



*French Agency for the Safety  
of Health Products*

**ANALYSIS OF THE RISK OF TRANSMISSION OF  
VARIANT OF CREUTZFELDT-JAKOB DISEASE BY  
HEALTH PRODUCTS AND BY TISSUES  
AND FLUIDS OF HUMAN ORIGIN**

**UPDATE OF FINDINGS OF AD HOC GROUP  
REPORT OF DECEMBER 2000**

**REPORT OF FEBRUARY 2004**

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## **- SUMMARY -**

Scientific data available since the publication in December 2000 of the group of multidisciplinary and independent experts on the risks of transmission of the variant of Creutzfeldt-Jakob (v-CJD) by blood and its derivatives, have been regularly examined and have given rise to two update reports in 2002 and 2003. This report concerns the updated expert evaluation dated February 2004 by the multidisciplinary and independent expert group (*Annick Alperovitch, Marc Eloit (Chair), Claude Guérois, Jean-Jacques Hauw, Norbert Ifrah, Corinne Lasmézas, Claude Négrier, Armand Perret-Liaudet, Jean-Marie Seigneurin, Yvette Sultan*) brought together on the initiative of the Afssaps (French Agency for the Safety of Health Products) 3 February 2004 (*report drafted by J.F. Legras and E. Pouchol*).

There are few new data on the pathophysiology of v-CJD, modes of transmission, distribution and degree of infectivity in various tissues nor on estimation of a possible infectious load in blood. The possibility of transmission of the disease by blood remains a hypothesis, which is still not fully confirmed, and neither without there being any tangible evidence of the absence of risk. There are no new features which would significantly modify upwards or downwards estimation of the degree of risk as considered in the December 2000 report.

From an epidemiological standpoint, no increased incidence has been seen of v-CJD. There would seem to be no need to estimate the number of individuals susceptible of developing v-CJD and hence currently in the course of incubation.

No new risk factor which could be used as an exclusion criterion in the clinical selection of blood donors has been identified.

The case of v-CJD being notified in December 2003 in the United Kingdom, possibly linked to a transfusion history, should nevertheless be seen as an alarm signal so as to, with a conservative approach, consider the risk of transmission as being no longer theoretical but possible, and hence maintain rigorous alertness concerning the analysis of risk and the pertinence of measures taken.

No screening test is applicable in humans in the current state of development. It is exclusion criteria for donors concerning risk factors of classical CJD and of v-CJD, currently applied, which remain the most appropriate measure for the qualification of donations, at least as long as valid screening tests usable routinely and applicable throughout the asymptomatic period are not available.

There is no method for inactivation of the agent of v-CJD which can be applied to blood products. Regarding labile blood products, leukoreduction and reduction in the residual volume of plasma are precautionary measures which can only contribute to a further decrease in the risk of transmission. Similarly, regarding stable blood products (blood derived medicines), the separation/purification stages can contribute to elimination of the agent during their preparation by plasma fractionation.

Conclusions and recommendations of the December 2000 report remain valid. None of the points dealt with and discussed in this report requires modification. There are no new measures to suggest for further reducing the possible risk of transmission of v-CJD by blood products. Currently applied methods seem effective and in proportion to guarantee the risk-benefit ratio of blood products, as well as for grafts and cells, and for medicines extracted from human urine.

## **Introduction**

In the context of permanent alertness by the Afssaps regarding the risk of transmission of the variant of Creutzfeldt-Jakob disease (v-CJD) by blood or products derived from the human body, scientific data available since the publication in December 2000 of the report of multidisciplinary and independent (1) experts, designated as the "experts' group" throughout this report, have been regularly examined. Two update reports were published, in February 2002 (2) then in March 2003 (3). The present report deals with the updated expert evaluation of February 2004.

Only scientific aspects have been reviewed. No new feature required reopening the discussion on other aspects, and in particular ethical considerations.

Publications referred to in this report have been used as a basis for deliberation. These references are by no means comprehensive on the subject of v-CJD; in fact, the most useful articles in the context of assessment of the risk of transmission by blood products have been taken into consideration and discussed.

The field of expert evaluation has extended from blood products to all health products for therapeutic use so as to include cells, tissues and products derived from the fluids of human origin. It therefore deals with the safety of grafts (organs and tissues, obtained from dead or living donors) as well as substances extracted from human urine.

The objective of these experts was:

- to examine newly published data and discuss results,
- to propose, if necessary, measures likely to reduce risk and analyze the consequences of any new measure,
- to determine whether the conclusions and recommendations of the report of 11 December 2000 and of its successive updates required modification.

NB.: The same terms and abbreviations as those used in the December 2000 report and its updates will be adopted in this report, with no further explanation. A glossary of abbreviations is provided at the end of this report.

## **1. Infectivity**

### **1.1 *Infectivity in blood***

#### **1.1.1 *Presence of infectivity in blood***

The most recent studies concerning screening for infectivity in blood and involving a primate model were published in 2000 (4). It should be remembered that the BSE agent, adapted by passage in the macaque monkey, has been used to experimentally infect a microcebe (grey mouse lemur, a lemurian primate). The brain and buffy-coat obtained from this diseased animal was sampled and injected intracerebrally in healthy microcebes (two animals received a fraction of brain homogenate and one animal received buffy-coat). All three recipient animals developed the disease. This study thus showed that it was possible to transmit the BSE agent from blood in a primate model, an experimental model closer to humans than the sheep model mentioned in §1.3. It should nevertheless be emphasized that the study concerned only buffy-coat and envisaged only the intracerebral route of administration. Furthermore, in this study, the central nervous system was directly involved either by the material inoculated or by the route of inoculation, and positive results obtained in terms of infectivity and the capacity to transmit were therefore predictable. This study should at least be completed by intravenous administration of whole blood or buffy-coat in order to provide a level of information comparable to that of the sheep model study.

This study should be viewed together with that of Brown et al., as yet unpublished (5), reporting transmission to the squirrel monkey of the familial CJD agent by intracerebral injection of purified leukocytes obtained from a chimpanzee.

The most pertinent studies, which have envisaged inoculation of blood from individuals suffering from v-CJD in primates or in susceptible transgenic mice are not yet complete. However more than 30 months after the injection of samples of human blood, no recipient animal has developed the disease. Similarly, no transmission to primates has been shown following inoculation of blood from a primate infected experimentally with BSE.

### *1.1.2 Distribution of level of infectivity in blood*

The most recent estimates of the distribution and level of infectivity in blood come from rodent experimental models.

A first study of Rohwer et al., unpublished (6), shows that infectivity is distributed less preferentially in the buffy-coat than had been accepted in the report of December 2000 (90%) and already reviewed downwards in the March 2003 update (50%). It now seems that the buffy-coat carries about one-third of infectivity of whole blood, while 50% of infectivity is present in plasma. The remainder is presumed to be associated with red cells without it being possible to specify the form and nature of this association. These new data concerning the distribution of infectivity in blood did not bring into question the potential usefulness of leukoreduction (see §4.3). In contrast, there are no new data concerning the presence of possible infectivity in platelets.

Another study has confirmed unpublished data and included in the March 2003 update regarding the infectious load of whole blood (7). This is indeed considered to be less in relation to the infectious load which had been estimated in the December 2000 report, i.e. about 20-30 rather than 100 intracerebral infectious units per ml of whole blood.

These new findings do not actually modify estimation of the infectious load of de-leukocytized plasma, expressed as infectious titer by intracerebral administration, which remains about 10 Inf-ic U/ml.

In contrast, another study suggests that IV administration would be similarly effective to intracerebral administration as route of transmission of infectivity (see §1.2 reference (9)).

However all these findings again raise the question of the precise physico-chemical nature of the v-CJD agent (8) and in consequence that of the infectious form circulating in blood and in particular plasma. This question is of particular interest for validation studies of procedures for the preparation of blood products (see §4.2).

## *1.2 Intravenous transmission of infectivity*

Two recent studies using experimental primate models have documented the capacity of intravenous administration to transmit the agents of human TSSE.

A first study consisted of experimental infection by intravenous or oral administration of macaques with the BSE agent present in a brain homogenate (9). This study was compared with a similar earlier study in which macaques infected intracerebrally developed a v-CJD type disease. This study tended to confirm that v-CJD in humans resulted from the transmission of BSE, also showing the usefulness of this macaque model (10). In the present study, PrP<sup>Sc</sup> was sought in various tissues at the end of the disease. The incubation time of the disease is much shorter following intravenous transmission than per os, and is very close to that seen for intracerebral administration in the princeps study.

Another study (5) consisted of experimental intracerebral infection of chimpanzees with various human TSSE agents (GSS, familial CJD, v-CJD) present in a brain homogenate. During the clinical phase of the disease, the brain was collected and injected IV and by intracerebral administration to squirrel monkeys. In the current state of this ongoing study, only one case of transmission of the GSS agent has been seen in a recipient animal, by both intravenous and intracerebral administrations.

