ANALYSIS OF THE RISK OF TRANSMISSION OF THE VARIANT CREUTZFELDT-JAKOB DISEASE BY MEDICINAL PRODUCTS OF HUMAN ORIGIN AND LABILE BLOOD PRODUCTS

DATA UPDATE OF THE AD HOC GROUP REPORT DATED DECEMBER 2000

MARCH 2003 REPORT

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- SYNTHESIS -

The scientific data available since the publication in December 2000 of a report by a multidisciplinary and independent group of experts on the risk of transmission of the variant Creutzfeldt-Jakob disease (vCJD) by blood and blood derivatives, have been regularly reviewed and were revised in a first update report in February 2002. The present report expounds March 2003 expertise update.

There exist no new data on the variant Creutzfeldt-Jakob Disease (vCJD) physiopathology, modes of transmission, distribution and level of infectivity in the various tissues or on the determination of a possible infectious load in blood. The possibility of transmission of the disease by blood remains a hypothesis. There is no new piece of information modifying upward or downward the estimation of the level of risk considered in the report dated December 2000.

On the epidemiological level, no significant increase in the incidence of vCJD was observed. The estimation of the number of people likely to develop vCJD doesn't seem to be modified.
No new risk factor, which could be used as an exclusion criterion on the clinical selection of blood donors, was identified.

No detection test in the present state of development is applicable to humans. The donor exclusion criteria with regard to the CJD risk factor, in force at present, will remain the most appropriate measure taken for the qualification of blood donations, at least as long as validated detection tests usable on a routine basis and applicable during the whole asymptomatic period will remain unavailable.

Leucoreduction remains a precaution to be considered and is a measure which will but contribute to reduce the risk of transmission. It is reminded that there exists no vCJD agent inactivation method applicable to blood products.

There is no reason why recommending a measure of exclusion of the donors who stayed in the British Islands, which is more stringent than that currently in place.

The conclusions and recommendations established in the report dated December 2000 remain valid. None of the items dealt with and discussed in the report needs to be modified. There is no new measure to propose in order to further reduce the possible risk of transmission of vCJD by blood products. The measures in force at present seem to be effective and proportionate so as to ensure the right benefit-risk ratio to blood products.

Introduction

Within the context of the permanent surveillance by the Afssaps over the risk of transmission of the variant Creutzfeldt-Jakob Disease (vCJD) by blood, urine and their derivatives, the scientific data available since the publication in December 2000 of a report by a multidisciplinary and independent group of experts (*1*), designated as "the group of experts" in the present report, have been regularly reviewed. A first update report was published in February 2002 (*2*). The review conducted by the group of experts continued all along the year 2002. The present report expounds March 2003 expertise update.

Only the scientific aspects were reviewed. There was new piece of information requiring to reopen the discussion on the other aspects, such as ethic considerations.

The publications referenced in this report were used as a support for reflection. This list of references doesn't intend to be exhaustive on the subject but the articles considered as the most useful in reviewing the risk of transmission of vCJD by blood products were discussed. The review also covers products extracted from human urine.

The experts' objective was to:
- review the data newly published and discuss their results,
- propose, if necessary, measures likely to reduce further the risk of transmission and analyse their consequences,
- determine whether the conclusions and the recommendations established in the report dated December 11th, 2000, updated in February 2002, should be modified.

Note: the same terms and abbreviations as those used in the report dated December 2000 and in February 2002 update will be used in the present report, and will consequently not be explained. As a reminder, an abbreviation lexicon is included at the end of this report.

1- Infectivity

1.1. Infectivity in blood

The work conducted by Houston et al. on the experimental transmission of the BSE agent in the sheep model was the subject of a second publication, reporting the transmission of BSE in three additional animals (*3,4*).
For the record, the study consisted in experimentally infecting sheep with the BSE agent by oral route, then collecting blood from infected animals, during both the preclinical and clinical incubation period , then injecting whole blood or buffy coat (leuco-platelet layer) by IV route, to healthy sheep genetically sensitive to scrapie (19 recipient sheep were transfused). The first transmission case noted in one of the animal recipients had been published in 2000 (*5,6)*. This result had shown the possible transmission of BSE both by the oral route and by blood during the asymptomatic phase and within a same species. This sheep model was judged as more representative of the human situation than the rodent models where these types of transmission cases had already been described. It is appropriate to remind that it is on the basis of this preliminary result that the report dated December 2000 had, already and as a precautionary measure, considered the hypothesis of the presence of infectivity in blood for vCJD and that the group of experts had consequently carried out an evaluation of the risk of transmission. The risk

