numerous European countries (Austria, Czech Republic, Finland, Germany, Greece, Holland, Italy, Luxembourg, Poland, Slovakia, Slovenia, Spain), as well as in Japan, Canada, and the USA. They have also shown that many countries with no recorded case up to 2000 (e.g. Germany, Italy, and Spain), and which denied the presence of BSE, had an incidence equivalent to or higher than that of France and Switzerland, which have been recording cases since the early 1990s. Lastly, rapid tests allow much more precise epidemiological follow-up, allowing the measurement of trends at low prevalence, and which has more clearly shown a spectacular decline in the BSE epizootic in Europe.

Active monitoring of TSE in small ruminants (sheep and goats) was set up in Europe in March 2002, essentially to gather epidemiological data, and obliges member states of the European Community to test a quota of animals slaughtered normally or from at-risk populations. Between 2002 and 2006, nearly three million tests were performed, which led to the detection of over 13 000 cases of scrapie[4]. Note that this active surveillance resulted in the detection of a great many cases of so-called atypical scrapie among European livestock. This form of scrapie, which very likely corresponds to strain Nor98 [7] identified in 1998 in Norway, now accounts for over 50% of TSE cases in small ruminants in many countries (France, Germany, Portugal, UK, etc.). The $PrP^{Sc}$ associated with this strain is characterised by increased sensitivity to proteinase K, which makes its detection more difficult and explains why numerous rapid tests perform poorly in diagnosis. In practice, the vast majority of cases of atypical scrapie were identified using the tests from Bio-Rad (TeSeE since 2002) and IDEXX (post-mortem test, since 2005).

In view of the diversity of TSE strains present in small ruminants, the European Commission set up biochemical typing in 2005, mainly designed to identify the BSE strain in small ruminant populations[5]. Testing, performed exclusively in national reference laboratories, is based on Western blotting techniques [74, 76]. It is worth noting that, to date, only a single case of BSE, in a goat, was observed in France [21] before the implementation of this new regulation.

## 3. NEW APPROACHES TO ANTE-MORTEM TESTS

As we have seen, diagnosis of prion diseases depends principally on the detection of the abnormal form of PrP ($PrP^{Sc}$ or $PrP^{res}$). This approach has been very useful in reacting to the BSE epizootic and in setting up active surveillance for TSE in ruminants, but to date has not met all the requirements of the diagnosis of prion diseases. These tests are only applicable to tissues collected after the death of the animal and so cannot be used for early preclinical diagnosis. So far, no test can give a reliable diagnosis using a readily available sample from a living animal or person, such as blood or urine. The problem is particularly acute for blood transfusion, insofar as it is now well established that vCJD can be transmitted by blood. Considerable effort has been devoted to the search for alternative markers enabling earlier diagnosis of TSE (for a review see Parveen et al. [55]).

### 3.1. The search for new markers

The search for alternative markers has grown greatly in recent years, boosted by the development of postgenomic approaches, which can be used for large-scale parallel analysis of the transcriptome, proteome, and metabolome of tissues. Attention naturally first turned to neuronal markers, which include protein 14-3-3 [28, 78], neurone-specific enolase [1], the protein S100B [5, 29], glial acidic fibrillar protein [44, 50], Tau protein [51], and prionins [59]. However, none of these markers has proved usable as a basis for a sufficiently sensitive and specific test allowing early preclinical diagnosis.

[5] Commission Regulation (EC) No 36/2005 of 12 January 2005 amending Annexes III and X to Regulation (EC) No 999/2001 of the European Parliament and of the Council as regards epidemio-surveillance for transmissible spongiform encephalopathies in bovine, ovine and caprine animals: http://ec.europa.eu/food/food/biosafety/bse/legisl_en.htm [consulted 11 January 2008].

Metabolic markers, such as fatty acid-binding proteins, interferon γ, prostaglandin E2, C-reactive protein, interleukin 6, cystatin C, and corticosteroids, have also been studied, but with no more success (for a review see Parveen et al. [55]).