These studies of IV transmissivity of infectivity of cerebral origin are highlighted by the pertinence of the animal (primate) model used, although the second study was not carried out within the same species (species barrier effect). The second study (5) directly concerns human TSSE agents including the v-CJD agent; the first study (9) concerning the BSE agent in macaques nevertheless seems to also be a good model of v-CJD in humans.

It should be pointed out that these two studies, involving the inoculation of nerve tissue rather than whole blood or plasma, provide no information as to the presence of infectivity in blood and its fractions, nor directly on the possibility of intravenous transmission of infectivity present in blood.

In the present state of the study (5), no case of transmission of v-CJD agent has been reported.

These studies show the ability of the intravenous route to transmit, in certain experimental models, infectivity when infectious material is of cerebral origin. In contrast, they show the relative effectiveness of the digestive barrier against transmission, which is consistent with the small number of cases of v-CJD seen up till now in the United Kingdom. The intravenous route hence appears to be 1 to 5 times less effective than the intracerebral route, subject to the reservation that the use of lower inoculation doses similar to those presumed to exist in blood may possibly have resulted in better documentation of the comparative efficacy of these two routes.

On the basis of these new findings, and as a precautionary measure, the experts recommend that the IV route should be considered to be as effective as the intracerebral route in the case of infectivity present in blood. For update of the calculation of residual risk, it is suggested that 1 log should no longer be subtracted between the intracerebral route infectious titer and that for intravenous administration, i.e. to consider that  $1 \text{ Inf-ic U} = 1 \text{ Inf.iv U}$ .

In view of the infectious load estimated for plasma on the basis of the studies mentioned in §1.1, the infectious load of de-leukocytized plasma would finally be 10 Inf-iv U/ml.

This new finding requires confirmation and refinement in the context of studies of the transmissivity of blood infectivity, if possible in the preclinical phase and using v-CJD agent in primate models in an intra-species context (see §1.3).

However it does not significantly modify the estimation of residual risk as calculated in the December 2000 report (see §7.2).

It finally renders more probable the hypothesis of a transfusion origin of v-CJD in the patient who died in December 2003 in the United Kingdom (see §2.5), a hypothesis which was taken into account in the December 2000 report.

### ***1.3 Presence of infectivity in intravenous blood transmissivity***

The works of Houston et al. on the experimental transmission of BSE agent in the sheep model have not formed the basis of any new publication since the second article which appeared in 2002 (11, 12, 13, 14).

It may be remembered that this study consisted of experimental oral infection of sheep with the BSE agent, the drawing of blood either during the preclinical incubation period or during the clinical phase, IV injection of the whole blood thus drawn or of buffy-coat (leuko-platelet layer) in 19 healthy sheep (not affected by scrapie) but genetically susceptible. The first case of transmission seen in one of the recipient animals was published in 2000. This result showed the possibility of oral transmission of BSE then secondarily via blood in the asymptomatic phase and within a given species. This sheep model was then considered more

representative of the human situation than rodent models in which said transmission had already been described. It should be borne in mind that it is on the basis of this preliminary result (11) that the December 2000 report had considered, already and a precautionary measure, the hypothesis of the existence of infectivity in blood for v-CJD. The experts' group hence evaluated the risk of transmission of this agent by blood products and concluded that any such risk was slight or even theoretical.

The three new cases of transmission seen in the same study and published in 2002 (13) confirmed, in this experimental model, the presence of infectivity in whole blood and in buffy-coat starting from half of the incubation period of BSE in sheep. This also confirmed the possibility of transmission of the agent by blood within a given species. Continuation of this study also eliminated uncertainties as to its methodological quality as well as to answer the criticisms expressed (12, 14) (by its verification of nature of agent responsible; appearance of disease in positive controls).

Furthermore, the second article reported that the same type of experiment had been repeated with a scrapie transmission model. This second model implied, as blood donor, sheep in the process of incubation of this natural sheep disease (scrapie) and, as recipients, sheep with a genotype susceptible of developing this disease. In this model, scrapie was transmitted in five of the 21 sheep infected experimentally. These results showed for the first time the possibility of blood transmission of a natural TSSE within a given species, in this case the scrapie agent in the sheep. However the route of exposure of recipient animals (blood) was not natural but experimental. This result nevertheless raised the more general question of the inter-human transmission of natural TSSE, notably classical CJD in humans. It should nonetheless be pointed out that epidemiological data have not always enabled the report of cases of blood transmission of the classical CJD agent in humans. Results seen could be interpreted as reflecting the particular susceptibility of the ovine species to the scrapie agent. It must be remembered that as a precautionary measure, people with a risk factor for developing classical CJD are excluded from blood donation.

At the time of the present report and since this second publication, several other animal recipients have developed the disease, in each of the two models of transmission studied, leading to blood transmission rates of more than 20% for BSE agent and close to 50% for scrapie agent.

All these results are interim and will require finalization.

However these results do not modify the comments already made in the December 2000 report on the limitations of interpretation of this experiment. The ovine model, although more representative than rodent models, and even if it is related to the situation of the natural disease concerning the study involving scrapie, is less pertinent than a primate model. Existence of the transmission of BSE agent and of scrapie in a given species and using the same route could therefore reflect particular susceptibility of the host ovine species in this type of experiment. These results remain incomplete and do not enable refinement of risk analysis insofar as they *i*) concern only whole blood, *ii*) do not enable the quantification of infectivity nor assessment of its distribution in the various sectors of whole blood (buffy-coat and plasma in particular). There is no precision as to the kinetics of appearance of infectivity in blood, nor on its degree over the course of time. It nevertheless appears that the level of infectivity in blood is low.

These limitations of interpretation will remain the same when final results are known. Beyond limitations of interpretation, it must be remembered that these studies show that infectivity in blood and peripheral tissues may indeed be found, provided that the experimental model is properly chosen, with the use of a pertinent methodology and appropriate methods of detection.

Although these additional results show the usefulness of these experimental models as research approaches, in any event they must be interpreted cautiously (15) and enable neither the establishment nor demonstration of the presence of infectivity in the blood of a patient with v-CJD, nor the possibility of transmission by blood products. The possibly different

nature of the infectious form circulating in blood in relation to the cerebral form, evoked in §1.1, might also influence its actual IV transmissivity.

These findings require the availability of results of studies using primate models, as extension of those mentioned in §1.2, some of which are underway, investigating the IV transmission of blood infectivity (whole blood and blood fractions) of the v-CJD agent during the preclinical phase.

#### **1.4 Infectivity in other tissues**

In light of available human studies, the infectivity of v-CJD is presumed to be circumscribed to a limited number of organs and tissues (brain, retina, optic nerve and secondary lymphoid structures, i.e. tonsils, spleen, lymph nodes). All tissues studied up till now proved to be negative. In particular, no abnormal protein nor infectivity has been found in blood and buffy-coat. These studies are nevertheless of limited range considering *i*) the same number of patients tested and *ii*) the weak sensitivity of detection methods used in view of the human/mouse species barrier imposed by infectivity tests, which nevertheless remain the only methods of detection.

There are few new data on the distribution of infectivity in the peripheral tissues of v-CJD patients available since work in 2001 which evaluated many tissues (16, 17).

A study involving various tissues of the buccal area in patients with v-CJD revealed no pathological protein (18).

It may be mentioned that the work of Kopereck et al. published in 2002 (19) reported the detection of abnormal prion protein in the media and more rarely in the intima of intracranial blood vessels as well as in the media and intima of the carotid and aorta extracranially, in patients who died from v-CJD. These studies may take on a certain degree of relief in the light of the cases of v-CJD possibly transmitted by transfusion and reported in the United Kingdom in December 2003.

A recent study of the distribution of infectivity in peripheral tissues, involving nine v-CJD patients, confirmed its presence in the reticulo-endothelial system (20). Studies on the ways of diffusion of TSSE agents within the peripheral nervous system (21) and reticulo-endothelial system (22, 23) may contribute to focusing the implication of the blood compartment (24).

In contrast, one study found no evidence within the central nervous system of patients suffering from v-CJD of a spatial correlation between blood vessels and the presence of vacuoles or of pathological protein (25).

Screening of PrP<sup>Sc</sup> in operative specimens of lymphoid organs (essentially tonsils) obtained from a high number of individuals in the United Kingdom, led to the detection of one positive case published in 2002 (26). These interim results merely confirm the presence of infectivity in lymphoid tissues and follow along with the hypothesis of asymptomatic carriers, a hypothesis already taken into account in estimations of risk factors for blood donation. Methodological problems of this study nevertheless make delicate any estimation of the number of cases of v-CJD still in incubation in the general population (see §2.6).

