SHORT COMMUNICATION

Specific diagnosis of transmissible spongiform encephalopathies

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Transmissible spongiform encephalopathies (TSE) are a group of lethal neurodegenerative diseases that include bovine spongiform encephalopathy (BSE) in cattle, Creutzfeldt–Jakob disease (CJD) in humans and scrapie in sheep and goats. According to S. Prusiner (4) the major or even the only etiological agent of TSE is pathologically modified prion protein which is partly resistant to the effect of proteases (PrPres). This pathological form of the protein is detected in all cases of human and animal TSEs. Prion protein (PrP) is part of normal cell membrane and it is coded by one gene. The entire open reeding frame (ORF) of PrP gene (PRNP) is encoded in a single exon. PrPres is formed by post-translational conformational modification of the normal PrP protein.

The common characteristics of these diseases includes:

1) Long incubation period – (asymptomatic preclinical phase which can last for ten or more years especially in humans)

2) Spongiform neurodegenerative changes in the brain.

3) Undetectable humoral or cellular immunological response (by available methods).

At present we distinguish 5 human (Kuru, CJD, GSS, FFI and vCJD) and a growing number of animal TSE diseases (Scrapie, Transmissible mink encephalopathy, chronic wasting disease of mule deer and elk, Bovine spongiform encephalopathy, Feline spongiform encephalopathy etc.)

In principle, the diagnosis of TSEs is the same in both humans and animals. Typical clinical signs indicate suspect CJD case. In humans we differentiate diagnosis as:

1. definitive, 2. probable and 3. possible. The typical clinical signs of CJD (the most frequent human TSE) are: progressive dementia, cerebellar, pyramidal and extrapyramidal symptoms (ataxia, myoclonus) as well as typical EEG and MRI (high signal changes in the putamen and caudate head) findings. Characteristic clinical symptoms and typical EEG indicates probable CJD. Patient with clinical symptomps without typical EEG is considered as possible CJD. The clinical diagnosis is verified by specific and some non specific laboratory methods.

There are two non specific laboratory diagnostic tests, which can be performed in vivo:

– Detection of protein 14-3-3 in cerebrospinal fluid (CSF) sample of suspected CJD case. In an attempt to find early diagnostic method, several researchers had tried to find a specific protein marker of CJD in CSF samples (5). Several non specific enolases (protein 130, 131, 14-3-3 and others) had been detected in CSF samples and their specificity and sensitivity of the detection methods were intensively studied. It is now proved that detection of these proteins in the CSF is not specific to TSE. However detection of protein 14-3-3 in CSF with combination to typical clinical findings is a valuable diagnostic tool especially where autopsy can't be performed (Fig. 1).

– Detection of anti-neurofilamentous autoantibodies in sera of suspect CJD cases.

Both methods can give false positive results since both, the autoantibodies and non specific proteins can be found in other neurodegenerative diseases and chronic neuroinfections (Alzheimer disease, herpetic encephalitis). The only way how to confirm definitive diagnosis of CJD in vivo is by detecting PrPres in biopsy brain sample.

Definitive diagnosis of majority of TSEs can only be confirmed post mortem by analysing formalin fixed or frozen autopsy CNS samples.

Non-specific methods:

The oldest, however very reliable non-specific diagnostic method is based on evaluation of neuro-histopathological findings in the CNS. Pathological lesions in the brain are represented by the typical trias : 1. Vacuolar degeneration of neurones and spongiform changes of the neuropil, 2. Astrocytic reaction (hypertrophy and hyperplasy) especially in the grey matter and 3. Neuronal death (mainly apopthotic).

Specific methods:

– The oldest specific TSE diagnosis is based on transmission of the disease to experimental animals. For high expenses and long incubation period it is at present used more for research

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This work was presented at the Meeting of the Slovak Medical Society which took place on May 7, 2001.