Transcriptomic studies have revealed potential markers [70, 85], but to date none has proved of practical use in the diagnosis of prion diseases. Erythroid differentiation-related factor, for example, initially seemed highly promising (downregulation [43]), but its value was not confirmed in subsequent work [25].

Finally, some research groups have developed an approach based on serum analysis by Fourier transform infrared spectroscopy combined with data processing by the neural network method [13, 39, 40, 69, 77]. This approach has shown high (> 90%) sensitivity and specificity in cattle populations, but it remains to be seen whether it is usable under routine conditions, and can be used to make an early diagnosis of TSE.

### 3.2. Protein misfolding cyclic amplification

To facilitate preclinical detection of prions in peripheral tissues, notably blood, Claudio Soto's group developed an original approach in which the $PrP^{Sc}$ in a sample is amplified by means of protein misfolding cyclic amplification (PMCA) [62]. In this approach, which seeks to mimic pathological processes and is akin to the polymerase chain reaction used to amplify DNA (but without addition of exogenous polymerase enzyme), $PrP^{Sc}$ is incubated in the presence of excess $PrP^{C}$ to allow expansion of aggregates of $PrP^{Sc}$ which are then dispersed by sonication to generate smaller units and to encourage the formation of new aggregates. The quantity of $PrP^{Sc}$ formed depends on the number of expansion/sonication cycles performed. In early articles [62, 72], amplification was modest (10- to 50-fold), but optimisation and automation subsequently enabled amplifications of several million fold [61]. In most studies, amplification is achieved by using as a source of $PrP^{C}$, a brain extract from the same species as that which produced the $PrP^{Sc}$ to be amplified. Recent works [18, 19] have shown that $PrP^{Sc}$ can be replicated in a more controlled "minimal" system in the presence of highly purified $PrP^{C}$ (the only identified contaminant being lipids) and polyanions (polyA RNA in these studies).

Although most of the work by Soto's group concerns a hamster model infected by strain 263K, significant amplification has been achieved with the $PrP^{Sc}$ produced by various mammalian species, including mice [47, 72], sheep, goats and cattle [72], cervids [38] and humans [36]. The $PrP^{Sc}$ newly formed by PMCA has all the properties of the original $PrP^{Sc}$, notably its infectious character [14, 82]. Lastly, early detection of $PrP^{Sc}$ in hamster blood fractions (buffy coats) was achieved at a sensitivity ranging between 0 and 89% and a specificity of 100% [15, 60].

PMCA has great potential and is certainly the most promising approach from the viewpoint of developing a blood test. It is, though, hampered by various fundamental and technical difficulties. Given the requirements imposed by a blood test (see paragraph below), notably in terms of practicability, sensitivity, and specificity, several technical improvements are needed. For adaptation to routine analysis, there is a need for simplification, reduction of the duration, and better control. Moreover, the obligatory requirement for a concentrated source of $PrP^{C}$ (brain extract or purified $PrP^{C}$) of the same species as the target to be amplified constitutes an important practical handicap. This specific problem could be resolved by the use of recombinant PrP and accelerated procedures as recently shown [4], assuming the results obtained with the hamster model can be extended to other mammalian species. However, PMCA must also prove effective in terms of diagnosis (sensitivity and specificity close to 100%) using blood sample series more representative than those obtained with the hamster model. Finally, recent results from Supattapone's group show that infectious $PrP^{Sc}$ can be generated de novo and stochastically by PMCA [18] in the absence of pre-existing prions, and this raises concerns about the specificity of this approach when used in routine conditions.

