diagnosed with pathological prion in the lymphatic tissue. He died of rupturing aortic aneurysm 5 years after the transfusion without any evidence of a neurodegenerative disorder. This case suggests that individuals not homozygous for methionine at codon 129 can be infected. It remains unclear whether those heterozygous individuals would stay as a potential asymptomatic vCJD carrier, or develop the disease only with delay.

The existence of a possible "carrier status" is supported by a retrospective serial analysis of appendix and tonsil material in the UK, where 3 of 12,674 appendices tested revealed pathological prion [47,48]. Recently, the genotype of two of these individuals who had not developed vCJD at the time of the examination was sequenced (the third sample was not available for analysis) and found to be homozygous for valine at codon 129 of the prion protein gene [49]. Immunohistochemical examinations revealed a different prion distribution in two out of the three cases from that found in the lymphatic tissue of vCJD patients. It is currently unclear whether this might be indicative of the outcome of the disease. Methodological problems in the evaluation with regard to sensitivity and specificity might play a role. In addition, it must be borne in mind that the studied patients were not representative of the general population due to age distribution. If this random sample of histological examinations is used as a basis, and 100% sensitivity and specificity of the test used is assumed, the estimated prevalence of undetected vCJD infections per 1 million inhabitants in the UK would amount to 235 (49–692). This would mean a higher prevalence of vCJD than previously estimated on the basis of the decreasing figures of clinical cases.

The mathematical models were adapted to these new findings; wider genetic susceptibility and a possible carrier status were assumed for the disease. Taking into account the remaining uncertainties on the length of the incubation period, the estimated number of clinical vCJD cases by the year 2080 is 70 (10–190) based on the existing calculation model and a model for a carrier status [50], as opposed to 363 cases (no confidence interval indicated), based on a more pessimistic assumption. If the data on the examinations of the appendices [48] are taken into account, the estimation is 133 (32–3,780) cases [50].

The model published by Clarke and Ghani in 2005 provides estimates for the number of individuals with subclinical and preclinical infection with the vCJD pathogen [50]. The histological data of the appendices were taken into account in this assessment, and 50% sensitivity of the tests for subclinical infection was assumed. Based on these assumptions, a far greater number of individuals infected but without clinical manifestation (1,130–13,440) can be assumed. The number of these carriers and the question of whether they would be infective are important for possible iatrogenic transmission and may markedly influence the absolute number of future vCJD cases. Besides transmission by blood products, incomplete disinfection of surgical instruments might also play a part.

The above model calculations refer to the UK. For countries without or with only a small number of vCJD cases, the estimate is even more uncertain. The decisive parameter is the extent of exposure to food stuffs produced from infected beef. A synopsis of the peak incidence of the BSE epidemic in various countries, as well as the assumed period of exposure to BSE (2001 report, endnote 1) clearly show that the extent of the BSE epidemic in the UK is a multiple of that of other countries, even if differences in the reporting criteria are taken into account. A risk of exposure for countries with no or only few BSE cases can only be estimated by the extent of imports of beef cattle from the UK within the relevant period of time. Fig. 2 shows imports of beef from the UK between 1990 and 1995.

The mathematical models from the UK on estimating the vCJD epidemic were used in Ireland and France, taking into account the actual situation in these countries. In Ireland, where four cases of vCJD have occurred up to now (two of them were residents of the UK for a considerable period of time), an estimation was performed on the basis of the model developed in the UK with adaptations for conditions in Ireland [51]. The estimation considers potentially contaminated Irish cattle, cattle imports from the UK, and the consumption of British beef during visits to the UK. This model, too, takes into account only the group of individuals who are homozygous for methionine at codon 129. It was estimated that 1–2 (0–46) more clinical cases of vCJD would occur in Ireland. Apart from the above limitations, the adapted model is suitable for performing estimation for countries with few or no cases of vCJD if the basic data are known. In France, 20 vCJD cases have been reported so far. In a current model calculation, also based on the epidemiological data from the UK, it was estimated that after 2004, another 33 vCJD cases (12 of them in 2004 and 2005) would occur [52]. The model calculation takes into account imports of British beef to France, beef consumption and travel to the UK. The estimate of the case numbers for France has decreased by two thirds compared with the previous forecasts from 2000 [53].