of transmission of this agent by blood products (blood for transfusion and blood-derived components such as plasma-derived medicinal products) was judged as low, even theoretical.
In this experimental model, the three new cases published in 2002 confirm the presence of infectivity in whole blood and in the buffy coat as early as in the middle of the incubation period of the disease after inoculation by oral route. It also confirms the possible transmission by blood within a same species. The continuation of the study also further clarifies some of the uncertainties relative to its methodological quality (verification of the nature of the agent involved; appearance of the disease in positive control animals).
Furthermore, in this article the same type of experience was repeated with a scrapie transmission model. This second model involves sheep as blood donors in which the incubation of the natural sheep disease (scrapie) is in progress, and sheep as recipients, from scrapie-free herds, but with a scrapie-sensitive genotype. In the report, scrapie has already been transmitted to 5 out of the 21 sheep experimentally infected by blood. These results show, for the first time, the possible transmission of a natural TSE by blood within a same species, in this case the scrapie agent in sheep, acknowledging however that all experimental conditions were meet (intra-species, large volume of blood injected, sensitive animals) to observe the case. However, the route of exposure of the recipient animals is not natural but experimental.
This result raises the more general question of the inter-human transmission of natural TSE, particularly of the classical form of CJD in humans. Still, it is reminded that no case of transmission of the classical CJD agent by blood or blood products in humans has ever been reported. The results observed could also be explained and interpreted as a particular sensitivity of the ovine species to the scrapie agent. Finally, it is appropriate to remind that, as a precautionary measure, any subject bearing any risk factor to develop a classical form of CJD are excluded from blood donation.
At the time of the present report and since the second publication of Houston's report, several additional animals have developed the disease, in each of the two transmission models studied (data not published yet).
All these results are interim results and require to be finalized.
Notwithstandingly, these results do not modify the remarks previously raised in the report dated December 2000 on the limits of interpretation of such an experiment. Beyond the interpretation limits, it is necessary to bear in mind that these studies show that infectivity in blood, and in the peripheral tissues, can be observed provided that a well chosen experimental model is used, according to a relevant methodology using adapted detection methods. Additionally, even if the sheep model is more representative than the rodent models and if, for the scrapie part of the study, it is closer to the natural situation, the ovine model is less relevant, for extrapolating to the human situation, than any primate model. Besides, the effective transmission of the BSE and the scrapie agents within the same species and by the same route could reflect the possibility of a particular sensitivity of the ovine host species in this type of experience. In the same way, this work studied infectivity in whole blood and in the buffy coat only, not in plasma. Finally, it does not allow any further conclusion as regards the kinetics of the disease and infectivity level in blood or buffy coat, even if it is reasonable to assume that the infectivity level in blood is low.
Consequently, even though these additional results show the relevance of such experimental models as a support for research, they still haven’t in any case demonstrated the presence of infectivity in blood in patients with vCJD and the possibility of its transmission by blood products.
Another experimental transmission study was conducted in a lemur primate model (*7*). The BSE agent, adapted after transition in the macaque monkey, was used to experimentally infect a lemur primate. Homogenates made with brain and buffy coat from the lemur primate were injected by intracerebral route to 2 and 1 healthy lemurs respectively. The three recipient animals developed

the disease. Therefore, the study shows the possibility of transmission of the BSE agent by blood between primates, in an experimental model closer to humans than the sheep model.
However, these are again experimental transmission cases of TSE agents in an animal model while the presence of infectivity in blood has never been demonstrated up to date for natural TSSE except in the previously discussed study. Furthermore, the study is conducted on brain homogenate and on buffy coat, not on plasma. In the same way, the study uses the intracerebral route, not the intravenous route. These results, directly involving the central nervous system either via the inoculated material or via the route of inoculation, were predictable. The article conclusions precisely mention it: " *This observation represents the first documented transmission of BSE from the blood of an experimentally infected primate which in view of rodent buffy coat infectivity precedents and the known host range of BSE is neither unexpected nor cause for alarm.* " This work should at least be completed with the administration of whole blood or buffy coat by the intravenous route to provide a level of information comparable to that of the study in the sheep model.

The most relevant studies of the inoculation of blood from subjects with vCJD to primates or sensitive transgenic mice are not completed. However, considering the present state of our knowledge, no animal recipient has developed the disease so far.

Some work using an experimental sensitive transgenic mice model showed the appearance of a vCJD-type disease after the inoculation of the BSE or the CJD agents using cerebral tissue, which confirms previous studies tending to demonstrate that vCJD in humans results from the transmission of BSE (*8*). Otherwise, the appearance of a sporadic CJD-type disease in certain animals inoculated with the BSE agent raised the hypothesis of the existence of a second BSE strain, which could be at the origin of a few human cases labelled as sporadic CJD. It was suggested that this could explain the high incidence of sporadic CJD in some countries. Later, the appearance of an epidemic due to this other supposed BSE strain in the hypothesis of a longer incubation period, was also put forward (*9*). However, no piece of information confirms these hypotheses in the present state of our knowledge (see §2).

Finally, studies in progress and not published yet in rodent experimental models confirm that infectivity is preferably distributed in the buffy coat. However, it seems that only half of the infectious load approximately would be associated with the buffy coat, instead of 90% as previously admitted in the report dated December 2000, in view of the studies available by then, the rest of it being distributed between red blood cells and plasma. It also appears that the whole blood infectious load would be lower, corresponding to a factor of 5 compared to that which had been estimated in the Dec. 2000 report (20 instead of 100 intracerebral infectious units per ml of whole blood). Therefore, the conjunction of these two new pieces of information doesn't question, *in fine*, the calculations of the residual infectious load for the various categories of blood products. It doesn't question either the possible relevance of leucoreduction (see §4.2).
There are no new data concerning the potential presence of infectivity in platelets (*10*).