### 3.3. Blood tests: state of the art

As we have emphasised several times in this review, the development of a blood test is the top priority in prion disease diagnosis, notably to ensure the safety of blood transfusion in humans. Numerous difficulties, however, have to be overcome, which explains why no test is yet operational. Whereas blood from vCJD infected patients is clearly infectious [41, 56, 84], its concentration of infectious material is very likely much lower than that in the central nervous system, and its concentrations of $PrP^{Sc}$ are estimated to be in the range of pg/mL [8, 9]. Given the efficacy of disease transmission by the intravenous route, and the large volume (commonly > 400 mL) of packed red blood cells transfused in humans, transmission can occur with very low levels of infectious material, and, as a consequence, candidate tests must have excellent analytical sensitivity. Also, blood is a complex tissue rich in cells and proteins, and little is known of the distribution of prions (and of $PrP^{Sc}$). Several studies indicate that the bulk of the infectious material is in the white blood cells, but the plasma is also clearly infectious [8, 9]. In a healthy individual, significant levels of $PrP^{C}$ are present in white blood cells, red blood cells, platelets, and plasma, probably at much higher concentrations than $PrP^{Sc}$. A candidate test must therefore also be very selective. Also, we know very little about the biochemical properties of the $PrP^{Sc}$ in the different blood fractions. Given its low concentration and its environment, it is not certain, for instance, that it can form aggregates resistant to proteinase K, the treatment on which most current rapid tests are based.

In terms of the risk of vCJD infection by blood transfusion, because the incidence of the disease is assumed to be very low, a highly specific test is needed, or it could lead to more false-positive results than detection of real cases. Such a situation would be very difficult to manage ethically, given that vCJD is a fatal disease for which at present there is no treatment. There is clearly a great need for at least one very specific confirmation test, which does not exist today.

Due to the above mentioned difficulties it is not surprising that very few publications report on blood tests for TSE.

The first promising results were obtained, as early as 1996, by the group of Mary-Jo Schmerr, which combined capillary electrophoresis with a competitive immunoassay to detect a PK resistant C-terminal sequence of PrP in the blood of sheep infected with scrapie [66–68]. The technique was subsequently improved and applied to more relevant series of scrapie infected sheep [34, 35, 42, 86], but despite achieved improvement, the method appeared insufficiently robust for routine use [22].

In recent years, many research groups or companies have developed original strategies to try to overcome the intrinsic difficulties associated with the blood test. These include:

- The use of ligands for a specific capture of $PrP^{Sc}$ possibly present in blood fractions, which include the 15B3 antibody produced by Prionics [37, 48] and the Seprion resin of the Microsense company (already used in the IDEXX test for post-mortem diagnosis). In both cases, the idea is to concentrate abnormal PrP by immunoprecipitation, taking advantage of its polymerisation state (aggregate? polymers? oligomers?) to allow a more sensitive and more specific detection by ELISA or flow cytometry. Another approach developed by the bioMérieux company (Marcy l'Étoile, France) involves binding and aggregation of abnormal PrP in plasma by streptomycin [45], followed by a specific capture on calyx-Arenes "molecular basket" immobilised onto a solid phase, and final detection with an appropriate anti-PrP antibody.
- The development of immunoassays designed to detect polymerised PrP (AS-ELISA, for aggregate specific ELISA) and based on the use of the same monoclonal antibody for capture and detection [52]. The sensitivity of AS-ELISA was increased by combining signal amplification (fluorescence) and target amplification (prion amplification using a simplified PMCA like procedure). Using

this approach (named Am-A-FACTT) the group of Man-Sun Sy succeeded in detecting prion aggregates in plasma from mice or deer infected with scrapie or CWD respectively [16]. A similar approach has been developed by the Korean company PeopleBio (Seoul, Korea, Multimer Detection System (MDS)) without amplification of signal and target but details remain unpublished.

– The use of fluorescence labelled palindromic PrP peptides to detect misfolded PrP (MPD for misfolded protein diagnostic). In this approach, when the labelled peptide is in contact with $PrP^{Sc}$, it undergoes a large coil to a β-sheet conformational change which largely modifies the fluorescence properties of the pyrene label [30]. This method allowed discrimination between TSE infected and uninfected animals, albeit on a rather small series of blood samples [53].

However, even if some of these approaches seem promising, for the moment none of these tests has fulfilled the very strict analytical and diagnostic requirements described above. With the passage of time (some of these approaches were initially described a few years ago) it becomes apparent that they are facing real difficulties in establishing routine and robust assays, and that much more time and development is needed to achieve the goal of an operational blood test for TSE.