However these findings should be placed in perspective with the study mentioned in §1.2 consisting of experimental oral or intravenous infection of macaques with BSE agent present in a brain homogenate (9). PrP<sup>Sc</sup> was sought in various tissues at the end of the disease and was found only in the brain, lymphoid tissue, digestive tract (from duodenum to rectum) and the peripheral nervous system, after both IV and oral transmission. Considering that oral experimental transmission in this macaque model may be a model representative of the transmission of BSE in humans, the wide distribution of the agent in the macaque in the digestive tract and peripheral nervous system is a finding needing to be taken into account for endoscopic and surgical procedures done in humans, notably regarding the decontamination of reusable medical and surgical equipment. This subject, which does not fall within the designated area of this report, has also been the object of guidelines (27).



As far as the risk of transmission linked to blood donors with a surgical or endoscopic history is concerned, decontamination of medical and surgical equipment, according to recommended protocols, is the basic measure. Permanent exclusions from blood donation, which already exist for a certain number of types of surgical past history, notably neurosurgery, are merely complementary to this basic measure. This study provides no information on the possible presence of PrP<sup>SC</sup> in blood.

The high amount of PrP<sup>SC</sup> found in the tonsils confirms the usefulness of this tissue for diagnostic purposes.

### **1.5 *Distribution of infectivity in bovine and ovine tissues***

There are no new data on the distribution of BSE agent in naturally infected bovine tissues nor that of scrapie in ovines.

However in view of the results of experimental transmission studies in rodents and sheep models, the WHO has modified the classification of tissues according to their level of infectivity, transferring blood from the category of tissues without detectable infectivity to that of tissues with low infectivity. It should nevertheless be remembered that at present infectivity of the BSE agent has not been found in the blood of bovines affected by the disease, and if it is present, it must be at a very low titer.

There are no new data on the level and nature of risk of dietary exposure to BSE to which the United Kingdom and hence France have been exposed (see §2.2). This risk remains at the level of that considered in the December 2000 report.

### **1.6 *Passage of BSE in ovines***

Possible recycling of the BSE agent in sheep remains a question, in particular for the British national herd (28, 29). However no clinical case suggestive of BSE has been seen up till now in herds of sheep. The absence of any significant modification of the incidence of scrapie in the British national herd would suggest the absence of massive transmission of the BSE agent in sheep. It should nevertheless be emphasized that epidemiology surveillance systems to scrapie remain relatively dubious in certain European Union countries. The question therefore remains and in view of possible consequences on the food chain, merits particular attention. However, models predicting recycling of the BSE agent in sheep have fairly limited consequences on the epidemic level of v-CJD (30).

### **1.7 *Conclusions***

There is little new information concerning the distribution and level of infectivity in various tissues, in particular concerning the presence or not of infectivity in blood. The existence of transitory or permanent infectivity in the blood of individuals affected by v-CJD has still not been confirmed. Nonetheless, results of more pertinent studies regarding the route of inoculation of samples and the nature of the biological material injected, in primate models, are still not yet available. In the meantime, *i*) the presence of the infectious agent in blood throughout the preclinical phase of incubation and *ii*) the ability of the infectious agent to be transmitted by blood are two hypotheses, theoretically pessimistic in view of infectivity studies, but which cannot be definitively ruled out and must also be included in working hypothesis for risk analysis, as was the case in the December 2000 report.

Most recent data do not significantly modify estimation of a possible infectious load in blood and its distribution between the various blood constituents. In the hypothesis of the existence of infectivity in blood, analysis of these findings continues to suggest that infectious load would be low.

The nature of the infectious form in blood possibly differs from that in brain, which must be taken into account for validation studies of procedures used to prepare blood products (see §4.2).

The existence of infected but as yet asymptomatic individuals, who could be candidates for the donation of blood, cells or grafts, cannot be ruled out. It is also necessary to envisage as a maximalist hypothesis, that certain individuals may have a very long incubation period with their disease not becoming evident before their death, making it impossible to identify a risk for recipients of blood donations given previously by this donor who has remained asymptomatic throughout his or her life. This is a further argument to justify the definitive elimination of blood donation by individuals previously transfused.

Studies in primate experimental models show in particular the efficacy of the intravenous route, which must be taken into account in a conservative approach for estimation of the level of risk of blood products. However these studies on efficacy of the IV route as compared with the IC route do not demonstrate the presence of infectivity in blood in a v-CJD patient nor the possibility of its transmission by blood products.

Finally, the possibility of transmission of the disease by blood remains a hypothesis which must always be considered in risk analysis. Apart from the possibly comparable efficacy of the intravenous route with the intracerebral route, but the impact of which is limited on the estimation of residual risk of blood products, there are no new features enabling the modification (upwards or downwards) of the level of risk considered in the December 2000 report.

## **2. Epidemiology**

### **2.1 *Situation of BSE***

Evolution of the BSE epidemic in the British national herd shows that the decrease in the number of cases continued in 2003 (31) (see appendix) and should continue in the same way (32). Since 2002, most cases have been identified by active monitoring, i.e. at slaughter, while no clinical sign had indicated the animal's condition. Estimation of the number of cases according to the birth year of the cohort of animals indicates continued decrease in the number of cases in the direction of very low values in the coming years, in view of gradual disappearance of the contribution of animals born before effective measures were taken to totally banish animal feeds in 1996 in the United Kingdom (33). However a very small number of cases remain in animals born after 1996. The explanation, which would be the most satisfactory in terms of controlling the epidemic, would be that of the fraudulent use of imported animal feeds.

In France, the number of cases also decreased in 2003, since the apparent peak seen in 2001, which resulted of the setting up of a routine screening program in slaughter houses of animals more than 24 months old. Most of the cases recorded annually now result from the application of this screening program, the part played by clinical cases having decreased markedly since 2001. The 2003 tendency is also that of a decrease in cases resulting from screening, hence the global decrease found (31).

In other European Union countries, the setting up of routine screening has also led to a moderate and transitory increase in the number of cases reported around 2001. Screening has also enabled a better European epidemiological overview in which many countries appear to be concerned, some with an incidence in 2003 slightly greater than that in France. However, in general the number of cases is small and is in the direction of a fall since 2003. These countries also banned (each at different dates) materials with specific risks from the human diet.

The first case of BSE in Canada was confirmed 20 May 2003. The case of BSE confirmed in the United States 25 December 2003 was in fact of Canadian origin (34). The presence of BSE on the North American continent has always been envisaged as a possibility in view of the limitations of the monitoring system. Presence of another TSSE in wild mammals,

chronic wasting disease (CWD) had already fed the discussion on possible exposure of the North American population to animal TSSEs transmissible via the diet (35). However the very small number of cases of BSE recorded on the North American territory shows that exposure of the human population is negligible. In the current state of knowledge, the level of exposure to BSE risk via the food chain should have no impact on the safety of blood-derived medicines obtained by fractionation of donations in the United States, nor would it justify measures against donors having spent time in North America. It should also be remembered that there is no over-exposure of the French population to any possible dietary risk resulting from the importation of American bovine products, because of embargo measures linked to the use of hormones in the United States.

In other developing countries (Eastern Europe, Asia, etc.), the situation is probably not as well controlled as in the European Union, but the number of cases of BSE apparently remains very low.

As a result, pressure of risk of dietary origin, but also pharmaceutical in view of specific measures in this area, on blood donors, is currently slow and is decreasing.

## 2.2 *Situation of v-CJD*

The number of cumulative cases of v-CJD continues to progress in the British Isles, with 146 cases in February 2004 as against 130 cases in February 2003, 114 cases in February 2002 and 85 cases in November 2000 (36) (see appendix). However this progression slowed in 2001-2002 then stabilized in 2002-2003. Deaths in 2003 were essentially the same as in 2002 and as a result less than in 2001 and even more so than in 2000: 28 deaths because of confirmed or probable v-CJD were recorded in 2000 and only 20 in 2001, 17 in 2002 and 18 in 2003. Annual incidence is no longer increasing in the United Kingdom and there also has been no increase in the number of probable cases currently progressing.

In France, the number of cumulative cases has not increased in 2003, i.e. in all still six cases of definitive or probable v-CJD as of 31 December 2003, already counted at 31 January 2003, as against five cases as of 1 February 2002 (37). Considering not the date of death of the patient but the date of onset of first symptoms, which is in this situation more variable since it depends upon the ability to go back in time and accurately date the first symptomatology attributable to the disease, it may be considered that there have been no new cases in France since early 2002. In terms of the origin disease, it has been confirmed that the first five patients had never spent time in the United Kingdom and that the last patient had spent very short stays (three or four days in all), and after 1995. It therefore seems that these are actually endogenous French cases for which the origin of contamination (consumption of bovine meat imported from the United Kingdom or bovine meat of French origin) has not been identified.