1.2 Tissue infectivity

It is reminded that in view of the available studies, infectivity is supposed to be confined to a limited number of organs and tissues (brain, retina, optic nerve, secondary lymphoid organs, i.e. tonsils, spleen, lymphatic ganglions). All the other tissues which have been studied so far proved to be negative. In particular, neither abnormal proteins nor infectivity were found in human blood or in the buffy coat. However, these studies are limited considering *i)* the small number of

patients tested and *ii)* the poor sensitivity of the detection methods used due to the human/mouse species barrier of the infectivity tests which nevertheless remain the only means of detection.

Since the studies conducted in 2001 by Wadsworth *et al.* and Bruce *et al.* who had examined a large number of tissues, there are few new data on the distribution of infectivity in the peripheral tissues of vCJD cases.
Other pieces of work confirmed the presence of PrPSc at the level of the retina and the optic nerve in subjects with vCJD (*11*).
Another study detected the abnormal prion protein in the tunica media and more rarely in the tunica intima vasorum of intracranial blood vessels in patients deceased of vCJD or of the sporadic form of CJD. The protein was also detected in the tunica media and the tunica intima vasorum of the carotid artery and ascending aorta at the extracranial level, in patients deceased of vCJD (*12*). However, these results require to be confirmed considering the cell typing difficulties. They don't enable to extrapolate the potential presence of the abnormal protein in circulating blood.

The detection of the abnormal protein at the level of the nasal mucous membrane in subjects with sporadic CJD doesn't bring any further piece of information relatively to the tissue distribution of vCJD (*13*). It may provide a direction for the diagnosis of the classical forms of CJD and it could lead to add the nasal cavity surgery and endoscopy antecedents to the exclusion criteria for people with a risk to develop a classical CJD.

In the United Kingdom, the examination of surgical specimens of lymphoid organs (tonsils and appendices mainly) coming from a large number of subjects without any clinical sign of vCJD at the time of surgery, looking for the presence of PrPSc, led to the detection of a positive case (*14*). Methodological problems and the absence of information on this case do not allow to draw conclusions for the moment. These interim results however confirm the presence of infectivity in the lymphoid tissues and is coherent with the hypothesis of the existence of asymptomatic carriers already taken into account. They don't either modify the last estimations of the number of cases of vCJD.

1.3 Infectivity distribution in bovine tissues

There are no new data on the distribution of the BSE agent in the tissues of naturally infected cattle or on that of scrapie in the ovine species. For the present time, the recent studies on muscles and on the milk of bovine origin do not lead to reconsider the classification of tissue infectivity. In the same way, the experimental transmission of the BSE agent to a bovine after the administration by intracerebral route of tonsils coming from a bovine which had itself been infected by oral route may have a selective consequence in the alimentary field, but it doesn't bring any new piece of information on the vCJD epidemiology and infectivity in humans.

1.4 BSE transition in the ovine species

The question of a possible recycling of the BSE agent in the ovine species remains. However, no clinical case evocative of BSE was observed in the sheep up to the present. Furthermore, the absence of a significant modification in the incidence of scrapie in the British livestock is a point in favour of the absence of massive transmission of the BSE agent in the sheep. However, it is necessary to point out that the scrapie epidemiosurveillance systems are still little effective in

some countries of the European Union. Thus, the question remains, and considering the possible consequences, it deserves particular attention. However, models predict that the BSE agent recycling in the ovine species would have quite limited consequences on the vCJD epidemic level (*15*).

1.5 Conclusions

Therefore, there exist no new piece of information relating to the distribution and the infectivity level of vCJD in the various tissues, particularly as far as the presence of infectivity in blood is concerned. The presence of temporary or permanent infectivity in the blood of subjects with vCJD hasn't been demonstrated yet. Nevertheless, the results of more relevant studies as far as the route of inoculation of samples is concerned, using primate models, are still unavailable. In the meantime, *i)* the presence of the infectious agent in blood during the whole preclinical incubation phase and *ii)* the capability of the infectious agent to be transmitted by blood, are two, *a priori* pessimistic, hypothesis which cannot be formally excluded and which shall always be taken as working hypothesis, for the risk analysis, as this had been the case in the Dec. 2000 report.

There exist no new data capable of significantly modifying the estimation of a possible infectious load in blood and of its distribution between the various blood compounds. In the hypothesis of the presence of infectivity in blood, the analysis of the most recent data still suggests that the infectious load would be low.

The existence of asymptomatic infected subjects cannot be excluded. It cannot be excluded either that, due to the incubation period, subjects cannot clinically declare the disease before their death. This is one more argument to justify the permanent deferral from blood donation, for subjects previously transfused.