No case of vCJD has so far been diagnosed in Germany. Since the epidemiological situation in Germany is hence markedly different from that in the UK, and, in addition, the extent of exposure to potentially BSE contaminated beef cannot be accurately quantified, no primary data are available, allowing a valid use of models for estimating the incidence of primary vCJD cases in Germany. Based on estimates for France and Ireland, where only a few vCJD cases have been diagnosed, it can be assumed on the basis of the current state

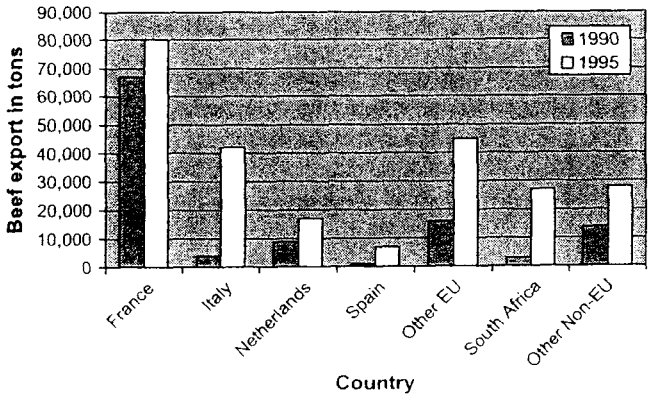

Fig. 2. Beef imports from the UK in tons.

of knowledge that only isolated cases of vCJD will occur in Germany.

7. Risk of vCJD transmission through blood (secondary infections)

Various approaches, such as animal experiments and epidemiological and case control studies, as well as the observation of individual cases, address the question of whether blood and blood products constitute a risk of vCJD transmission.

A number of experiments explore the possibility of prion transmission by blood and its components, with great variety of combinations of TSE agents and animal species [42,54,55]. Many results, however, must be interpreted with a number of restrictions. The investigational material of the donor animals (blood, serum, cells etc.) was usually given to the indicator animals by i.c. administration. This permits a more sensitive detection of the pathogen, but conclusions regarding i.v. administration are difficult. Secondly, many experiments were performed with animals infected in a "non-natural" manner, which makes the extrapolation of the corresponding results more difficult. In addition, the tests often involve a species barrier, which means a decrease in sensitivity.

Despite many contradictory results, the following can be concluded from small rodent experiments:

- In principle, infectivity can be detected in the blood of experimentally infected animals.
- Titers of infectious TSE agent in the blood of artificially infected animals were found to be very low (1–100 infectious units/ml) in sensitive detection systems. The question arises in some experiments as to what extent the detected infectivity really reflects replicated agent and not just residual inoculum.
- While in various experiments i.v. application has led more rarely to infection of the indicator animal than i.c. inoculation, in a recent experiment using mice infected with either mouse adapted strains of GSS, a special familial form of CJD, or vCJD, no major difference was found between i.v. and i.c. inoculation. Infectivity of approx. 20–30 IU/ml was found in the preclinical and the symptomatic phases 17 and 23 weeks after inoculation in both the buffy coat of the blood and in the plasma [56].

A transfusion experiment, where blood for transfusion was drawn from sheep in two series of experiments, pointed to a potential transmissibility of prion diseases by blood [57]. The donor sheep had been either orally infected by brain material from BSE infected cows or were sheep with particular genetic susceptibility for scrapie. After transfusion of whole blood or buffy coat of those donor animals that later died of the orally induced "BSE" or scrapie, definite TSE developed in a number of recipient animals. The identification of the pathogen confirmed transmission in this experiment. It must be noted that the recipient animals were genetically sensitive to TSE.

Formal retrospective epidemiologic studies and case control studies did not reveal any evidence of transmission of human TSEs by blood or blood products. In contrast to a number of viruses (HIV, HCV, HBV), no suspected case of transmission of classical CJD to a hemophilia patient has become known [58]. Since this disease can barely be overlooked in this well monitored group of patients, this is reassuring.