The ratio of incidence between the two countries has changed little and is still around the 1/20 ratio which had been considered for the dietary exposure factor in the December 2000 report, which would tend to validate this initial estimation. In early 2004, it was nearer to 1/25 but the very small number of cases in France explains these fluctuations. It should be remembered that a study concerning experimental transmission of the BSE agent between primates has notably confirmed that cases of v-CJD seen in France had, as British cases, the BSE agent at their origin, even though certain articles still question dietary origin (38). The December 2000 report was based in particular on the consumption in France of imported British bovine products in order to estimate the number of people who might potentially develop v-CJD and the theoretical risk associated with blood products.

Each of the cases reported outside the United Kingdom, i.e. one case in the United States, Canada, Hong Kong and Ireland respectively, concerned patients who had long resided or stayed in the United Kingdom. Hence these cases cannot be considered as being of native origin. In contrast, the case reported in Italy concerned a woman who had never spent time in

the United Kingdom. This would seem to fall in a context analogous to that of French cases. However it is not possible to precisely state whether the origin of dietary contamination was endogenous or linked to the importation into Italy of British meat.

### **2.3 *Situation of sporadic v-CJD***

The incidence of cases of sporadic v-CJD appears to have increased in some countries. This applied to the United Kingdom in 2002 (36, 39). Similar findings had been reported in the previous update concerning Switzerland in the years 2001-2002 (40).

Among suggestive hypotheses, the existence of cases of v-CJD which might be confused with sporadic v-CJD have been put forward. These cases could be a special form of v-CJD with clinical manifestations very similar to sporadic v-CJD in fairly elderly individuals. However there is no argument to validate this hypothesis. This special form of v-CJD could also be linked to transfusion or surgical origin (see §2.5).

This increased incidence is probably the result of "notoriety bias" since 1996 with improvement in the diagnosis and notification of cases of sporadic v-CJD. Furthermore, and notably regarding certain countries such as Switzerland, it is important to emphasize the small numbers involved, which would amplify variations seen from one year to another. Aging of the population also predisposes to the appearance and diagnosis of sporadic forms in increasingly elderly individuals.

There has been no identification of an increased risk via the use of blood-derived medicines obtained from plasma collected in countries where the incidence of cases of sporadic v-CJD appears to have increased (remembering that in the United Kingdom plasma has no longer been collected for the preparation of blood-derived medicines since 1998).

### **2.4 *Characteristics of cases of v-CJD and identification of risk factors***

The major characteristics of cases of v-CJD are stable: young adults (mean age 29), Met-Met genotype on codon 129 of the PrP gene.

Stability of mean age at the time of onset of the disease would tend to confirm the hypothesis of greater susceptibility of young individuals at the time of exposure to risk. In contrast, the hypothesis according to which the duration of incubation might be longer in individuals exposed at an older age is not consistent with the high degree of stability of mean age of those affected.

Genotyping, when done (123 cases out of 146) has identified up till now only Met-Met codon 129 homozygous individuals. Uncertainty remains as to the possibility of the subsequent onset of v-CJD in individuals genotypes Val-Val or Met-Val on this codon, as has been reported in iatrogenic cases of CJD (use of extracted growth hormone) as well as in Kuru and which might modify current projections of the number of cases. However these cases should be less in number.

No cluster, apart from that in Leicestershire, nor any particular distribution of cases, apart from north-south socioeconomic regionalization, have been shown in the United Kingdom (41).

Hence no risk factor has been identified which could be used as an exclusion criterion for blood or graft donors.

### **2.5 *Hemovigilance and pharmacovigilance reports***

On 17 December 2003, British health care authorities reported the special case of a 69-year old individual (Met-Met on codon 129) who died 8 December 2003 from v-CJD and with a past history of blood transfusions. In 1996, this patient had received a concentrate of non-de-leukocytized packed red cells obtained from a 24-year old donor who himself developed v-CJD in 1999 (42). This report immediately raised the question of possible transmission of v-CJD by transfusion (43).

The donor and recipient, both residents in the United Kingdom, were exposed to the risk of dietary transmission of BSE. However statistical calculation showed that the probability of these two cases being independent and both due to dietary contamination via the BSE agent was very slight, of the order of 1/15,000, and even 1/30,000 with integration of the unusual age of the recipient (44). As explained above, cases of v-CJD occurring preferentially in young individuals could be interpreted as particular susceptibility of a population within the young population concerning the dietary route. Older individuals would not be, or only slightly, susceptible to such dietary transmission. A case of v-CJD in an individual much older than the general mean for the disease could be explained by another route of exposure, such as transfusion or a surgical procedure.

According to this hypothesis, a transfusion origin would be more probable than a dietary origin. This would then be the first case of transmission of v-CJD within the cohort of 48 British patients who had been transfused with blood products obtained from 15 donors who subsequently developed v-CJD (44, 45).

If the hypothesis of blood transmission should prove to be correct, this would raise a number of points for consideration.

- i) The fairly short duration of incubation of v-CJD in the recipient, of the order of 6.5 years, could indicate relatively high efficacy of the intravenous route for transmission of the agent and/or possibly the more pathogenic nature of the infectious form present in blood, or even particular susceptibility of the recipient.
- ii) Onset of cases of v-CJD in a post-transfusion population, often elderly and fragile, could raise the problem of the detection of such cases.
- iii) As the donor died from v-CJD only 3.5 years after the donation, this could confirm the existence of asymptomatic carriers, with a fairly long contaminant silent period. It should nevertheless be remembered that this is currently the only case reported among the cohort of recipients of blood products obtained from donors later developing v-CJD. However, the number of years between transfusion in recipients who are still alive remains too short in relation to the presumed duration of incubation of v-CJD, even when it might be estimated as being shorter following intravenous contamination, to be able to draw any conclusion (46).
- iv) Absence of de-leukocytation of the packed red cells used could explain why there was transmission from human material, the infectivity of which had not been reduced, presuming that de-leukocytation is sufficiently effective.

The absence of reported cases of transmission of the sporadic CJD agent by blood products might be explained by a markedly different nature and distribution of this agent as compared with that of v-CJD. If not, this raises the question of transfusion cases of sporadic CJD passing unnoticed, or at least considering that the case reported by the British authorities in December was finally merely of dietary origin. This sporadic CJD agent is present essentially in the central nervous system, with a peripheral distribution found only rarely and at low levels (47). In the case reported in France a few years ago of a case of sporadic CJD in an individual who had received blood products from a donor who subsequently developed sporadic CJD, the hypothesis of chance was preferred. The above discussion shows that the interpretation of such a case should not be definitively closed. Scientific monitoring is required concerning classical CJD agents, as well as measures for the exclusion of donors and of reminders of CJD linked with this criterion (see §5).

No case of transmission to humans of v-CJD via LBP or BDM has been reported in France by hemovigilance and pharmacovigilance systems, the six patients with v-CJD having received no transfusion nor being blood donors themselves.

## **2.6 *Modelization of cases and estimation of number of expected cases of v-CJD***

Evolution of the number of deaths by v-CJD in the United Kingdom does show a fall in annual incidence, even if overall a plateau seems to have been reached in the 2001-2003 period. Statistical interpretation based upon the date of onset of symptoms reveals a peak in

2000 (48). However the greatest possible caution is required concerning interpretation of the curve of cases seen up till now, in view of the small numbers and as yet limited follow-up period. A further number of years of observation are required in order to be able to state that incidence is currently beginning to fall.

Successive estimations of the number of cases expected in the coming years envisages on each occasion a fall in the estimated number. The most recent estimations (49, 50, 51, 52, 53, 54) are in line with the least pessimistic hypotheses of the modelization of Ghani et al. (*Nature*, 2000) who used the December 2000 report as a reference. These more precise estimations would lead, according to an as yet unpublished study, to a maximum number of a few tens of cases in France during the coming 20 years (55), with the hypothesis that the mean duration of incubation would be of the order of 10 to 15 years (52, 53, 54). Taking a conservative approach, and in view of the margin of inaccuracy of these estimations, the number of 300 cases in France used to estimate risk in the December 2000 report can be maintained.

However these estimations must be considered with caution, since they do not take into account certain parameters which remain in the area of hypothesis, notably the appearance of cases in codon 129 Val-Val or Met-Val subjects. It cannot be ruled out that the 2001-2003 period plateau (or 2000 peak) might not be followed in the more or less short term by another or even several peaks leading to a finally higher total number of cases. However cases of iatrogenic CJD seen in France following administration of extracted growth hormone show that following peaks or waves, made up of individuals with other genotypes, were less high than the first peak, thereby indicating less susceptibility to infection and to development of the disease.

These estimations generally do not take into account secondary transmission cases, if they exist, resulting from transfusion, transplant or surgical procedures. However they should not significantly modify projections.