## 4. CONCLUSIONS

The successive "mad cow" crises of 1996 and 2000 have clearly boosted very significantly research in the field of prion diseases, and more data have been accumulated during the last ten years than during the previous century. This has considerably improved our knowledge on prion biology, but also provided much more relevant tools including: transgenic mice ($PrP^{0/0}$ or over-expressing various forms of wild-type or mutated PrP), cellular models of TSE infection, a large series of well characterised monoclonal antibodies and, of course, much more relevant analytical methods and diagnostic tests. As far as diagnosis is concerned, very significant progress has been made in the post-mortem detection of $PrP^{Sc}$, with the development of reliable and very sensitive methods suitable for routine analysis (results available within less than three hours, more than 20 000 tests performed every day throughout the world), having the capacity of diagnosing TSE before the onset of clinical signs. These tests have been used efficiently for managing the mad cow crisis, and are still very useful for monitoring the BSE epizootic as well as the various forms of TSE in small ruminants and cervids. The analytical sensitivity of these tests can now be considerably improved by coupling PMCA amplification with the appropriate detection techniques (ELISA, CDI, Western-blot), and this allows detection of minute amounts of $PrP^{Sc}$ in the brain or in peripheral lymphoid tissues. However, so far, there is no test that delivers an early and specific diagnosis of TSE in live animals or patients, i.e. a test which can be easily applied to a body fluid like blood or urine. This is particularly critical for ensuring the safety of blood transfusion in countries that have experienced a large BSE epizootic (UK and Western Europe). We have seen that PMCA has shown a good potential, in terms of sensitivity, for achieving such an aim but its use in routine conditions and its actual specificity are questionable. There is thus a place for another approach, and the development of a blood test for TSE diagnosis remains the most important challenge for the years coming in this field of prion research.

## REFERENCES

[1] Aksamit A.J. Jr., Preissner C.M., Homburger H.A., Quantitation of 14-3-3 and neuron-specific enolase proteins in CSF in Creutzfeldt-Jakob disease, Neurology (2001) 57:728–730.

[2] Andréoletti O., Berthon P., Marc D., Sarradin P., Grosclaude J., van Keulen L., et al., Early accumulation of PrP(Sc) in gut-associated lymphoid and nervous tissues of susceptible sheep from a Romanov flock with natural scrapie, J. Gen. Virol. (2000) 81:3115–3126.

[3] Arnold M.E., Ryan J.B., Konold T., Simmons M.M., Spencer Y.I., Wear A., et al., Estimating the temporal relationship between PrPSc detection and incubation period in experimental bovine spongiform encephalopathy of cattle, J. Gen. Virol. (2007) 88:3198–3208.

[4] Atarashi R., Moore R.A., Sim V.L., Hughson A.G., Dorward D.W., Onwubiko H.A., et al., Ultrasensitive

detection of scrapie prion protein using seeded conversion of recombinant prion protein, Nat. Methods. (2007) 4:645–650.

[5] Beekes M., Otto M., Wiltfang J., Bahn E., Poser S., Baier M., Late increase of serum S100 beta protein levels in hamsters after oral or intraperitoneal infection with scrapie, J. Infect. Dis. (1999) 180:518–520.

[6] Bendheim P.E., Bolton D.C., A 54-kDa normal cellular protein may be the precursor of the scrapie agent protease-resistant protein, Proc. Natl. Acad. Sci. USA (1986) 83:2214–2218.

[7] Benestad S.L., Sarradin P., Thu B., Schonheit J., Tranulis M.A., Bratberg B., Cases of scrapie with unusual features in Norway and designation of a new type, Nor98, Vet. Rec. (2003) 153:202–208.

[8] Brown P., Blood infectivity, processing and screening tests in transmissible spongiform encephalopathy, Vox Sang. (2005) 89:63–70.