However, three case reports published in the UK demonstrate the possibility of vCJD transmission by transfusions. No available diagnostic system could prove or exclude vCJD transmission in any individual case. Joint consideration of the published cases, however, does not permit any other conclusion than transmissibility of the vCJD agent by blood transfusion. A monitoring system, the National CJD Surveillance Unit, was established in the UK in 1990, which, among other things, was designed to identify blood donors among vCJD patients and locate their donations. The recipients of these donations were observed, and in the event of their death appropriate tests including an autopsy with histopathological identification of the vCJD agent were performed. 15 of the vCJD patients who were diagnosed up to December 2003 had donated blood. They had donated a total of 55 labile blood products of which 48 had been transfused. At that time 17 living recipients were identified and monitored. This surveillance system has so far identified three cases: In 1996, a then 62-year-old patient received a total of 5 RBCC within one operation. One of these concentrates (non-leukocyte reduced) originated from a donation of a 24-year old individual who was healthy at the time of donation but died of confirmed vCJD in 2000 (3.5 years after the donation). The recipient developed symptoms 6.5 years after the transfusion and died of vCJD 13 months later; the diagnosis was confirmed by a post mortem [59]. Since the recipient lived in the UK, thus also exposed to a risk for contraction of vCJD via the food chain, transmission by transfusion could not be proven but was highly likely considering the low statistical probability of coincidence, i.e. an infection via the food chain not related to the transfusion, rated as 1:15,000–1:30,000. The second case was another elderly patient who received a non-leukocyte reduced RBCC from a donation of an individual who developed symptoms 18 months later and died of confirmed vCJD in 2001 [60]. The recipient died of a non-related cause (rupturing aortic aneurysm) 5 years after the transfusion without any signs of a neurologic-psychiatric disorder. Within the above-described surveillance, a post mortem was carried out. The vCJD agent was found by histopathological examination in the spleen and neck lymph nodes. The agent was obviously transmitted; however, an involvement of the CNS was not detectable—neither clinically nor histopathologically. In contrast to all previously identified vCJD patients, this patient was heterozygous M/V at codon 129. The infection was subclinical at the time of death; whether vCJD would have developed in this case must remain open. A third patient in the UK was reported in 2006 who developed symptoms of vCJD after receiving non-leukocyte depleted RBCC 7 years and 10 months before. The patient is a M/M homozygote at codon 129. The donor of the concentrate developed vCJD 21 months after the donation

[61,62]. A fourth probable case was announced in January 2007 (http://www.hpa.org.uk/hpa/news/articles/press_releases/2007/070118_vCJD.htm).

As long as a blood test for vCJD does not exist, assessment of cases of suspected transmission would be possible only to a limited extent; this applies either if the individual affected by vCJD donated blood or had received transfusions of blood components. Recommendation 33 of the AK Blut (National Advisory Committee 'Blood'), 2006, provides guidance in this context [63]. A detailed statement regarding the safety of blood products in view of vCJD has recently been published by the Scientific Committee on Emerging and Newly Identified Health Risks of the EU Commission, SCENIHR [64].

Of crucial importance for the vCJD risk from transfusion is the number of individuals within a given population who are infected and may carry the agent in their blood. Histological evidence of prions in appendices [48] indicates a higher frequency of infection than previously estimated based on the occurrence of vCJD. As described above, one would now hypothesize that all individuals are susceptible to the vCJD agent, not only those homozygous for M/M, who represent 40% of the population. In another human TSE, Kuru, cases with extremely long incubation periods of up to 56 years have been reported, and all whose PrP gene could be analyzed were not M/M homozygotes [65]. Thus a higher number (maximally double) of infected individuals should be assumed than up to now. If this was conceivable for the British population, we would have to expect one subclinical case in roughly 4,000 people.

8. Reduction of TSE in the manufacture of blood products

In view of the limited knowledge, in assessing the effectiveness of methods the following partly speculative and pessimistic assumptions and remarks of reservation have to be made:

- The amount of infectivity in blood is estimated on the basis of data from animal experiments. The French authority AFSSAPS [66] had previously assumed as worst case scenario that infectivity in whole blood is 100 IU i.c./ml (infectious units/ml in case of intracerebral administration), and lower for intravenous inoculation at 10 IU i.v./ml (infectious units/ml in the case of intravenous administration). However, in primates survival rates after i.v. and i.c. inoculation were similar [29,67]. In addition, in recent comprehensive studies, 13.6 IU i.c./ml were measured in the blood of scrapie infected hamsters [68], and approx. 20 IU i.c./ml in the plasma of mice infected with adapted vCJD or GSS pathogens [56,69]. AFSSAPS now assumes an infectivity of 20 IU i.v./ml in the blood based on this new data. For leukocyte depleted plasma, a pathogen reduction by 50% is assumed, thus 10 IU i.v./ml in leukocyte depleted plasma instead of previously 1 IU i.v./ml plasma [66]. A study performed for the British Health Ministry (DNV-Consulting, 2003) [70] assumes a pathogen content of 10 IU i.c./ml in the plasma and a 5-fold reduced infectivity in the case of i.v. inoculation, thus 2 IU i.v./ml.
- This is extrapolated to vCJD cases even though no infectivity has been found in their blood so far [71,72].
- There are no accurate data during which times infectivity could be present in the blood of individuals during the incubation period and the course of disease.
- The form of infectious prions (association with cells, monomers, multimers, aggregate, fibrils) in the blood of "naturally" infected creatures is unknown. Based on animal experiments [73], it had been assumed that 90% of the infectivity of whole blood would be present in the cellular fraction and 10% in the plasma. More recent studies [56], however, point to an approx. equal distribution of the amount of pathogen in the plasma and in the leukocyte fraction.