The additional results relative to the experimental work carried out by Houston *et al*. on the transmission of BSE from sheep to sheep after an IV injection, confirm the validity of the model as a support for research. However, they don't demonstrate the presence of infectivity in blood of patients with vCJD or the possibility of its transmission by blood products.

As a conclusion, the risk of transmission of the vCJD by blood remains a hypothesis which shall always be considered in the risk analysis. There is no new piece of information modifying (upward or downward) the level of risk considered in the Dec. 2000 report.

2- Epidemiology

The evolution of the BSE epidemics in the British livestock shows that the decrease in the number of cases continued in 2002.
In France, the number of cases decreased in 2002. Additionally, the largest part of the cases currently registered results from the implementation of the screening programme, the part of clinical cases reducing gradually; this evolution had already significantly started in 2001 (*16,17*).

The number of vCJD cumulated cases keeps increasing in the British Islands, with 130 cases in February 2003 for 114 cases in February 2002 and 85 cases in November 2000 (*18*). However, the annual progression slowed down again in 2002. As a result, mortality was less in 2002 than

in 2001 and *a fortiori* than in 2000: 28 confirmed or probable vCJD deaths were registered in 2000 for only 20 in 2001 and 17 in 2002. The annual incidence doesn't increase any longer in the United Kingdom and there is no more increase in the number of probable cases under evolution either.

In France, the number of cumulated cases further increased in 2002, with a total of 6 certain or probable cases of vCJD by January 31, 2003, for 5 cases by February 1st, 2002 (*19*). The first five patients never stayed in the United Kingdom and the last case had only very short stays there (3 or 4 days in total), and this was after 1995.

The incidence ratio between both countries was little modified and is close to 1 to 20, a ratio that had been considered for the alimentary exposure factor in the report dated December 2000, which tends to confirm this estimation. It is reminded that a study relative to the experimental transmission of the BSE agent between primates notably confirmed that the cases of vCJD observed in France had for origin the BSE agent via the food chain, like the British cases. The report dated December 2000 was particularly based on the consumption of contaminated British bovine products in France in order to determine the number of people likely to develop nvCJD and the theoretical risk presented by blood products.

Each one of the cases respectively reported in the United States (*20*) and in Canada (*21*) in 2002 concerns patients who lived or stayed long in the United Kingdom. Therefore, these 2 cases cannot be considered as being native.
On the contrary, the case reported in Italy concerns a woman who never stayed in the United Kingdom (*22*). It seems that it lies within a context analogue to that of the French cases.

Apparently, the incidence of cases of sporadic CJD noticeably increased in Switzerland in 2001 and this increase continued in 2002 (*23*). It goes hand in hand with an incidence of sporadic CJD already high in Switzerland (*24*). Among the hypothesis advanced, the existence of cases of vCJD labelled as sporadic CJD was put forward. It could be a particular form of vCJD, with a clinical expression very close to that of sporadic CJD in the elderly subject (see §1). However, no argument validates this hypothesis and in particular no observation of this nature was made in the United Kingdom. The evolution of the incidence observed in Switzerland would more probably result from an improvement of the diagnosis and the notification of cases of sporadic CJD, and the small number of cases would amplify the variations observed from one year to the next. An increase in the incidence of sporadic CJD associated with the improvement of case notification was noticed for other countries (*25*). Therefore, no overrisk bound to the use of plasma-derived medicinal products that would be derived of plasma collected in Switzerland was identified.

The major characteristics of the cases of vCJD are stable: young adults (the mean age is 30 years old), Met-Met genotype at codon 129 on the PrP gene.
The mean age stability at the beginning of the disease would tend to confirm the hypothesis of a greater sensitivity in very young subjects. The hypothesis according to which the incubation period could be longer in people at a higher age is not in line with the significant stability associated with the mean age of the subjects affected.
So far, when carried out, genotyping could only identify Met-Met subjects at codon 129. Uncertainty on the possibility that vCJD appears later in subjects genotyped Val-Val or Met-Val at the same codon remains, as this was observed in the iatrogenic cases of CJD caused by the administration of an extractive growth hormone, similarly as in Kuru, uncertainty which could

modify the present projections of the number of cases. However, the number of these cases should be inferior.
A study showed the influence of the expression of the gene encoding for the protein on the disease incubation period in transgenic mice. The modification of the expression of the PRNP gene could be a factor of individual sensitivity to CJD (*26*).
Furthermore, it was postulated that the genetic sensitivity wouldn't only be limited to the Met-Met genotype at codon 129 on the PrP gene, but also on a marker of the HLA system. This combined sensitivity (genotype x HLA) could explain in part that only a very small fraction of the Met-Met homozygote population, supposed to have been exposed to the disease by oral (alimentary) route, developed vCJD. However, recent studies don't confirm the initial work results (*27,28*).

It is reminded that no case of transmission of vCJD to humans by LBP or PDMP was ever reported by haemovigilance and pharmacovigilance networks. As far as the British recipients of blood products derived from donors who developed vCJD later, are concerned, no case of transmission of vCJD was ever reported up to the present. However, the number of years passed since these recipients were transfused is still too short compared to the supposed vCJD incubation period to draw any conclusion (*29*).