Screening for pathological protein in operative specimens (tonsils in particular) from individuals apparently free of the disease at the time of excision, undertaken in the United Kingdom, could provide information on the number of asymptomatic characters, though the conception of the study, with no predefined denominator, is such that any extrapolation to the population as a whole is rather hazardous (26).

Very small numbers in France make it difficult to make any pronouncement on a possible shift in the peak of the epidemic curve in relation to that of the United Kingdom. Such a shift is nevertheless possible, in view of a peak of consumption of at-risk bovine products (notably coming from the United Kingdom) later in France than in the United Kingdom (56).

In light of the Italian case, appearance of v-CJD in other European countries exposed to the dietary risk of BSE (endogenous risk or risk by importation of British meat) cannot be ruled out. However exposure overall remains slight in comparison with the United Kingdom (4,278 cumulative cases of BSE in Europe versus 183,496 cases in the United Kingdom, October 2003 data). Estimation of cases of v-CJD in Ireland results in very low values (57). Probably slight exposure does not lead to identification of countries in which measures, such as a stay for a given period, could be justified as exclusive criterion for blood donation.

## **2.7 Conclusion on epidemiological data**

The case of v-CJD occurring in an individual who had received a blood product from a donor who subsequently developed v-CJD could be explained by a transfusion origin, though without dietary origin being totally ruled out. It is hence essential that a highly effective epidemiological surveillance system, concerning both variant and sporadic forms of CJD, as there exists in the United Kingdom and in France, should be able to report and document all cases.

Epidemiological findings show for the first time a possible relation between v-CJD and transfusion, though without giving proof. A precautionary approach suggested in the December 2000 report, which already envisaged this risk although then theoretical, should be continued considering this risk to be possible. Projection of the number of cases of v-CJD in France remains at a maximum that mentioned in the December 2000 report. No epidemiological data justifies upward revision of the conclusions of earlier reports on the risk level considered in the December 2000 report.

### **3. Tests**

Tests now used routinely in France for the screening of BSE in bovines have been developed and validated for this sole purpose. They cannot be used in humans for v-CJD screening and are not applicable to blood donors and even less for checks and verification of blood products.

It remains difficult to describe the current status of development of tests for v-CJD screening in humans which might be used routinely on blood, or possibly urine. In view of industrial aspects, a few data are published and since update of the report in March 2003, there is no new information available on methods already known nor on new research and development approaches.

The development of screening tests must overcome a number of obstacles.

Uncertainties as to the precise nature of the infectious form of the agent possibly present in blood makes validation of a test difficult, notably in terms of positive or negative predictive value.

Absence of any national European or even worldwide organizing structure, the creation of biological libraries for human samples and conditions for their use also makes problematic the access of research teams to samples necessary for validation.

Development of a validated and actually usable routine screening test will require reopening of the discussion on ethical aspects, more or less invasive collection of samples, linked with the possibilities of management and treatment of diagnosed patients.

Availability of a screening test will probably not provide the sole and effective solution for qualification of donations and/or the safety of blood products. Exclusion criteria for donors will probably remain the primary elementary measure, even if effective and routinely-usable tests become available. Screening tests could complete exclusion criteria and above all be useful for epidemiological purposes in order to better target and identify at-risk groups. Similarly, in view of capacity for elimination of procedures for preparation of BDM, subject to reservation of the representativity of available validation studies, the additional contribution made by screening tests might be totally relative concerning BDM. It is important in the short- and mid-term to emphasize that inclusion criteria initiated are not measures taken by default in the absence of a test, but rather that they represent the most appropriate measure and most proportional to risk.

### **4. Elimination and inactivation methods**

#### **4.1 *Elimination and inactivation methods for BDM***

With regard to methods of elimination of v-CJD agent in procedures for the preparation of BDM, recently published data are few in number and the studies referred to here do not provide any new information (58, 59). However some manufacturers of BDM have started to validate certain stages in their preparation procedures, this information being accessible in the Marketing Authorization files of the medicines concerned (see §4.2).

There is no method for inactivation of v-CJD agent which would be applicable to blood products. Procedures for inactivation of the agent with duly established efficacy (autoclaving, oxidation, treatments with urea, normal caustic soda, etc.) are incompatible with the fragility and relative stability of proteins extracted from blood. These methods can be envisaged rather for operations used to decontaminate reusable production materials and equipment (60, 61). It should be remembered that these effective decontamination methods are used whenever needed by LFB. No particular problem has been identified in this area, LFB not fractionating plasma from countries with an incidence of v-CJD higher to that of France.

There has been no publication on new specific methods for elimination or inactivation in course of development. Use of nanofiltration as a specific method for the elimination of prions, progressively developed by the LFB, remains a potentially useful approach, notably because it is unlikely to interfere with the integrity of active substances.

#### **4.2 Validation studies for elimination methods used for BDM**

In its Marketing Authorization files, the LFB has undertaken validation of the preparation procedures for the various BDM which it markets.

Validation concerned stages presumed to be particularly effective and reproducible in the light of data from the literature. Validation also involved stages shared by several BDM, notably those most used and/or for those with the highest theoretical residual level of infectivity according to estimations proposed in the December 2000 report.

Validated stages concerned different types of precipitation, absorption, filtration and separation by chromatography. They also looked at nanofiltration, used in particular to specifically eliminate prions.

Products concerned are factor VIII, von Willebrand factor, fibrinogen, factor IX, IV polyvalent immunoglobulins and albumin.

Studies carried out and results obtained are consistent with data from the literature, notably in terms of overload material, strains and detection methods. In particular, different strains of the infectious agent and detection methods were used in order to correlate and consolidate results together. Results coincide with estimations adopted in the December 2000 report concerning the efficacy of different stages, reduction factors method being the same as or better than those which had been used as a low hypothesis in the calculation presented in the report. These findings support estimations of theoretical residual risk as had been calculated and presented in the December 2000 report.

These studies were undertaken in accordance with recently proposed European guidelines (62). However, in principle there remains uncertainty as to the representativity of all of the validation studies carried out in accordance with these guidelines. These by necessity involve overload with the infectious agent in a physico-chemical form (brain homogenate from animals with experimental TSSE) which may not precisely correspond with the form of the infectious agent circulating in blood (see §1.1). The circulating infectious form of v-CJD could have physico-chemical properties (and in particular conformation, hydrophobicity or even truncated form or modified glycosylation) and hence behavior regarding mechanisms of partition/purification which differ from those of cerebral forms commonly used for validations by overload. The actual capacity of elimination of certain stages could differ from that measured by validation. In particular, the size of the agent could be smaller, leading to overestimation of elimination capacity of filtration stages, including nanofiltration. Comparative validation studies by fractionation of blood from infected animals (endogenous infectivity) should provide a level of correlation, but these studies are much more difficult to carry out, and the experimental nature of the infection might also leave questions as to the representativity of the agent finally studied.

It is nevertheless important to emphasize that since the 2000 report, validation data published in the literature or available in Marketing Authorization files accumulated. Although results differ in their order of grandeur concerning the effectiveness of different procedures, all of



these data converge. Regardless of the type of overload material, the animal species of origin, strain, method of detection and operative conditions of these stages studied, reduction factors obtained are consistent and justify accordance of a minimum of credit to validation studies. None of these findings would justify a downward revision of the conservative estimation of the elimination capacity of preparation procedures of BDM adopted in the December 2000 report.

In addition, the agent, regardless of its actual form in blood, will partition and dilute throughout the fractionation procedure, unless specific co-purification with one of the proteins derived from fractionation is envisaged. Calculations in the December 2000 report, based notably on cumulation of the elimination effects of fractionation stages, led to a hierarchical distribution of substances which remains valid in principle (partition/dilution effect attributed to each fraction resulting from the alcoholic fractionation of plasma).

With the intention of updating calculation of residual risk, a conservative approach could consist of considering only the low hypothesis of the December 2000 report, avoiding in particular addition of reduction factors in individual stages, with the exception of those involving sufficiently different physico-chemical mechanisms. On this basis, validation studies of a combination of several successive stages could provide useful information on the possibility of the cumulation of reduction factors. Some data available to the LFB appear to go along these lines and it is possible that stages of similar mechanism but different operative conditions could finally have an additive effect. Nevertheless with a very conservative approach, calculation must remain cautious concerning the additivity of stages with a similar mechanism as well as the taking into account of filtrations. It must rather be based on the most representative stage.

Results of these studies applied specifically to procedures for the preparation of BDM corroborate and support estimations on the elimination capacity of these procedures, these estimations being made in December 2000 and then based upon only generic data from the literature. Remaining uncertainties concerning the precise conformation and size of the agent circulating in blood nevertheless indicate the need to maintain the conservative approach of the December 2000 report concerning update and interpretation of the calculation of residual risk (see §7.2).