9. Blood components for transfusion, leukocyte depletion

Leukocyte depletion (LD) became compulsory (among other reasons) as a precautionary measure against a possible transmission of vCJD by blood components in various countries, including Germany. Treatment of whole blood (2.5×10^9 leukocytes/ml) results in a reduction of leukocytes by 3–4 log steps with residual numbers limited to 10^6 leukocytes per blood component. An experimental study has been conducted into the capacity of LD to remove the TSE pathogen using 500 ml blood of scrapie infected hamsters [74]. The concentration was reduced from 13.1 IU_{50}/ml in whole blood to 7.6 IU_{50}/ml, i.e. 42% of the pathogen were removed during leukocyte depletion. Since the actual pathogen concentration in human blood is unknown, it is difficult to assess to what extent this reduction would represent a gain in safety. No LD of RBCC was carried out in either of the three transmission cases. However, no conclusion may be drawn that such treatment of the components would have prevented transmission.

9.1. Red blood cell concentrates (RBCC)

In Germany, RBCC predominantly originate from whole blood donations. Before LD was enforced (October 2001), buffy coat-free RBCC were the standard preparations which, in an average volume of 250 ml, may contain up to 1.2×10^9 leukocytes, according to the applicable national guideline [75] and the Council of Europe Recommendations. Even after the LD has become mandatory, a potential transmission risk of RBCC must be assumed.

9.2. Platelet concentrates (PC)

Eighty percent of the PC in Germany is manufactured from whole blood donations (WB-PC, e.g. buffy coat, usually pooled from 4–6 donations) and approx. 20% from apheresis (A-PC). Titers of approx. 10 IU/ml [56] were measured in the thrombocyte fraction of mice. Residual infectivity must be assumed even after 42% pathogen reduction by LD of the

whole blood. A preference for A-PC is not justified at present. Assessment of a residual infectivity is difficult since in apheresis high blood volume is processed and the behavior of vCJD infectivity in the apheresis system is difficult to predict.

9.3. Plasma for transfusion ("Fresh Frozen Plasma, FFP")

In Germany, quarantined plasma (Q-P) and solvent/detergent treated plasma (SD-P) are currently available. The market share of SD-P is approx. 10%. SD-P is manufactured by pooling approx. 700–1,200 individual donations. The volume for a unit of Q-P in Germany is approx. 230–280 ml, and for SD-P it is 200 ml.

In a previous assessment (2001 report, footnote 1), the content in cell free plasma had been estimated to be 1 IU i.v./ml; 250 ml of quarantined plasma would contain 250 IU i.v. cell free plasma. Two calculations had been made for SD-P:

(a) Based on the assumption that infectivity is distributed homogenously in the pool, 200 ml individual plasma containing approx. 200 IU i.v. (residual cells neglected, see above) would enter a pool; assuming a low number of 500 donations this would result in the dilution to 0.4 IU i.v. per plasma bag in the SD-P separated after treatment.
(b) Based on the assumption that infectivity is in principle not evenly distributed in portions <1 IU i.v., an infectious donation containing 200 IU i.v. could be distributed to a maximum of 200 plasma bags, i.e. 200 of 500 SD-P would be infectious. Assuming 1 out of 120,000 donations were infectious (AFSSAPS, 2000) and a pool size of 500 donations, the risk would be 1 out of 240 SD-P batches. The risk of an infectious SD-P would thus be approx. 1 in 600 (240 times 2.5), which would be less favorable compared with 1:120,000 for Q-P from an individual donation.