No new risk factor, which could be used a an exclusion criterion on the clinical selection of blood donors, was identified.

The most recent estimations of the number of cases expected in the next few years are more precise than the model by Ghani *et al*. (*Nature, 2000*) and follow the same direction as the less pessimistic hypotheses of this modelling. They predict the distribution of the disease incubation period based on the cases of vCJD observed between 1995 and 2001 and not on the basis of theoretical scenarios. For the record, according to the last models developed by Huillard d'Aignaux *et al*. and by Valleron *et al*., the average estimation of the number of cases of vCJD in the United Kingdom would be of the order of 200 to 300 cases, with a 95% confidence interval, the upper limit of which varies between 400 and 2000 cases according to the model. The average incubation period would be about 15 years. Therefore, it is confirmed from the most recent discussions that the expected number of cases is revised downward (*30,31,32,33,34,35*).
It is reminded that the less pessimistic hypotheses had been considered as being the most realistic in the report dated December 2000 and taken into account to perform the risk analysis. The decrease in the number of deaths in the United Kingdom during the last two successive years (2001 and 2002) is in line with one of the conclusions of the last models which placed the peak of the vCJD epidemics around the year 2000.
In France, the number of cases is too small to give an opinion on a possible deviation of the peak from the epidemic curve.

As a conclusion, the epidemiological data do not warrant revising the previous report conclusions.

3 - Tests

It is reminded that the tests now used systematically in France for the detection of BSE in the

cattle were developed and validated for this sole purpose. Therefore, they are cannot be used in humans for the detection of vCJD and they are not applicable to blood donors or even less to the control of blood products.

It is now difficult to draw a statement of the development of tests for the detection of vCJD in humans considering the industrial stakes, in particular as far as tests likely to be used on a routine basis are concerned.

The PrPres amplification method in the presence of PrPc, proposed by Soto et al (*36*), was successfully repeated in other laboratories. Consequently, this piece of work continues to be potentially interesting for the development of a detection test. It is reminded that the method could be proposed, as a preliminary step to sample preparation, to amplify low quantities of PrPres existing in the samples to be analysed. This method shall therefore be combined with a detection test.

The existence in urine of a form of PrP resistant to proteinase K but presenting a molecular weight differing from that of PrPSc, named «UPrPSc » and showed by Gabizon et al. (*37)*, was confirmed. The physiopathological significance of «UPrPSc » is still unknown. It is reminded that if it turned out that this form of PrP appeared specifically during the incubation period of some forms of CJD, it could lead to the development of a detection test to be performed on urine.

No new data was published since the February 2002 report update on the other already known methods or on new research avenues.

As a conclusion, research progresses towards the development of detection tests although it cannot be predicted whether and when this could lead to the development of validated tests (especially in terms of predictive positive or negative value), effectively applicable on a routine basis in humans.

Experts draw the attention on the fact that the development of a detection test is probably not the only most efficacious solution for the qualification of donations and the purification of blood products. The donor exclusion criteria will probably remain the basic measure, even if effective routine tests are available. Detection tests will be employed as a complement to the exclusion criteria and will be especially useful to better target and identify risk populations. As a consequence, it is important to point out that the exclusion criteria in force are not default measures on the short and medium terms in the absence of a test but they represent the most appropriate measure.

4 - Elimination and inactivation methods

4.1 Elimination/inactivation methods for PDMP

As far as the elimination methods of the vCJD agent in the PDMP preparation processes are concerned, the referenced studies do not bring any new piece of information (*38,39,40*). They provide limited data on the filtration and precipitation steps, confirming their contribution to the elimination of the TSE agents. These publications also remind the influence of the operating conditions and nature of the spiking material on the results obtained. The comparative results obtained with various models do not question the supposed validity of the most frequently used

models in the studies previously published.

There is no method for the inactivation of the vCJD agent which is applicable to blood products. The various inactivation processes, the efficacy of which is duly established (autoclave, oxidation, precipitation with urea) are incompatible with the fragility and the relative stability of the proteins extracted from blood. Gamma irradiation at a dose of 50 kGy, far less likely to cause protein degradation, was studied for albumin; the modification of albumin was limited but the inactivation of the TSE agent model used was very low (*41*).

No new specific elimination or inactivation method is being developed.

4.2 Leucoreduction

The potential relevance of leucoreduction, as acknowledged in the report dated December 2000, rests on the observation that, in the experimental models of animal TSE, blood infectivity is essentially associated (90%) with leucocytes. It is appropriate to remind that in humans, the presence of the vCJD agent could be established neither in whole blood nor in its fractions.
The most recent experimental data confirm the preferential distribution of infectivity in leucocytes, but at a level possibly inferior to that previously reported, that is to say of the order of 50% (see §1.1). As a precautionary measure, this could lead to assign to leucoreduction a capacity of elimination of the vCJD agent of half a log instead of one. This correction by half a log doesn't however induce any significant modification for the calculation of the theoretical blood product residual infectious load. Additionally, it is appropriate to note that these experimental studies revised downward the whole blood infectious load. Consequently, experts consider that the general calculation used in the report dated December 2000 remains valid.