### **4.3      *Leukoreduction***

The potential usefulness of leukoreduction, recognized in the December 2000 report, was based upon the findings that in animal TSSE experimental models, blood infectivity is essentially (90%) associated with leukocytes. It should be remembered that in humans, it has not been possible to confirm the presence of v-CJD agent in either whole blood nor its fractions, though subject to reservation of the interpretation of the case notified by British health care authorities 17 December 2003 (see §2.5).

Experimental data subsequently confirmed the preferential distribution of infectivity in leukocytes, though possibly to a lesser degree, of about 50%. This decreased distribution in buffy-coat led to attribution to leukoreduction, as a precautionary measure, in the previous update of the report, of an elimination capacity for v-CJD agent of half a log rather than one log.

Most recent findings are suggestive of a distribution of about one-third in buffy-coat, half in plasma and the remaining third in red cells, with possible existence of a small and only minimally hydrophobic infectious form in plasma (see §1.1). The actual distribution in blood fractions of the infectious form of v-CJD in the hypothesis of its presence in blood nevertheless remains uncertain, in particular for RBC where infectivity might actually be due to associated plasma. The case of v-CJD notified in December 2003, in the hypothesis of a transfusion origin, does not justify any conclusion as to the nature of the infectious blood fraction. The packed RBCs received by this patient being neither de-leukocyted nor plasma reduced, infectivity could also have been derived from leukocytes, plasma or RBC themselves.

However the distribution of infectivity, now estimated as being about 30% in buffy-coat, is not such as to require the half-log correction suggested in the last update of the report. This half-log correction does not lead to any significant modification of calculation of theoretical residual infectious load in blood products. The same experimental studies led to downward revision of infectious load in whole blood. The global calculation in the December 2000 report remains valid.

There are no new data on the proportion and nature of leukocytes destroyed by the filters used. This effect of leukocyte lysis could even be harmful if a high proportion of B lymphocytes and dentrite cells was destroyed, leading to possible release of infectious particles. While awaiting these studies, there is nothing to suggest that this effect could be such as to bring into question the potential benefits of leukoreduction. The only available data, obtained with equipment used in the United Kingdom, would not appear to show any significant cell lysis.

In conclusion, in the context of a precautionary measure, leukoreduction of initial material (cells, plasma) remains a necessary approach which even if not sufficient in itself alone, can only contribute, via a reduced infectious load, to a decreased risk of transmission of v-CJD by blood products.

## **5. Measures introduced in France since December 2000**

The following measures have been introduced, notably during 2001, following recommendations of the December 2000 report.

These measures have been added to those already existing concerning the exclusion from blood donation of individuals at risk of development of classical CJD (family history, neurosurgery, treatment with extracted pituitary hormones).

There is also a permanent exclusion measure where there is any history of transfusion, taken since September 1997. This measure, in the current context of the case reported by British health care authorities 17 December 2003, proved to be an effective measure for the prevention of possible inter-human secondary transmission.

BDM derived from a plasma pool for fractionation containing a donation from a donor subsequently diagnosed with classical CJD or v-CJD will form the basis of reminder measures. This reminder measure in the case of classical CJD, which is not applied elsewhere in Europe, may seem to be an extremely cautious measure, but it nevertheless is well-founded in view of the discussion arising around the case reported by British health care authorities 17 December 2003 (see §2.5).

### ***5.1 Exclusion of donors having spent time in the British Isles***

The measure for exclusion of donors having spent time in the British Isles for a year or more (cumulative period) between 1980 and 1996 has been effective since January 2001.

Similar exclusion measures have also been taken in several European countries. These measures are not in harmony as to the cumulative duration of stay, which is explained by the different situation from one European country to another in terms of the relative degree of exposure to the risk of BSE between each of these countries and the British Isles, and the distribution of cumulative durations of stay of donors from these countries in the British Isles. It may be remembered that the stricter exclusion measures taken by Canada and the United States, introduced in 2002, have no rational scientific basis nor are they based on any new findings.

There is no new argument, either epidemiological, concerning modes of transmission or arising from calculations of infectivity described in the report, to justify reconsideration of the strategy for exclusion of donors introduced in France. It is useful to recall that in terms of feasibility, this measure has led to only a very small reduction in the number of donors (about -2%) which has not created any difficulty in terms of self-sufficiency in transfusion blood products.



## 5.2 *Leukoreduction*

The principle of maximum leukoreduction of all plasmas (for direct therapeutic use and for fractionation) has been adopted, even if it has been recognized that leukoreduction beyond  $10^6$  residual leukocytes per liter would bring about only a minimal and non-measurable reduction in potential infectious load. However this extremely cautious measure has been proposed in view of uncertainties as to the nature of cells carrying infectivity in blood and the efficacy of filters in their specific elimination (see §4.3).

Generalized use of leukoreduction of plasma (safety FFP, FFP for preparation of VAB, FFP for preparation of SCE, PFF) has been effective since 15 April 2001, with a limit then provisionally fixed at  $< 10^6$  residual leukocytes/L.

Following an experimental phase, the standard in terms of residual leukocytes was permanently determined:

- at  $1.0 \times 10^4$  residual leukocytes per liter for de-leukocyted homologous plasmas for therapeutic use and products derived from their transformation (safety fresh-frozen plasma, fresh-frozen plasma solidarized for the preparation of de-leukocyted reconstituted blood for pediatric use, fresh-frozen plasma viro-attenuated by detergent-solvent, safety cryodesiccated plasma). Checks must show that for a calculation with a 95% confidence interval and using a sampling plan appropriate for the site and rhythm of production, this content must be complied with in at least 95% of products prepared on each site,
- for plasma for fractionation, de-leukocytation procedures used by establishments for the collection and/or preparation of plasma must guarantee that the residual leukocyte content is less than or at the limit of  $1.0 \times 10^6$  per liter of de-leukocyted plasma.

In view of production times, BDM prepared exclusively using leukoreduced plasmas at  $< 10^6/L$  were made available by the LFB in January 2002 for all medicines; the validity of medicines prepared before the generalized use of plasma leukoreduction expired at the latest in December 2002.

Generalized use of leukoreduction has not led to any major difficulty.

This standard was reflected in the Decree of 29 April 2003 defining the list and characteristics of labile blood products.

It has been pointed out that there is no harmonized European position, the routine leukoreduction of cellular LBP and/or plasmas having been introduced in only certain countries. At the time of this report, there is also no European position on the benefits of leukoreduction of plasma for fractionation, regarding the decrease in risk of transmission of the pathogenic agent by BDM.

## 5.3 *Reduction of amount of plasma in cell LBP*

Since 2003, platelet concentrates stored in an additive solution have been available, which reduces the amount of plasma, thus following a recommendation in the December 2000 report. In various categories of cell LBP (platelet, RBC concentrates), it is now possible to also use plasma-poor preparations.

These two measures (leukoreduction and reduced plasma volume) contribute to a decrease in possible infectious load in an LBP.

## 5.4 *Revision of recommended guidelines concerning the use of LBP*

These guidelines produced by the ANAES in 1997 have been revised by an ad hoc working party of the Afssaps. They were published in August 2002 for plasma and for packed red cells, and in June 2003 for platelet concentrates and granulocyte concentrates.

### **5.5 *Improvement in procedures for preparation of BDM of LFB***

Factor IX (BETAFACT) and factor XI (HEMOLEVEN) were already nanofiltered (15 nm) at the time of the December 2000 report.

Regarding factor VIII (FACTANE), the 35+15 nm nanofiltered product has been available since 28 January 2001.

Concerning IV polyvalent immunoglobulins (TEGELINE), the 75+35 nm nanofiltered product has been available since February 2002.

Concerning von Willebrand factor (WILFACTIN), the 35 nm nanofiltered product has been available since January 2004.

Concerning the association factor VIII-von Willebrand factor (WILSTART), the nanofiltered product (35+15 nm and 35 nm) became available in March 2004.

Validation studies of stages in the nanofiltration of factor IX, factor VIII, von Willebrand factor (and hence the association factor VIII-von Willebrand factor) and IV polyvalent immunoglobulins were presented by the LFB in December 2003.

### **5.6 *Availability of imported BDM***

Regarding BDM identified in 2000 as showing the lowest level of safety (factor VIII, antithrombin III, factor VII, fibrinogen and fibrinogen for biological glue), the Afssaps sought the availability of products derived from plasma collected in countries which theoretically had a lower risk of BSE or v-CJD and which could be imported into France.

At present, no medicine has been imported in this specific context, files of the medicines identified failing to meet all quality, safety and efficacy requirements. It should be remembered that the group of experts in December 2000 encouraged the supply of BDM derived from plasmas collected in countries with a theoretical lower risk of BSE or of v-CJD. It was nevertheless clearly recommended that this precautionary measure should not be taken to the detriment of the intrinsic quality of the products proposed.