Assuming 10 IU i.v./ml instead of previously 1 IU i.v./ml in the contaminated plasma donation [66], the risk becomes higher to the disadvantage of the pooled plasma. Based on this assumption, the above calculation (a) for a pool of 500 donations and 2,000 IU in a donation would result in an average burden of 4 IU in all plasma bags of a batch. If it was assumed that infectivity in principle is not distributed in units <1 IU i.v. (b), an infectious donation would contain 2,000 IU i.v. in 500 donations so that all 500 plasma bags from a pool of SD-P could be infectious. However, since these calculations contain many unknowns (e.g. reduction effects) and are based on unproven hypotheses, no recommendations are given here as to the preferred type of plasma.

Another question is whether infectivity in the plasma can be reduced by further measures. It has been considered to prepare plasma cell free to the greatest possible extent and to remove cell fragments by filtration through a membrane with appropriately small pores, an approach pursued in France. No experimental evidence is available on whether this could effectively reduce the infectivity of plasma. Furthermore, it is not clear whether the quality of the plasma (e.g. activation of coagulation factors, neoantigen formation) might be impaired. Therefore, a decision in favor of introducing such membrane filtration seems currently premature.

10. Industrial products from pool plasma, nanofiltration

The evaluation of individual fractionation and inactivation steps in the manufacture of plasma derivatives (e.g. factor concentrates, immunoglobulins, albumin), regarding vCJD pathogens and the risk for the recipient is still fraught with uncertainties:

- Some assessments are based on the assumption that existing vCJD infectivity can be pushed below a presumably safe threshold dose by means of several dilution and reduction steps. It has not yet been determined whether an infectious threshold dose administered once would cause infection of the recipient, and whether several doses "below the threshold" would have a cumulative effect.
- Opinions are divided as to whether the size of the fractionation pool plays an important part (analogous with the SD-P):
  - Using a large pool, in case of possible contamination of the products a large number of recipients could be at risk. This would suggest that small pools would have to be used.
  - On the other hand, a freely distributed infectivity (e.g. if prion monomers were present) would be diluted considerably by pooling. Therefore, larger pools could present less risk.

For a reliable assessment of the influence of the pool size, more knowledge would be required on the infective dose in humans, the degree of aggregation of infectivity, its dispersibility, and the pathogen concentrations which can occur in the blood of asymptomatic donors. Calculations about the relation between pool size and transmission risk (Appendix (A) of [1]), assuming that the pathogen would behave like a virus, show that if a recipient requires life-long treatment, a reduction of the pool size would not contribute to minimizing the risk. The current situation is relatively heterogeneous for products on the German market, with different manufacturers, different countries of origin of the starting plasma, various import products and a great variability of the manufacturing methods.

10.1. Effectiveness of the plasma fractionation steps

Usually, infectious material from brains of scrapie or BSE infected hamsters or mice is used to assess the capacity of process steps to remove the vCJD pathogen. The question is to what extent such material is representative of the potential vCJD pathogen in human blood. In a comparative study, no differences in removal of PrP^{Sc} were observed between material from the brain of humans who had developed vCJD, sCJD or GSS, and material from the brain of scrapie infected hamsters [76]. So far, no major differences of pathogen reduction

have been reported when different detection methods (PrP^{Sc} detection versus bioassay) were used [68,77,78]. However, preparation of infectious material from brain can influence pathogen removal: highly purified PrP^{Sc} can aggregate into high molecular fibrils, which may behave differently than dispersed brain material or infectivity in the microsomal fraction [79]. The degree of aggregation is particularly important for pathogen retention in nanofiltration [80] and precipitation and separation by means of centrifugation and depth filtration. It was shown that PrP^{Sc} tends to aggregate in the alcoholic production intermediates during plasma fractionation [81,82]. Despite the above mentioned uncertainties in the interpretation of the experimental data, a reasonably homogenous picture is revealed for plasma fractionation.

Several publications are available for the conventional alcohol fractionation steps of plasma derivatives [68,79,82–86], which state that the pathogen is removed successively from the albumin and immunoglobulin fractions. For coagulation factors, however, such a generalization is far more problematic since individual production processes may differ considerably. This is why the EMEA position paper of 23 June 2004 (EMEA/CPMP/BWP/2879/02) required manufacturers to assess their production methods specifically and to carry out their own experimental trials if suitable published results were not available. PrP^{Sc} reductions by at least 4 log steps have been reported so far for Factor VIII [77,85,87].

