Research Letters

Diagnosis of new variant Creutzfeldt-Jakob disease by tonsil biopsy

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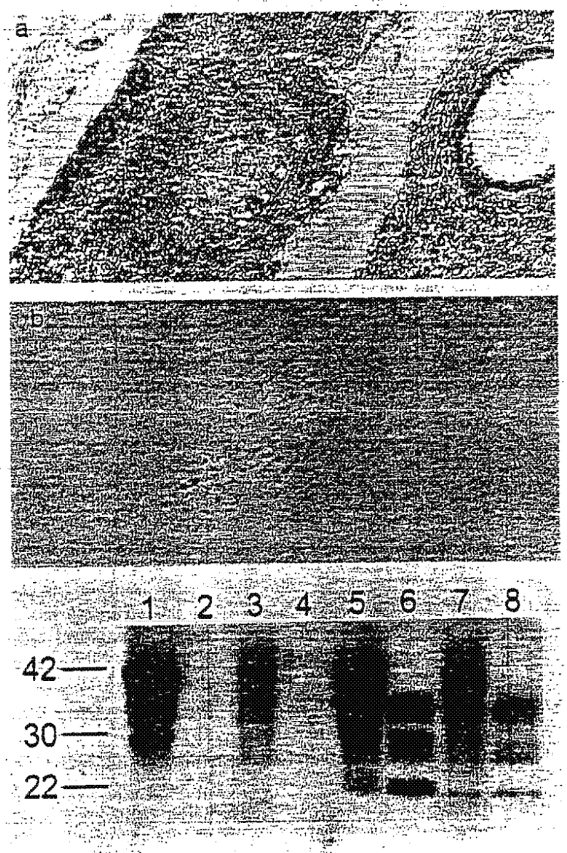
The diagnosis of Creutzfeldt-Jakob disease (CJD) can only be confirmed by brain biopsy or at necropsy, although a rapidly progressive dementia, myoclonus, other neurological signs, and a characteristic electroencephalogram allows confident ante-mortem diagnosis in typical cases, albeit at a relatively advanced clinical stage. A new clinicopathological type of CJD, new variant CJD (nvCJD) has been reported in the UK and putatively linked on epidemiological grounds with dietary exposure to bovine spongiform encephalopathy (BSE).[1] Evidence in support of a causal link between BSE and nvCJD is provided by experimental transmission of BSE to macaques, revealing neuropathological similarity to nvCJD, and by the demonstration that nvCJD is associated with a molecular marker that distinguishes it from other forms of CJD and which resembles that seen in BSE and transmitted BSE in a number of other species.[2] To date, 14 nvCJD cases have been neuropathologically confirmed in the UK and one in France. It is at present unclear how many further cases of nvCJD are likely to appear. In nvCJD, confident diagnosis depends on neuropathology, either from a brain biopsy or at necropsy because the clinical course is atypical and the characteristic electroencephalogram of CJD is absent.[1] Furthermore, brain biopsy carries a notable morbidity and may give a falsely negative result if the area of brain sampled is unaffected or lacks the specific pathological features of nvCJD.[1] If nvCJD is caused by BSE, and an epidemic ensures, early diagnostic markers will be essential for differential diagnosis as the presenting clinical features (depression and sensory disturbance) are non-specific and many may be concerned that they have developed this condition.

Recently, we reported that nvCJD is associated with a specific pattern of protease-resistant prion protein (PrP) on Western blot analysis.[2] This marker can already be used to aid differential diagnosis on brain biopsy samples.[3] However, since PrP is widely expressed outside the central nervous system, we investigated whether an alternative and more accessible tissue might be biopsied to allow a diagnosis of nvCJD before death and to avoid brain biopsy. PrP is expressed in the lymphoreticular system and prion replication is known to occur in the spleen and other lymphoreticular tissues in experimental rodent scrapie models;[3] prion infectivity has also been reported in human lymphoreticular tissues.[4]

We have studied PrP in tonsillar tissue obtained at necropsy using both immunohistochemistry on periodate-lysine-paraformaldehyde and formalin-fixed tissue, and Western blot analysis of frozen tissue. The patient was a 35-year-old woman who died after a 14-month illness with depression at onset followed by ataxia, hyper-reflexia, memory loss and dementia; a diagnosis of nvCJD was made by neuropathology. Abnormal PrP staining was present within tonsillar germinal centres (figure, top a and b). Western blot analysis revealed the presence of protease resistant PrP (figure, bottom); confirming the diagnosis of prion disease. Furthermore, the sizes and intensity ratios of the three PrP bands (representing diglycosylated, monoglycosylated, and unglycosylated PrP) were similar to those seen in brain from the same patient (designated a type 4 pattern[2]) suggesting that it may be possible to make the specific diagnosis of nvCJD by this technique. However, tonsillar tissues from types 1–3 CJD[2] were not available for comparison. Such study of protease-resistant PrP in lymphoreticular tissue may be particularly relevant in iatrogenic CJD after peripheral prion inoculation (for instance after treatment with human cadaveric pituitary-derived growth hormone) where lymphoreticular involvement would be expected.