Factor VIII is now nanofiltered and fibrinogen for biological glue is no longer on the market.

### **5.7 *Information for prescribers, patients and donors***

The most recent update of data in comparison with the December 2000 group of experts, in the form of a report dated March 2003, has been added to the Afssaps web site.

## **6. European positions**

The European position, expressed by the EMEA in 1998, has been updated by the same organization, published in 2003 (63). This concerns BDM and medicines of urinary origin. Its guidelines and conclusions are consistent with those of the December 2000 report and its updates.

At the time of this report, a new update is in process of publication, and is expected to coincide with the findings of the present report.

Directive 2002//98/EC of the European Parliament and of the Council of 27 January 2003 concerning the establishment of quality and safety standards for the collection, quality control, transformation, storage and distribution of human blood and of blood constituents, and modifying Directive 2001/83/EC (64), and the current draft directive concerning implementation of that Directive, will create a regulatory context at European level for LBP, and the beginning of harmonization of criteria for the exclusion of blood donors.

A European Council Guideline had already expressed a harmonized basis for these criteria in 1998 (65).

It is important to emphasize the difficulty in reaching harmonization, notably in relation to France where criteria are particularly strict. It must be remembered that almost all LBP used in France are fractionated by the LFB using plasmas collected almost exclusively in France

(with the exception of specific anti-D immunoglobulins which are 97% prepared from hyper-immune plasma collected in the United States).

## **7. Update of estimate of residual risks and measures**

New data concerning infectivity, epidemiology and studies for validation of procedures used for preparation of blood products lead to updates of the estimation of residual risks as stated in the December 2000 report.

In essence, hypotheses and parameters enabling estimation of the level of risk, considered in the December 2000 report, have very little changed.

Furthermore, reasoning on the calculation of residual risk and its interpretation remain valid. Discussion of general measures which could be envisaged, their usefulness, limitations and disadvantages, as described in §3 of the December 2000 report, which readers are advised to refer to, remains pertinent. There is no new information justifying a downward revision of the risk of appearance of inhibitors with recombinant blood-derived medicines. No new applicable measure has emerged since 2000.

The current update will deal specifically only with revision of numerical values of estimation of residual risk, and results and conclusions concerning update of measures suggested in 2000 and regularly reviewed since.

### **7.1 *Labile blood products***

Estimation of residual risk is based upon epidemiological data, i.e. the maximum number of individuals likely to develop the disease in France, which then determines the potential incidence of blood donors carrying the disease at the time of the donation.

The most recent estimations tend to a downward revision of the maximum number of cases of v-CJD. However, with a conservative approach, and bearing in mind the margin of inaccuracy of these estimations, the number of 300 cases in France used to estimate risk in the December 2000 report must be retained.

This leads to retention of the hypothesis that at the most one blood donation out of 120,000 could be contaminated.

As a result, analysis of risk for various LBP (PRC, MPC, APC, FFP, SCP and VAP) presented in §3.1 of the December 2000 report is unchanged.

### **7.2 *Blood-derived medicines***

Hypotheses taken into account for estimation of the degree of risk are as follows, some of which being slightly modified:

- in France, one blood donation out of 120,000 could be infected (no change),
- infectious load of whole blood is 20-30 Inf-ic U/ml (instead of 100 Inf-ic U/ml considered in 2000), corresponding to 20-30 Inf-iv U/ml (instead of 10 Inf-iv U/ml considered in 2000),
- distribution of infectious load in blood fractions and the effect of leukoreduction lead to an infectious load in plasma of about 10 Inf-iv U/ml (instead of 1 Inf-iv U/ml considered in 2000),
- residual infectivity of 1 Inf-iv U or more per dose of finished product, leads to consideration that it is potentially infectious by intravenous administration (no change),
- nanofiltration has started to be used for several products,
- preparation procedures for several products have been validated, leading to confirmation or upward revision of the elimination efficacy of procedures,
- extraction yields and the size of initial plasma pools have undergone minimal variations linked to variations in techniques,
- the maximum annual dose for each active substance (no change),

- the method of calculation which, in a very conservative approach, is based upon the low hypothesis of the December 2000 ratio and with very limited addition of reduction factors in preparation procedures.

The updated level of residual risk for products currently available is hence as follows:

|                       | log <sub>10</sub> |
|-----------------------|-------------------|
| Factor VIII           | -3.21             |
| Factor VII            | -1.65             |
| Factor IX             | -5.51             |
| Factor XI             | -3.04             |
| von Willebrand factor | -7.18             |
| Fibrinogen            | -3.63             |
| PPSB                  | -3.57             |
| Antithrombin III      | -1.43             |
| C protein             | -4.85             |
| Albumin 4%            | -3.25             |
| Albumin 20%           | -3.55             |
| Alpha 1 antitrypsin   | -4.81             |
| Polyvalent Ig         | -6.03             |
| IV anti-B Ig          | -2.97             |
| IM anti-B Ig          | -7.46             |
| Anti-D Ig             | -5.79             |
| Antitetanus Ig        | -7.58             |

The method of calculation adopted is much more conservative than that considered in the December 2000 report, notably for integration of significant improvements in preparation procedures and their validations, while taking into account as accurately as possible uncertainties concerning the results of validation studies and the efficacy of nanofiltration. For this same reason, comparison of the level of residual risk by product cannot be done based only on calculations in the present report and those of the 2000 report, but must take into consideration all changes in initial hypotheses as well as the various different modes of calculation used.

Hence this very conservative calculation leads to a residual risk level which is globally of the same order of grandeur as that considered in the December 2000 report. At any event, residual risk remains very low for all of these products.

### 7.3 Conclusion concerning updated measures

Recent data on infectivity and epidemiology have boosted the hypothesis of the existence of an infectious form of the v-CJD agent in blood and its transmission by blood products. However the current state of scientific knowledge does not provide objective evidence of the actual existence of this danger. Nonetheless, this risk of transmission cannot be ruled out and in a conservative approach it must be considered to be no longer a theoretical but rather a possible risk. While awaiting advances in scientific knowledge which might enable the confirmation or ruling out of this possibility, and in the situation of confirmation, of determination of the actual probability of onset, the approach expressed in the December 2000 report remains valid and must be maintained.

Recent data have also specified hypotheses and parameters used to estimate residual risk, by consolidating and confirming estimations made in 2000. These finally lead to estimation that this level, possible if not actually proven, is of the same order of grandeur as that considered in the December 2000 report, bearing in mind the enhanced conservative approach used in 2004 to estimate this level of risk.

On the risk scale, the respective positions of LBP and BDM remain unchanged. LBP seemed to be more at risk, preparation procedures not being sufficient to confirm the safety of the product if an initial donation should prove to be contaminant. It should nevertheless be remembered that there are no reasonable alternatives and that in most instances these products are used in vital indications.

BDM manufactured from plasma undergo, during the course of fractionation, a number of stages which increase their safety level. Continued improvement of procedures used for the preparation of products which are in the upper part of the risk scale should be ensured, according to technological possibilities, with completion of studies used to validate procedures.

The various measures introduced since 2000, described in §5, can all be envisaged and are appropriate, currently available, and no new measure has been identified.

## **8. Medicines extracted from urine**

No new data have been published confirming the initial scientific results (66) describing the existence of a form of PrP resistant to proteinase K but with a molecular weight different from PrP<sup>SC</sup>, called "UPrP<sup>SC</sup>" and found in animals and in humans affected by TSSE, particularly those with familial forms of CJD. The pathophysiological significance, origin and potential infectivity of this "UPrP<sup>SC</sup>" are still not known. With regard to blood products, the existence of "UPrP<sup>SC</sup>" in urine does not indicate the presence of PrP<sup>SC</sup> in blood, whether in classical forms of CJD or in v-CJD. It nevertheless offers a possible research approach for the development of a screening test usable on a routine basis.

Reciprocally, suggestion of possible infectivity in blood in v-CJD may support the hypothesis of the actual presence of an infectious form in urine, at least for this type of CJD, linked to the glomerular filtration of blood.

Regarding medicines extracted from urine, i.e. gonadotrophins and urokinases, the existence of "UPrP<sup>SC</sup>" has led to the adoption of contrasting positions, taking into account the various aspects of risk-benefit ratio<sup>1</sup> and the existence of alternative sources of production (recombinant proteins). Taking into account the strict evaluation of risk, it is useful to remember that these medicines are prepared from "donors" who cannot be selected clinically because of the particular conditions surrounding the "donation" of urine (high frequency of donation, very large number of "donors"). Clinical selection, in particular in the case of sporadic forms of CJD, would nevertheless not eliminate from the "donation" individuals at the stage of end of incubation during which "UPrP<sup>SC</sup>" is present in urine. In the hypothesis of the actual existence of "UPrP<sup>SC</sup>" in all forms of TSSE, including sporadic CJD, and its infectious nature, it should be noted that there have been no pharmacovigilance reports of cases of transmission with gonadotrophins. This absence of any observed transmission is all the more important to note since urine is obtained essentially from post-menopausal women, i.e. elderly and more at risk of being at the end of incubation of the disease, and that recipients are young women, monitored and with a normal life expectancy in whom the disease would be easily detectable. Furthermore, procedures for the preparation of gonadotrophins and urokinases are complex extraction and purification procedures which potentially include several stages capable of eliminating or inactivating TSSE agents, such as treatments at high pH, adsorptions, chromatographies. In the case of v-CJD, an additional safety criterion is the fact that "donors" do not come from countries with a high incidence of v-CJD or BSE.