10.2. Nanofiltration

Considerable reduction factors are reported by filter manufacturers and plasma fractionators for nanofiltration. However, studies were carried out with differing TSE spiking materials (e.g. fibrillary material, detergent treated material, brain homogenate). The infectious form(s) of the vCJD pathogen is (are) currently still unknown. What the effect of the nanofilters on smaller prion aggregates would be remains open. It is assumed that for prion monomers, no mechanical exclusion by pore size would be given. However, reduction on the basis of other interactions with the filter materials cannot be excluded. The actual benefit of nanofiltration for the removal of vCJD pathogen, therefore, remains fraught with some uncertainty. Until recently, the view prevailed that nanofiltration was not possible with large sensitive molecules such as factor VIII. This option, however, has been implemented by the French manufacturer LFB (pore sizes 35 nm and 15 nm). Since problems might occur that cannot be assessed in laboratory tests, e.g. the development of neoantigenicity, clinical testing should be discussed before marketing authorization. The change in France was effected without clinical trials. However, no additional adverse effects have so far been observed after the change. A detailed discussion of nanofiltration can be found in Appendix (B) of [1].

Hence, several manufacturing steps of plasma products can considerably reduce vCJD infectivity from the starting material, but the extent of this reduction must be further tested and validated. The risk of infectious fractionated plasma products should be markedly lower compared with blood components. The assessment of the safety of recombinant products is not the subject of this report. Therefore, reference is made only briefly to a few aspects. Human plasma derivatives, essentially albumin, may be used as a stabilizer during production of certain recombinant products. In eukaryotic cell cultures, materials of bovine origin are sometimes used. The risk of primary infections with the BSE pathogen must be considered in principle; however, such theoretical risk is minimized [88,89], e.g. by purchasing materials from BSE-free countries. Individual tolerability of different products in the patient and relative frequency of the development of inhibitors must also be considered in the overall assessment of safety. In the past, bottlenecks have existed in the supply of both recombinant coagulation factors and coagulation factors prepared from plasma. With the current state of knowledge, there is no need to advise against the use of plasma derivatives if the indication is established correctly. In hemophilia treatment, decision between coagulation factor products manufactured from plasma and recombination coagulation factors must be considered very carefully, taking into account the situation of the individual patient. A schematic recommendation cannot be given here.

11. Optimal use of blood products

Being "medicinal products from humans," blood products cannot be entirely risk-free, despite the great progress in safety. Critical indication and restrictive administration of blood products are therefore essential to reduce the patients' risk, which is particularly true for a potential transmission of vCJD by donor blood. In the Sanguis Study [90,91], noticeable differences were found in the frequency of transfusion among 43 hospitals from 10 European countries participating in the study. As extreme examples, the preoperative request for provision of RBCC in cholecystectomy was more than ten times that of the actually transfused units, and the frequency of transfusion of hemicolectomy patients ranged between 0% and 79%. Such differences can hardly be explained. Heterogeneous transfusion practice has not changed significantly in the past few years: a more recent Finnish study thus shows that contrary to international recommendation, the median pre-transfusion hemoglobin in transurethral prostate resection was 112 g Hb/l [92]. Various authors have stated unanimously that prospective transfusion criteria and consistent instructions of personnel would lead to a considerable reduction of the consumption of blood components [93,94]. Another possible approach would be autologous blood transfusion, which would avoid any de novo infection relating to allogeneic blood products (including vCJD). Autologous blood transfusions, however, can be performed only in elective surgery with a timely and reliably foreseeable transfusion requirement [95].

Under German EU presidency in 1999, a meeting was held in Wildbad Kreuth [96] with experts in attendance from the EU member states. An assessment of the current situation concerning the use of the most important blood products was elaborated and questions of use, quality management, and economic aspects of transfusion medicine were summarized. It

would be desirable to continue this Kreuth initiative. In regards to therapy with blood components and plasma derivatives, an interdisciplinary working group of the Bundesärztekammer (German Medical Association), summarized basic principles for a clinically indicated use of all important blood products with special consideration of the international literature, national and international consensus conferences and clinical experiences [97]. An essential contribution in Germany has been the requirement laid down in the Transfusion Act of 1998 to establish in all health care facilities a well-functioning quality assurance system for the use of blood products. An appropriately qualified physician responsible for transfusions must be designated and, in addition, transfusion representatives in each clinical unit. This was transposed into the German hemotherapy guidelines [75]. At a European level, too, appropriate recommendations were adopted [98]. Of crucial importance is how these guidelines are implemented and used by hospitals and doctors. It is necessary that such efforts are actively encouraged on the part of the top managers of health care facilities and the health policy makers and recognized by the health care providers who are supposed to finance them.