[9] Brown P., Cervenakova L., Diringer H., Blood infectivity and the prospects for a diagnostic screening test in Creutzfeldt-Jakob disease, J. Lab. Clin. Med. (2001) 137:5–13.

[10] Brown P., Will R.G., Bradley R., Asher D.M., Detwiler L., Bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease: background, evolution, and current concerns, Emerging Infect. Dis. (2001) 7:6–16.

[11] Bruce M.E., Will R.G., Ironside J.W., McConnell I., Drummond D., Suttie A., *et al.*, Transmissions to mice indicate that "new variant" CJD is caused by the BSE agent, Nature (1997) 389:498–501.

[12] Calavas D., Ducrot C., Baron T., Morignat E., Vinard J.L., Biacabe A.G., et al., Prevalence of BSE in western France by screening cattle at risk: preliminary results of a pilot study, Vet. Rec. (2001) 149:55–56.

[13] Carmona P., Monleon E., Monzon M., Badiola J.J., Monreal J., Raman analysis of prion protein in blood cell membranes from naturally affected scrapie sheep, Chem. Biol. (2004) 11:759–764.

[14] Castilla J., Saa P., Hetz C., Soto C., In vitro generation of infectious scrapie prions, Cell (2005) 121:195–206.

[15] Castilla J., Saa P., Soto C., Detection of prions in blood, Nat. Med. (2005) 11:982–985.

[16] Chang B., Cheng X., Yin S., Pan T., Zhang H., Wong P., et al., Test for detection of disease-associated prion aggregate in the blood of infected but asymptomatic animals, Clin. Vaccine Immunol. (2007) 14:36–43.

[17] Curin S., V. Bresjanac M., Popovic M., Pretnar H.K., Galvani V., Rupreht R., et al., Monoclonal antibody against a peptide of human prion protein discriminates between Creutzfeldt-Jacob's disease-affected and normal brain tissue, J. Biol. Chem. (2004) 279:3694–3698.

[18] Deleault N.R., Harris B.T., Rees J.R., Supattapone S., From the cover: formation of native prions from minimal components in vitro, Proc. Natl. Acad. Sci. USA (2007) 104:9741–9746.

[19] Deleault N.R., Lucassen R.W., Supattapone S., RNA molecules stimulate prion protein conversion, Nature (2003) 425:717–720.

[20] Deslys J.P., Comoy E., Hawkins S., Simon S., Schimmel H., Wells G., et al., Screening slaughtered cattle for BSE, Nature (2001) 409:476–478.

[21] Eloit M., Adjou K., Coulpier M., Fontaine J.J., Hamel R., Lilin T., et al., BSE agent signatures in a goat, Vet. Rec. (2005) 156:523–524.

[22] Everest D.J., Waterhouse S., Kelly T., Velo-Rego E., Sauer M.J., Effectiveness of capillary electrophoresis fluoroimmunoassay of blood PrpSc for evaluation of scrapie pathogenesis in sheep, J. Vet. Diagn. Invest. (2007) 19:552–557.

[23] Fraser H., The pathology of a natural and experimental scrapie, Front. Biol. (1976) 44:267–305.

[24] Gavier-Widen D., Stack M.J., Baron T., Balachandran A., Simmons M., Diagnosis of transmissible spongiform encephalopathies in animals: a review, J. Vet. Diagn. Invest. (2005) 17:509–527.

[25] Glock B., Winter M., Rennhofer S.O., Brunholzl E., Troscher D., Reisacher R.B., Mayr W.R., Transcript level of erythroid differentiation-related factor, a candidate surrogate marker for transmissible spongiform encephalopathy diseases in blood, shows a broad range of variation in healthy individuals, Transfusion (2003) 43:1706–1710.

[26] Grassi J., Comoy E., Simon S., Creminon C., Frobert Y., Trapmann S., et al., Rapid test for the preclinical postmortem diagnosis of BSE in central nervous system tissue, Vet. Rec. (2001) 149:577–582.

