A newly developed assay for the presymptomatic detection of prions in blood

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this purpose.[9] It was predicted that PrP226* accumulates in diseased brain in aggregates, from which it can be released using chaotropic salts.

We found that PrP226* indeed accumulates in aggregates during the disease, but is also present naturally in brain in minute amounts. With our ELISA test we were able to follow the distribution of this fragment. PrP226* is most likely to accumulate in cerebellum, followed by cortical regions, but very rarely in spinal cord or olfactory bulb.[10] Comparison of the results for PrP226* and PrP^{Sc} revealed that the distribution of both proteins correlates well, and strongly indicates that PrP226* is part of PrP^{Sc} aggregates (Lukan and Černilec, et al. under revision).

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P.224: Evaluation of assays intended for the diagnosis of variant CJD

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Keywords: variant CJD, diagnostic test, evaluation

Tests intended for the diagnosis of potentially life threatening disorders are listed in Annex IIA of the EU directive and have associated minimum requirements outlined in a common technical specification. Variant CJD diagnostic tests were added to Annex IIA in 2011.

Typically tests are evaluated for sensitivity using a large number of clinically relevant samples, for vCJD this is not possible due to the small number of clinical cases and the very limited number of relevant samples. To help ensure fair and appropriate access to the small number of rare samples held at the UK CJD Resource Centre its oversight committee have developed a process to assess sensitivity and specificity (http://www.nibsc.org/Spotlight/CJD_Resource_Centre/CJD_Tests.aspx).

This process involves analysis of 1. vCJD tissue homogenates spiked into the relevant blood component (to determine analytical sensitivity) 2. Blood components from experimentally or naturally infected animals (diagnostic sensitivity) 3. Blood components from normal donor samples (for diagnostic specificity).

Each stage of the evaluation process is reviewed by the oversight committee and if minimum requirements are met samples from vCJD clinical cases are provided for testing.

Two test developers have completed the evaluation process and tested blood components from clinical cases of vCJD held at the UK CJD Resource Centre.[1]

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P.225: A newly developed assay for the presymptomatic detection of prions in blood

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Introduction. Prion diseases or Transmissible Spongiform Encephalopathies (TSEs) are neurodegenerative diseases including variant Creutzfeldt-Jakob disease (vCJD) in humans. The central event of these diseases would be the conformational change of a normal cellular protein PrP^{C} into an infectious form PrP^{TSE}. It is now evident that TSEs are transmissible by blood transfusion and this has raised concerns that a reservoir of infectious asymptomatic people could exist in the blood donor population. A recent prevalence study based on stored lymphoreticular samples analyses led to en estimation of 1 in 2000 persons being potentially infected by vCJD prions in the United Kingdom. Until now, no screening test could detect the infectious agent in human blood before the onset of clinical signs of disease.

The objective of this study is to develop a sensitive and specific test that would enable the detection of PrP^{TSE} in the blood during the presymptomatic phase of TSE.

Materials and Methods. The detection assay comprises three major steps: (1) a ligand-coated bead pre-analytical step in order

to concentrate PrP^{TSE} from the different blood components and to remove inhibitory factors which can interfere with the amplification; (2) a PrP^{TSE} amplification by serial PMCA using transgenic mouse brain homogenate as substrate and (3) a specific detection of the amplified PrP^{TSE} by immuno-blotting after partial proteinase K digestion. The sample volume has been optimized for 500 µL of plasma and for 25 to 50 µL of buffy-coat. Whole blood samples from infected sheep collected during preclinical and clinical phases of scrapie were processed in buffy-coat, white blood cells (WBC) and plasma. Blood from humanised transgenic mice infected by vCJD was also tested in relation with the duration post inoculation.

Results. PMCA assay allowed detection of PrP^{TSE} in: (1) the WBC of four sheep at the acute phase of scrapie with a 100% sensitivity and specificity, (2) in the plasma and buffy coat collected in the asymptomatic phase of the disease (3) in the blood of vCJD-infected transgenic mice before the occurrence of clinical signs.

Conclusions. The expected level of sensitivity for the detection of prion in the blood was reached. This assay is currently evaluated as a confirmatory detection test for the presence of the vCJD agent in human blood. The next step will be to perform prevalence studies by analysing panels of at-risk populations.

P.226: Neurodegeneration and neurogenesis in the gastrointestinal tract of prion infected mice

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The presence of disease associated PrP^{Sc} in the gastrointestinal tract of patients affected by prion diseases represents a source of disease transmission through surgical procedures and may contribute to symptoms affecting their quality of life.

Following intracerebral inoculation of wildtype mice with prions, PrP^{Sc} was detected in the gastrointestinal tract during symptomatic disease (20 weeks post infection). PrP^{Sc} accumulation in the ileum was associated with high levels of infectivity, caspase activation, loss of neuronal subpopulations in the myenteric plexus and the loss of enteric glial cell integrity.[1, 2] In the current study a time course analysis indicated that PrP^{Sc} accumulation was detected in the ileum within 3 weeks of intracerebral inoculation, with highest levels detected 6-10 weeks post-inoculation, at which time mice demonstrated no neurological symptoms of disease. There was a quantitative decrease in PrP^{Sc} load 13 weeks post infection suggestive of a shedding of PrP^{Sc} from the gastrointestinal tract and a potential source of environmental contamination.

A significant loss of neurofilament (medium) immunoreactive neurons, reflective of intrinsic sensory neurons, was detected 10 weeks post inoculation and coincident with high PrP^{Sc} loads. This was followed by the apparent recovery of this neuronal population between weeks 13 and 17 post-inoculation and coincident with reduced PrP^{Sc} loads. These results suggest that the accumulation of PrP^{Sc} may directly contribute to the loss of this neuronal population and that a reduction in PrP^{Sc} burden enabled its regeneration.

We had previously observed the qualitative loss of neuronal nitric oxide synthase (nNOS) immunoreactive neurons, a marker of inhibitory motor neurons, in conjunction with regions of distorted glial fibrillary acidic protein (GFAP) immunoreactivity, indicative of enteric glial cell derangement at the terminal stages of disease. We now report a significant decrease in nNOS immunoreactive neurons between 10 and 17-week post inoculation and evidence of enteric glial cell derangement as early as 6 weeks post inoculation. These results support the view that glial cell dysfunction in the gastrointestinal tract of prion affected animals precedes and contributes to the loss of nNOS immunoreactive neurons.

This study has identified PrP^{Sc} accumulation and glial cell dysfunction as mediators of prion induced neurodegeneration of specific neuronal subpopulations within the gastrointestinal tract and the potential for regeneration of neuronal populations following a reduction in PrP^{Sc} load. The effect of neuronal loss on gastrointestinal dysfunction will be presented.

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P.227: Selective binding of high molecular mass assemblies of amyloid β-peptide to prion protein in patients with Alzheimer's disease

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In Alzheimer's disease the generation of oligomeric species of amyloid β-peptide is causal to disease initiation and progression. Oligomeric species of amyloid β-peptide bind to the N-terminus of plasma membrane-bound cellular prion protein (PrP^{C}). This binding may be associated to synaptic degeneration. Composition of bound amyloid β-peptide oligomers, binding domains within PrP^{C} and modifiers of this binding have mostly been studied in cell culture or murine models of Alzheimer's disease. Our