Optimal use of blood and blood products is an undisputed goal, especially as a safety measure in view of vCJD. Shortly after the first suspected case of transmission was disclosed, the EU Commission called in a "Technical Meeting of Blood Experts related to vCJD transmission" on 20 January 2004 in Luxembourg. One of the statements elaborated by this meeting reads as follows: "There was agreement that optimal use of blood may further reduce the risk of transmission of vCJD by avoiding unnecessary exposure to allogeneic blood transfusion. In addition, avoiding unnecessary transfusion may improve the availability of blood for transfusion; this in turn may facilitate the introduction by Member States of additional donor deferrals if required."

12. Diagnosing vCJD: screening tests

Clinical diagnosis of vCJD can, in principle, be carried out premortem in symptomatic individuals by screening for PrP^{Sc} in the tonsils [27,35], though such a biopsy presents a burden for the patient. At present, solid confirmation of vCJD by histological display of amyloid plaques or detection of PrP^{Sc} in brain material by Western blot is only possible by brain biopsy or post mortem. Clinical diagnosis is a laborious process consisting of various methods and is of subordinate importance for the safety of blood donations. A summary can be found in Appendix (C) of [1].

The development of screening tests is one of the key endeavors for the safety of blood donations. The principal goal of a vCJD screening test is to detect infections as early as possible before onset of initial symptoms in order to prevent possible further transmissions and, if appropriate, to allow therapeutic measures to be taken in an appropriate time frame. Though intensively pursued by a number of groups, so far no concrete success has emerged. The current approaches towards screening tests using blood or other easily accessible body fluids are based either on direct detection of PrP^{Sc} or on the detection of other markers associated with the infection (surrogate markers) [99]. One of the problems with detecting PrP^{Sc} in body fluids is the extremely low concentration at which it may occur in the periphery; estimates expect considerably less than 1 pg/ml PrP^{Sc} in the blood. The most sensitive antigen tests (e.g. for the detection of HBsAg of HBV or p24 of HIV, two proteins with a molecular size similar to that of PrP^{Sc}), after many years of development and improvement, are capable of detecting antigen only at levels of 10 pg and above per ml plasma or serum. In addition, physiological prion protein is present in approx. 10,000-fold excess, which makes the sensitive and specific detection of PrP^{Sc} considerably more difficult. Highly specific so-called "conformational" antibodies (for the recognition of PrP^{Sc} characteristic folding epitopes or conformation epitopes) therefore seem indispensable for a sensitive detection of this protein. The possible use of such an immunoassay (CDI; "conformation dependent immunoassay") for clinical diagnosis of infections is currently under discussion [100].

Research projects are pursuing different approaches to surmounting these limitations, e.g. attempts to increase the tests' sensitivity by means of spectroscopic techniques, enrichment steps to increase the PrP^{Sc} concentration by selective precipitation of PrP^{Sc} through its binding to "ligand" molecules, or cyclical amplification of the pathogen prion protein. The artificial in vitro replication of PrP^{Sc} by means of the PMCA method ("protein misfolding cyclic amplification") [101] has raised high expectations. However, demonstration of the possibility to replicate infectious PrP^{Sc} in one species by a factor of 10^3 [99,102] has not yet led to the development of test systems for routine screening.

The obvious difficulties with the sensitive detection of PrP^{Sc}, the only known specific marker of vCJD infection, have led to the exploration of alternative test concepts. A possible choice would be a screening strategy using single surrogate markers or a combination of them, which could be carried out both at the RNA level (differential display) and at the protein level (proteome analysis). Previously, analyses of differential gene expression in TSE infections have shown that a number of genes are over- or under-expressed in the course of the disease. Several groups have examined to what extent the differential gene expression in the course of the disease could contribute to a better understanding of the infection. Much attention was attracted by the publication of a peripheral marker detectable in blood cells ("erythroid differentiation factor, EDF") [103]. Follow-up tests, however, showed that it is subject to major fluctuations in healthy individuals [104]. Several candidate surrogate screening markers have been published, while proof for these remains to be provided.

Extensive examinations on well-defined populations and acceptable test features (sensitivity, specificity, high throughput) are indispensable preconditions for introducing a screening test, especially in blood donation screening. These conditions have so far not been met by any of the test procedures, and specific criteria should be established for validation of new vCJD tests. Evaluating them in healthy populations,