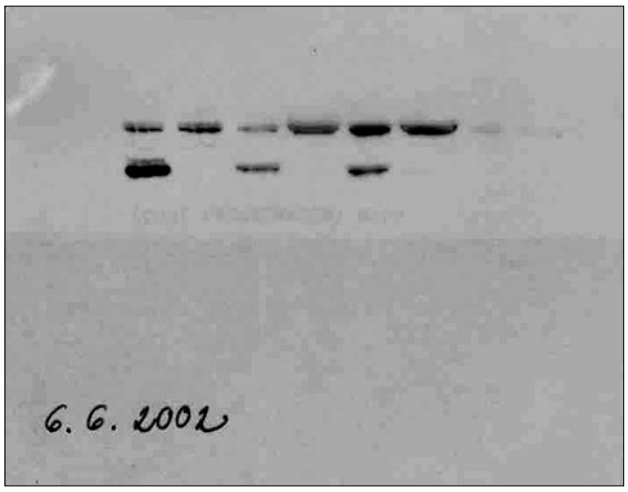

Fig. 1. Detection of protein 14-3-3 by Western blot. Col. 1 – positive control, cols. 3 and 5 shows positive reaction in patients.

than for diagnostic proposes. Transmission of sporadic form of CJD to primates is successful in 90 % cases. In comparison, only 10 % of mice infected with brain homogenate of CJD patient acquire clinically manifest TSE.

Due to an experience that mice are optimal experimental animals, the development of transgenic and knock out mice became a necessity in the study of TSE etiopathogenesis. Thanks to these transgenic mice it is now possible to prepare specific anti-PrP polyclonal and monoclonal antibodies which are applied to develop different highly sensitive and specific diagnostic methods.

– Electron microscopy – detection of scrapie-associated fibrils (SAF) (concentrated and partially purified PrPres protein) isolated from affected CNS tissue is a reliable method to confirm the diagnosis of TSEs (3). The advantage of this method is that the test can be performed even on partly autolyzed tissue. Results are highly specific (there are no false positive findings).

– Methods based on detection of PrPres protein.

Immunohistochemistry:

Direct detection of PrPres protein in formalin fixed and paraffin embedded histological sections of brain or lymphatic tissue (tonsils, appendix) in vCJD. This method can be applied in research and for diagnostic purposes not only in fresh samples but also for retrospective confirmation of TSE in archive material. Besides direct detection of PrPres protein, it enables to localise its distribution in the CNS. It serves as confirmatory test for diagnosis of human and animal TSEs. PrPres protein forms usually irregular granules and/or plaques, scattered or grouped in the brain (2).

Histoblot:

After blotting native brain sample on wet nitro-cellulose membrane and proteinase K digestion, PrPres is detected by specific antibodies. This method is used mainly in experimental studies.

Western blot:

It is a very reliable method of PrPres detection. The advantage of this method is its ability to distinguish among various types of PrPres on the basis of their migration profile on SDS polyacrylamide gel, which can be caused by structural conformational differences or by different degree of glycosylation (1). This method is now adapted to examine large number of samples simultaneously (routine diagnosis of BSE). The main disadvantage of western blot test is that because of a possible false positive result, it should be confirmed by other specific methods.

ELISA method:

A highly sensitive diagnostic method which enable to process large amount of sample with semi-automatic technology, especially in veterinary laboratories (BSE diagnosis).

Molecular genetic methods for analysis of PRNP gene:

More than eighteen various modification (point mutation, polymorphism, deletion and insertion) of the human PRNP gene had been described. Majority of these modification have direct or indirect influence on the susceptibility, risk and duration of TSEs. It is an important examination, used for detection of CJD-specific mutation in suspect TSE patients. It is helpful for in vivo diagnosis of genetic (familial) CJD. Analysis of the PRNP gene helps to select asymptomatic carriers of TSE-specific mutations, i.e., persons at TSE risk and to control animal TSEs, especially scrapie, by selecting resistant breeding animals.

The major priority in TSE research, especially from the aspect of human iatrogenic CJD (transfusion, transplantation, hormonal bio-preparates) and nvCJD should be to develop a highly sensitive and specific in vivo diagnostic method, which enables to determine definitive diagnosis of TSE in pre-clinical stage of the disease.

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Received June 7, 2003.
Accepted October 27, 2003.