Consequently, although there exist no harmonized European position and even though some authors discuss the benefit of this measure (*42*), experts maintain that leucoreduction is potentially interesting.

Different types of filters are used in the present context of the leucoreduction generalization. There are no existing numerical data on the proportion and the nature of leucocytes destroyed by the filters used. It is reminded that such an effect could be deleterious, at least if a significant proportion of B lymphocytes and dendritic cells were destroyed; however, the resulting infectivity will be less important than in the non-filtered product. While waiting for these studies to be completed, nothing indicates that this effect can be such that it calls into question the potential benefit induced by leucoreduction. Furthermore, recent data obtained with the material used in the United Kingdom don't seem to show any significant cell lysis (*43*).

As a conclusion, in the context of a precautionary measure, the leucoreduction of the starting material (cells, plasma) remains an approach to be taken into account since it contributes to reduce further the risk of transmission of vCJD by blood products.

5 - Measures taken in France since December 2000

The following measures were enforced after the recommendations established by the report dated December 2000. Most of these measures were already in force at the time of the report update in February 2002. For the record, these measures were added up to those already existing relative

to the exclusion of the subjects with a risk to develop a classical CJD (family antecedents, neurosurgery, treatment with extractive pituitary hormones) from blood donation.

- Exclusion of the donors who stayed in the British Islands:

The measure excluding the donors who stayed in the British Islands for a cumulated period of one year or more between 1980 and 1996 has been effective since January 2001.
Similar exclusion measures were also taken in several European countries. However, these measures are not harmonized regarding the cumulated duration of the stay. This can be explained by the situation which differs from one European country to another in terms of the relative level of exposure to the risk of BSE between each one of these countries and the British Islands, and the distribution of the cumulated durations of stay of the donors native from these countries in the British Islands(see §7). For the record, the more stringent exclusion measures taken by the United States and Canada and enforced in 2002, do not lie on any scientific rationale or any new data.
There is no new argument on the epidemiological level, the modes of transmission as well as the infectivity calculations developed in the report, leading to reconsider the donor exclusion strategy implemented in France. It is useful to remind that, in terms of feasibility, the measure resulted in a very limited reduction in the number of donors (about –2%), which didn't jeopardize the self-sufficiency in labile blood products for transfusion.

- Leucoreduction:

The principle of a maximal leucoreduction for all kinds of plasma products (for direct therapeutic use and for fractionation) was retained, even though it was acknowledged that leucoreduction beyond 10^6 residual leucocytes per litre only reduces the potential infectious load in a minimal and non-measurable fashion. However, this measure of extreme precaution was proposed, considering the uncertainties on the nature of the cells which carry infectivity in blood and on the efficacy of filters to eliminate them specifically (see § 4.2).
The generalization of plasma leucoreduction (purified FFP, FFP for the preparation of VAP, FFP for the preparation of FDP, PFF) is effective since April 15, 2001, with a limit temporarily set at $< 10^6$ residual leucocytes /L.
At the conclusion of an experimental phase, the residual leucocyte standard was finally set:
- at $1{,}0 \times 10^4$ residual leucocytes per litre for leucodepleted homologous plasma products for therapeutic use and the products derived from their transformations (quarantined fresh frozen plasma, fresh frozen plasma for the preparation of leucodepleted reconstituted whole blood for paediatric use, solvent-detergent viro-attenuated fresh frozen plasma, frieze-dried plasma). For a calculation made with a 95% confidence interval using an adapted sampling plan, controls shall show that this content is respected at least in 95% of the production.
- for the plasma for fractionation, the leucodepletion processes used by plasma collection and/or preparation centres shall guarantee that the content in residual leucocytes is inferior or equivalent to a limit of $1{,}0 \times 10^6$ per litre of leucodepleted plasma.

The standard is being translated through regulatory channels at the time of the present report.
Considering the production deadlines, the supply by the LFB of PDMP prepared exclusively from leucodepleted plasma products at $<10^6$/L took place in January 2002 for all medicinal products; the validity of the medicinal products prepared before the generalization of plasma leucoreduction expired in December 2002 at the latest.
The generalization of leucoreduction involved no major difficulty.
It was reminded that there is no harmonized position on the European level, the systematic

leucoreduction of cell LBP and/or plasma products being enforced in some countries only. There is no European position either on the benefit of plasma leucoreduction for the reduction of the risk.

- Revision of the recommendations on the use of LBP:

The recommendations issued in 1997 by the ANAES are currently under revision by an *ad hoc* working group from the Afssaps. For plasma and red blood cells, the revised recommendations were published in August 2002, whereas for platelet concentrates and white blood cells revision and publication will take place in 2003.

- Additional expertise on the safety level of the VAP compared to the unit of FFP:

The evaluation of the respective safety level for VAP and FFP respectively, needs a validation study of the VAP preparation process. This study (recommended by the experts in December 2000) is in progress.