[27] Grassi J., Creminon C., Frobert Y., Fretier P., Turbica I., Rezaei H., et al., Specific determination of the proteinase K-resistant form of the prion protein using two-site immunometric assays. Application to the post-mortem diagnosis of BSE, Arch. Virol. Suppl. (2000) 16:197–205.

[28] Green A.J., Cerebrospinal fluid brain-derived proteins in the diagnosis of Alzheimer's disease and Creutzfeldt-Jakob disease, Neuropathol. Appl. Neurobiol. (2002) 28:427–440.

[29] Green A.J., Jackman R., Marshall T.A., Thompson E.J., Increased S-100b in the cerebrospinal fluid of some cattle with bovine spongiform encephalopathy, Vet. Rec. (1999) 145:107–109.

[30] Grosset A., Moskowitz K., Nelsen C., Pan T., Davidson E., Orser C.S., Rapid presymptomatic detection of PrPSc via conformationally responsive palindromic PrP peptides, Peptides (2005) 26:2193–2200.

[31] Hibler C.P., Wilson K.L., Spraker T.R., Miller M.W., Zink R.R., DeBuse L.L., et al., Field validation and assessment of an enzyme-linked immunosorbent assay for detecting chronic wasting disease in mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), and Rocky Mountain elk (*Cervus elaphus nelsoni*), J. Vet. Diagn. Invest. (2003) 15:311–319.

[32] Hilton D.A., Ghani A.C., Conyers L., Edwards P., McCardle L., Penney M., et al., Accumulation of prion protein in tonsil and appendix: review of tissue samples, BMJ (2002) 325:633–634.

[33] Hilton D.A., Ghani A.C., Conyers L., Edwards P., McCardle L., Ritchie D., et al., Prevalence of lymphoreticular prion protein accumulation in UK tissue samples, J. Pathol. (2004) 203:733–739.

[34] Jackman R., Everest D.J., Schmerr M.J., Khawaja M., Keep P., Docherty J., Evaluation of a preclinical blood test for scrapie in sheep using immunocapillary electrophoresis, J. AOAC Int. (2006) 89:720–727.

[35] Jackman R., Schmerr M.J., Analysis of the performance of antibody capture methods using fluorescent peptides with capillary zone electrophoresis with laser-induced fluorescence, Electrophoresis (2003) 24:892–896.

[36] Jones M., Peden A., Prowse C., Groner A., Manson J., Turner M., et al., In vitro amplification and detection of variant Creutzfeldt-Jakob disease PrP(Sc), J. Pathol. (2007) 213:21–26.

[37] Korth C., Stierli B., Streit P., Moser M., Schaller O., Fischer R., et al., Prion (PrPSc)-specific epitope defined by a monoclonal antibody, Nature (1997) 390:74–77.

[38] Kurt T.D., Perrott M.R., Wilusz C.J., Wilusz J., Supattapone S., Telling G.C., et al., Efficient in vitro amplification of chronic wasting disease PrPres, J. Virol. (2007) 81:9605–9608.

[39] Lasch P., Beekes M., Schmitt J., Naumann D., Detection of preclinical scrapie from serum by infrared spectroscopy and chemometrics, Anal. Bioanal. Chem. (2007) 387:1791–1800.

[40] Lasch P., Schmitt J., Beekes M., Udelhoven T., Eiden M., Fabian H., et al., Antemortem identification of bovine spongiform encephalopathy from serum using infrared spectroscopy, Anal. Chem. (2003) 75:6673–6678.

[41] Llewelyn C.A., Hewitt P.E., Knight R.S., Amar K., Cousens S., Mackenzie J., Will R.G., Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion, Lancet (2004) 363:417–421.

[42] Lourenco P.C., Schmerr M.J., MacGregor I., Will R.G., Ironside J.W., Head M.W., Application of an immunocapillary electrophoresis assay to the detection of abnormal prion protein in brain, spleen and blood specimens from patients with variant Creutzfeldt-Jakob disease, J. Gen. Virol. (2006) 87:3119–3124.