In experimental murine and sheep scrapie, prion replication occurs initially in spleen and is only detectable in the central nervous system considerably later in the incubation period. Abnormal PrP immunostaining has been



Top: photomicrographs of tonsil
(a) tonsillar tissue germinal centre in sub-epithelial lymphoid tissue; (b) serial section following hydrolytic autoclaving using anti-PrP monoclonal antibody 3F4 shows strong staining of cells within the germinal centre which has the morphology of follicular dendritic cells. Tissue fixation was with periodate-lysine-paraformaldehyde. A similar staining pattern but with reduced intensity was present in formalin-fixed tissue. No reaction for PrP was seen in two age-matched control cases dying from other neurological illnesses.

Bottom: Western blot of tissue homogenates with anti-PrP monoclonal antibody 3F4
Horizontal lines indicate positions of molecular mass markers (in kilodaltons). Odd numbered lanes are before, and even numbers after, treatment with proteinase K. Lanes 1 and 2: normal human brain; lanes 3 and 4: normal human tonsil; lanes 5 and 6: brain from patient with nvCJD; lanes 7 and 8: tonsil from patient with nvCJD.

reported in tonsils of experimental scrapie-infected sheep long before the occurrence of clinical signs.[1] It is possible therefore that Western blot analysis of human tonsil material may allow early clinical, or possibly pre-clinical, diagnosis of CJD and nvCJD although extensive prospective studies of suspected cases will be necessary to assess the clinical usefulness of this investigation. Tonsil tissue can be easily obtained by biopsy under local anaesthetic in most patients and complications are most unusual. Although many adults will have had childhood tonsillectomy, lymphoreticular tissue may still be obtained from the lingual tonsillar remnants. As a result of the extreme resistance of prions to normal sterilisation procedures, current UK recommendations are that neurosurgical instruments from patients with CJD are destroyed. While infective titres of prions in tonsil may be much lower than in brain, similar precautions to avoid cross contamination and iatrogenic transmission of CJD would seem prudent at present. The development of a disposable tonsil-biopsy kit should be feasible.

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Absence of rigor mortis in Indian childhood cirrhosis

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Indian childhood cirrhosis (ICC) is an enigmatic liver disease which mainly affects rural Indian children under the age of 3 years. Around 1000 BC, Sushruta documented in his Samhita an ICC-like syndrome "Mukhamandika Graha": "The child has a yellow complexion, oedema over the face and limbs, network of veins on the abdomen, voracious appetite, urine-like smell of the body, fever, and dyspepsia."[1] Since its earliest description by Sen in 1887,[2] its aetiology has remained unknown.

During an epidemiological field study of ICC, an intriguing observation was made by a social worker who organised funeral ceremonies for bereaved families. He remarked on the total absence of body stiffness in children who died from ICC. A study was undertaken to validate this observation.

Parents of 32 patients who died from ICC between the ages of 10 months and 2 years in whom the funeral was delayed 12 hours were interviewed about postmortem stiffness in their children. Information was sought from social workers and health personnel about children with ICC and those dying of other unrelated causes. Observations of rigor mortis in five ICC patients in hospital were recorded. Visual impressions were confirmed by attempting passive flexion-extension movements at various joints including the spine (figure).

Parents and social workers reported absence of body stiffness in all the 32 patients for a minimum of 12 h to a maximum of 30 h postmortem. Children who died from other causes developed body stiffness within 4 to 6 hours. In five hospital patients, the authors observed absence of rigor mortis for from 22 to 36 h after death. Absence of rigor mortis in children dying of ICC has not been referred to in the many publications on ICC and on other liver diseases in children (Medline search to August 1996).

Photograph of child 36 h after death
There is no evidence of rigor mortis and the joints are mobile.

Absence of rigor mortis has been reported in obese patients and in fetuses less than 7 months old. A 66% fall in adenosine triphosphate (ATP) concentration in muscles initiates muscle contraction and precipitates rigor mortis.[3] Energy for resynthesis of ATP is derived from phosphocreatine with high-energy phosphate bonds; from carbrohydrates, fats, and proteins; as well as from breakdown of glucose and glycogen. Clinical features of ICC include the precirrhotic symptom complex, with irritability, disturbed sleep, sticky stools, and increased appetite with excessive glycogen storage in the liver.[4] There is no evidence for increased glycogen storage at other sites, but we consider that there probably is in skeletal muscles. Excess glycogen in muscles would facilitate resynthesis of ATP and so delay rigor. There may be long delay in the onset of rigor in well-fed animals with increased muscle glycogen.[5]

It would be interesting, to look at glycogen content of muscle biopsy specimens in patients with ICC before and after death and to see if rigor mortis develops in patients who died from glycogen-storage diseases.

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