- Improvement of the LFB PDMP preparation processes:

For factor VIII (FACTANE), the 35+15 nm nanofiltered product has been available since January 28, 2001, with the simultaneous withdrawal of the non-nanofiltered factor VIII.
For Polyvalent immunoglobulins IV (TEGELINE), the 75+35 nm nanofiltered product has been available since February 2002. It is reminded that the demonstration of the NCTA elimination efficacy through this type of filtration (75+35 nm) was not made in the M.A.A. dossier, and the results obtained for some viral families (in particular small-sized viruses) suggest a limited efficacy with respect to NCTA.

- Supplying imported PDMP:

Regarding the PDMP which had been identified as presenting the least relevant safety level (factor VIII, antithrombin III, factor VII, fibrinogen and fibrinogen for biological sealant), the Afssaps inquired about the availability of products derived from plasma collected in countries *a priori* with a lower risk of BSE or vCJD, which could be imported in France.
Up to date, no medicinal product was imported in this specific context, as the identified medicinal product dossiers do not meet one or several of the quality, safety and efficacy requirements. It is necessary to remind that the group of experts who met in December 2000 had encouraged the supply of PDMP derived from plasma collected in countries *a priori* with a lower risk of BSE or vCJD. However, they had clearly recommended that this precautionary measure couldn't be taken to the detriment of the intrinsic quality of the products offered.

- Physician, patient and donor information:

The data update of December 2000 expert group report, in the form of a report dated February 2002, was put on the Afssaps website.

6 – Medicinal products extracted from urine

The existence of a form of PrP resistant to proteinase K but presenting a molecular weight

differing from that of PrPSc, named «UPrPSc » and found both in animals and in humans with TSE, especially in subjects suffering from familial forms of CJD, has been confirmed since the initial publication (*37*). The physiopathological significance, the origin and the potential infectivity of «UPrPSc » are still unknown. Regarding blood products, the presence of «UPrPSc » in urine doesn't support the presence of PrPSc in blood, both in the classical forms of CJD and vCJD. However, it provides a possible research direction for the development of a detection test usable on a routine basis.

Regarding medicinal products extracted from urine, gonadotropins and urokinases, the existence of «UPrPSc » gave rise to contrasted positions, taking into account the various aspects of the benefit ratio and the existence of alternative production sources (recombinant proteins) (*44,45*). To stick to the simple evaluation of the risk, it is useful to remind that these medicinal products are prepared from « donors » who cannot be submitted to any clinical selection considering the particular urine « donation » conditions (high frequency of donations, very large number of « donors »). The clinical selection, particularly in the case of sporadic forms of CJD, wouldn't however discard from « donation » subjects in the final phase of incubation during which «UPrPSc » is present in urine. In the hypothesis of the effective presence of «UPrPSc » in all the forms of TSE, including sporadic CJD, and of its infectious nature, it is necessary to note that no transmission case with gonadotropins was observed within the context of pharmacovigilance; the absence of transmission case is all the more to be noted as urine comes from menopaused women among other donors, consequently elderly women who are more at risk to be in the final stage of the disease incubation period, and as the recipients are young women who are monitored and have a normal life expectancy, in whom the disease would be easily detectable. Furthermore, the processes used for the preparation of gonadotropins and urokinases are complex extraction and purification techniques which potentially include various steps capable of eliminating or inactivating the TSE agents, such as high pH treatments, adsorptions and chromatographic steps. In the case of vCJD, an additional safety criterion requires that «donors » do not originate from countries presenting a high incidence of vCJD or BSE.
As a conclusion, the safety of the medicinal products derived from urine with regard to the risk of transmission of human TSE agents doesn't seem to be questionable.

7 – Updated European position, issued by the EMEA in 2002

The European position, which had been issued by the EMEA in 1998, was updated by this authority in 2002 (*46*). It is about PDMP and urinary-derived medicinal products. The essential part of the recommendations and conclusions remained unchanged. For the information of all, the main details may be succinctly reminded:

- there is no justification for calling back the PDMP derived from the blood of a donor later diagnosed with sporadic, familial or iatrogenic CJD.

- the vCJD agent presents a larger peripheral tissue distribution and a higher level of infectivity than the sporadic CJD agent; the potential presence of infectivity in blood is unknown. Consequently, the precaution approach shall be continued.

- the PDMP preparation processes should reduce infectivity if it were present in the starting plasma. Manufacturers shall analyse the capacity of eliminating the vCJD agent for each one of their preparation processes.

- staying in the United Kingdom is an acknowledged risk factor for vCJD, which justifies to recommend the exclusion of the donors who stayed for a long cumulated period of 1 year or more in the United Kingdom between 1980 and 1996; each country shall determine the length of this period according to its specificities.

- it is recommended, as a precautionary measure, to call back the PDMP derived from the blood of a donor later diagnosed with vCJD.

- to avoid the recall of a large number of batches of medicinal products consecutively to the preceding precautionary measure, it is recommended to use albumin coming from plasma collected in countries exposing to the smallest possible probability to diagnose vCJD in the donor population.

