and genetic susceptibility.
- Epidemiologic surveillance studies including closer monitoring, targeted look-back, and prevalence among donor population when an effective and reliable test becomes available.
- Development of effective and reliable blood assays for *in vitro* detection of TSE infectivity on samples collected in preclinical stage. The specificity of these assays will be essential.
- Collect blood samples, particularly during the preclinical stage, from infected patients and animals in order to establish effective panels for evaluation of detection assays.
- Improve prion removal and/or inactivation technologies without compromising product quality.
- Develop simple and sensitivity cell culture based infectivity assays as a tool for research and to serve as a confirmatory test for blood screening.
- Improve the current donor deferral policies to reduce the risk of transfusion but yet minimizing unnecessarily deferral of qualified donors.