In conclusion, it would not seem necessary to bring into question the safety of medicines of urinary origin regarding the risk of transmission of human TSSE agents.

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\* Translator's Note : French original says "benefit-ratio". We thought that meaning was "risk-benefit ratio" and translated as such.



## **9. Grafts**

The presence of possible infectivity in grafts depends upon the distribution of the infectivity of v-CJD, presumed to be circumscribed to a limited number of organs and tissues (brain, retina, optic nerve, secondary lymphoid structures, i.e. tonsils, spleen, lymph nodes), which furthermore are not concerned by the graft. However recent data concerning infectivity (see §1.4) and epidemiology (see §2.5) indicate the need to consider, as a precautionary measure, the possible presence of the agent in other tissues and notably in blood, and hence in all grafts, and notably according to their blood supply.

With regard to grafts harvested in France, the risk of contamination can be estimated as being similar to that of LBP, i.e. for the recipient a risk of 1 in 120,000 per graft, on the basis of epidemiological data. This approach assumes that all grafts obtained from a donor with v-CJD may contain at least one infectious unit (plausible hypothesis considering theoretical infectious load in 1 ml of blood), and that the context of the graft enables transmission of the infectious agent. This approach may be conservative for grafts with a poor blood supply and containing little or no cells of the reticulo-endothelial system.

Measures are based upon the clinical selection of donors of organs, tissues and cells. There are no new measures to be proposed to reduce the possible risk of transmission. In particular, exclusive use of grafts obtained from countries with a lower level of risk of exposure to BSE cannot be envisaged in view of the constraints of donor-recipient compatibility, the general situation of penury and the emergency context which is materially difficult to manage. It should also be pointed out that grafts must often involve emergency or extremely serious situations of a life-threatening nature. Risk-benefit ratio remains widely favorable.

Grafts imported in France for reasons of availability come from countries with a lower degree of risk of exposure to BSE, with the exception of hematopoietic stem cells which are very occasionally imported from the United Kingdom in the context of the need to find a compatible donor. In this case, the recipient must be fully informed of the origin of the graft from a country where the incidence of v-CJD is higher than in France and of the possibility of transmission.

There have been no biovigilance reports in France of any case of transmission to humans of v-CJD by grafts.

## **Conclusions**

Information available since the first report (December 2000) of the group of experts on analysis of risk of transmission of v-CJD by blood and its derivatives provide little new scientific information nor any argument justifying modification, upwards or downwards, of initial conclusions (1).

Recent data on the pathophysiology of v-CJD, modes of transmission, distribution and level of infectivity in different tissues and estimation of a possible infectious load in blood provide little new information, but also offer no tangible proof of the absence of risk. As a result, the possibility of transmission of the disease by blood remains a hypothesis. Recent data do not lead to any significant modification of the level of risk as stated in the December 2000 report.

From an epidemiological standpoint, there has been no evidence in France and the United Kingdom of an increased incidence of v-CJD. Estimation of the number of people potentially developing v-CJD (and hence currently in course of incubation) does not seem to require modification.

No new risk factor, which might be used as an exclusion criterion in the clinical selection of blood donors, has been identified.

The case of v-CJD notified in December 2003 in the United Kingdom, possibly linked with a transfusion history, has nevertheless been viewed as an alarm signal in any conservative approach, with consideration of the risk of transmission as being no longer theoretical but possible, and hence to maintain strict monitoring of risk analysis and the pertinence of measures adopted.

No screening test is presently available for use in humans. Furthermore, criteria for the exclusion of donors used currently are and will probably remain, at least in the short term, the most appropriate measure for the qualification of blood donations, measures which will complete screening tests available for routine use when biotechnology permits.

Improvement in procedures for the preparation of BDM and validation studies now available confirm estimations on the ability of procedures used for the preparation of BDM to eliminate the v-CJD agent. Hence December 2000 data remain valid.

Regarding LBP, it should be remembered that no method exists for the inactivation of v-CJD agent which could be applicable to labile blood products. Leukoreduction remains a precautionary measure to be taken into account and which can only contribute to reducing the risk of transmission. Similarly, reduction of residual plasma volume in LBP may contribute to a further increase in the infectious load of LBP.

Conclusions and guidelines of the December 2000 report remain valid. None of the points raised and discussed in the report requires modification. There are no new measures to be suggested for reducing possible risk of transmission of v-CJD by blood products. Measures currently enforced seem to be effective and in proportion for guaranteeing the risk-benefit ratio of blood products. The same applies to medicines extracted from human urine and to grafts.

Updated data concerning the number of cases of BSE and of v-CJD are given in an appendix.

Scientific monitoring must be maintained, including in particular the observation of results in studies of experimental transmission in primate models and transgenic mice, possible onset of v-CJD in PrP gene codon 129 Val-Val or Met-Val genotype individuals, developments concerning the presence of a form of resistant PrP in urine or any detection of the agent in other tissues and biological fluids.

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## Glossary\*

|                     |   |
|---------------------|---|
| APC:                | apheresis platelet concentrates   |
| CBW:                | chronic wasting disease   |
| BSE:                | bovine spongiform encephalopathy  |
| TSSE:               | transmissible subacute spongiform encephalopathies  |
| PRC:                | packed red cells  |
| GSS:                | Gerstmann-Sträussler-Scheinker syndrome   |
| LFB:                | Laboratoire Français de Fractionnement et de Biotechnologies (French Fractionation and Biotechnology Laboratory)  |
| Leukoreduction:     | Operation consisting of aseptic subtraction of the majority of leukocytes from a labile blood product. For technical reasons, subtraction is most often incomplete. This being the case, the term leukoreduction is preferable to de-leukocytation. |
| CJD:                | Creutzfeldt-Jakob disease (sporadic, iatrogenic, familial forms)  |
| MCP:                | standard mixture of platelet concentrates   |
| BDM:                | blood-derived medicines   |
| SCP:                | safety cryo-dessicated plasma   |
| FFP:                | fresh-frozen plasma   |
| PFF:                | plasma for fractionation  |
| PrP <sup>SC</sup> : | abnormal form of the natural protein PrP  |
| VAP:                | viro-attenuated plasma  |
| LBP:                | labile blood products   |
| v-CJD:              | variant Creutzfeldt-Jakob disease   |

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\* Translator's Note: glossary left in French alphabetical order for easier checking



## APPENDIX

### Update of numerical data included in 11 December 2000, February 2002 and March 2003 reports

#### Number of cases of BSE

- United Kingdom (as of 16/01/2004): 183,616 cumulative cases (*182,802 cases in November 2002, 181,368 cases in November 2001 and 179,256 cases in October 2000*) with 425 cases reported for the year 2003 as of 30/09/2003.

#### Reminder:

- 2002: 877 cases.
- 2001: 1218 cases.
- 2000: 1443 cases.
- France: for the year 2003, 95 cases as of 31/08/2003, distributed between eight clinical cases, 59 cases resulting from the monitoring of at-risk bovines and 28 cases discovered by routine slaughterhouse screening.

#### Reminder:

- 2002: 239 cases in all distributed between 41 clinical cases, 124 cases resulting from the monitoring of at-risk bovines and 74 cases discovered by routine slaughterhouse screening.
- 2001: 274 cases in all distributed between 91 clinical cases, 100 cases resulting from the monitoring of at-risk bovines and 83 cases discovered by routine slaughterhouse screening.
- 2000: 161 cases in all.

#### Number of cases of v-CJD

- United Kingdom (as of 05/01/2004): 146 cumulative definite or probable cumulative cases.

#### Reminder:

- 2003: 18 cases (*140 cumulative cases*).
- 2002: 17 cases (*122 cumulative cases*).
- 2001: 20 cases (*105 cumulative cases*).
- 2000: 28 cases (*85 cumulative cases*).
- France (as of 31/12/2003: six definite or probable cumulative cases (*six cumulative cases in March 2003, five cumulative cases in February 2002 and three cumulative cases in November 2000*)).
- Other countries: one case in Hong Kong (counted among British cases), one case in Republic of Ireland, one case in the United States, one case in Canada (all these four patients had spent long periods in the United Kingdom) and one case in Italy.