- For medicinal products extracted from urine, there is no recommended donor exclusion measure until the presence of infectivity in urine is established.

These recommendations and conclusions are closely akin to those established in the report dated December 2000 and updates.

Conclusions

The information available since the first expert group report (December 2000) on the analysis of the risk of transmission of vCJD by blood and its derivatives doesn't provide any further scientific material or any argument likely to modify the initial conclusions (*1*).

Up to date, there exist no new data on the variant Creutzfeldt-Jakob Disease (vCJD) physiopathology, modes of transmission, distribution and level of infectivity in the various tissues or on the estimation of a possible infectious load in blood. For this reason, the possibility of transmission of the disease by blood remains a hypothesis too. Furthermore, no new piece of information allows to affirm that the level of risk considered in the report dated December 2000 is modified.

On the epidemiological level, no increase in the incidence of vCJD was observed in France and in the British Islands. The estimation of the number of people likely to develop vCJD doesn't seem to be modified.
No new risk factor, which could be used a an exclusion criterion on the clinical selection of blood donors, was identified.
No detection test is for the present applicable to humans. Furthermore, the donor exclusion criteria in force at present, are and will probably remain, on the short and medium terms, the most appropriate measure for the qualification of blood donations, measure which will be completed with detection tests usable on a routine basis when the available biotechnological tools allow it.

There is no new piece of information on the capacity of the PDMP preparation processes to eliminate the vCJD agent. Consequently, the data included in the report dated December 2000 remain unchanged.
For LBP, leucoreduction remains a precaution to be considered, a measure which will but contribute to reduce the risk of transmission.
It is reminded that there exists no vCJD agent inactivation method applicable to blood products.

The conclusions and recommendations of the report established in December 2000 remain valid. None of the items dealt with and discussed in this report needs to be modified. There is no new measure to propose in order to further reduce the possible risk of transmission of vCJD by blood products. The measures in force at present seem to be efficacious and proportionate so as to ensure the right blood product benefit-risk ratio.

Updated figures relative to the number of BSE and vCJD cases appear in the annexe

Finally, the scientific surveillance should be continued. Especially, it is appropriate to follow the possible appearance of vCJD in subjects genotyped Val-Val or Met-Val at codon 129 on the PrP gene. It is also appropriate to follow any possible development relating to the presence of a resistant form of PrP in urine. In the meantime, there is no reason for anticipating a reevaluation of the safety of medicinal products derived from urine with regard to the risk of transmission of the CJD and vCJD agents.

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Lexicon

NCTA:
Non-conventional transmissible agents

BSE:
Bovine Spongiform Encephalopathy

TSE:
Transmissible Subacute Spongiform Encephalopathy

GSS:
Gerstmann-Straüssler-Scheinker Syndrome

Leucoreduction:
Operation consisting in removing, under aseptic condition, the major part of leucocytes in a labile blood product. For technical reasons, the removal is most often incomplete; in such case, the term of "leucoreduction" is preferable to "leucodepletion".

CJD:
Creutzfeldt-Jakob Disease (sporadic, iatrogenic, familial diseases)

FDP:
Frieze-dried Plasma

FFP:
Fresh Frozen Plasma

LBP:
Labile Blood Products

PFF:
Plasma For Fractionation

PDMP:
Plasma-derived medicinal products

PrPSc:
Abnormal form of the naturally-occurring protein PrP

VAP:
Viro-Attenuated Plasma

vCJD:
Variant Creutzfeldt-Jakob Disease

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Annexe

Update of the numerical data appearing in the reports dated December 11, 2000 and February, 2002

Number of cases of BSE:

- United Kingdom (by 21/11/2002) : 182 802 cumulated cases (*181 368 cases in November 2001 and 179 256 cases in October 2000*) with 755 cases reported by 30/09/2002 for the year 2002 (*1202 cases for the year 2001 and 1443 cases for the year 2000*).

- France (by 21/03/2003) : 239 cases for the year 2002 with 41 clinical cases, 124 cases resulting from the surveillance of the risk cattle and 74 cases due to systematic screening at the slaughterhouse (*in total, 274 cases for the year 2001 with 91 clinical cases, 100 cases resulting for the surveillance of the risk cattle and 83 cases due to systematic screening at the slaughterhouse; in total, 161 cases for the year 2000*).

Number of cases of vCJD :

- United Kingdom (by 03/03/2003) : 132 certain or probable cumulated cases (114 cumulated cases in 2001 and *85 cumulated cases in November 2000*), with 17 cases for the year 2002 and 1 case by 3/02/2003 for the year 2003 (*20 cases for the year 2001 and 28 cases for the year 2000*). They include 1 case notified in Hong-Kong.

- France (by 06/03/2003) : 6 certain or probable cumulated cases (*5 cumulated cases in February 2002 and 3 cumulated cases in November 2000*).

- Other countries: 1 case in Italy, 1 case in Republic of Ireland, 1 case in the United States, 1 case in Canada (these last three cases are to be connected with the British ones).