[43] Miele G., Manson J., Clinton M., A novel erythroid-specific marker of transmissible spongiform encephalopathies, Nat. Med. (2001) 7:361–364.

[44] Missler U., Wiesmann M., Wittmann G., Magerkurth O., Hagenstrom H., Measurement of glial fibrillary acidic protein in human blood: analytical method and preliminary clinical results, Clin. Chem. (1999) 45:138–141.

[45] Moussa A., Coleman A.W., Bencsik A., Leclere E., Perret F., Martin A., Perron H., Use of streptomycin for precipitation and detection of proteinase K resistant prion protein (PrPsc) in biological samples, Chem. Commun. (Camb.) (2006) 9:973–975.

[46] Moynagh J., Schimmel H., Tests for BSE evaluated. Bovine spongiform encephalopathy, Nature (1999) 400:105.

[47] Murayama Y., Yoshioka M., Horii H., Takata M., Yokoyama T., Sudo T., et al., Protein misfolding cyclic amplification as a rapid test for assessment of prion inactivation, Biochem. Biophys. Res. Commun. (2006) 348:758–762.

[48] Nazor K.E., Kuhn F., Seward T., Green M., Zwald D., Purro M., et al., Immunodetection of disease-associated mutant PrP, which accelerates disease in GSS transgenic mice, EMBO J. (2005) 24:2472–2480.

[49] Oesch B., Doherr M., Heim D., Fischer K., Egli S., Bolliger S., et al., Application of prionics Western blotting procedure to screen for BSE in cattle regularly slaughtered at Swiss abattoirs, Arch. Virol. Suppl. (2000) 16:189–195.

[50] Otto M., Wiltfang J., Differential diagnosis of neurodegenerative diseases with special emphasis on Creutzfeldt-Jakob disease, Restor. Neurol. Neurosci. (2003) 21:191–209.

[51] Otto M., Wiltfang J., Cepek L., Neumann M., Mollenhauer B., Steinacker P., et al., Tau protein and 14-3-3 protein in the differential diagnosis of Creutzfeldt-Jakob disease, Neurology (2002) 58:192–197.

[52] Pan T., Chang B., Wong P., Li C., Li R., Kang S.C., et al., An aggregation-specific enzyme-linked immunosorbent assay: detection of conformational differences between recombinant PrP protein dimers and PrP(Sc) aggregates, J. Virol. (2005) 79:12355–12364.

[53] Pan T., Sethi J., Nelsen C., Rudolph A., Cervenakova L., Brown P., Orser C.S., Detection of misfolded prion protein in blood with conformationally sensitive peptides, Transfusion (2007) 47:1418–1425.

[54] Paramithiotis E., Pinard M., Lawton T., LaBoissiere S., Leathers V.L., Zou W.Q., et al., A prion protein epitope selective for the pathologically misfolded conformation, Nat. Med. (2003) 9:893–899.

[55] Parveen I., Moorby J., Allison G., Jackman R., The use of non-prion biomarkers for the diagnosis of transmissible spongiform encephalopathies in the live animal, Vet. Res. (2005) 36:665–683.

[56] Peden A.H., Head M.W., Ritchie D.L., Bell J.E., Ironside J.W., Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient, Lancet (2004) 364:527–529.

[57] Prusiner S.B., Prions, Proc. Natl. Acad. Sci. USA (1998) 95:13363–13383.

[58] Prusiner S.B., Scott M.R., DeArmond S.J., Cohen F.E., Prion protein biology, Cell (1998) 93:337–348.

[59] Ruth L., The quest for new prion tests, Anal. Chem. (2003) 75:32A–36A.

[60] Saa P., Castilla J., Soto C., Presymptomatic detection of prions in blood, Science (2006) 313:92–94.

[61] Saa P., Castilla J., Soto C., Ultra-efficient replication of infectious prions by automated protein misfolding cyclic amplification, J. Biol. Chem. (2006) 281:35245–35252.

[62] Saborio G.P., Permanne B., Soto C., Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding, Nature (2001) 411:810–813.