seen in normal tonsil and is attributable to weak cross reactivity of the secondary antibody with an immunoglobulin fragment. While this band is consistently observed after high sensitivity enhanced chemiluminescence of total lymphoreticular homogenate, it is not recovered after sodium phosphotungstic acid precipitation. We determined that the level of PrP^{Sc} present in the brain of the vCJD patient with PrP^{Sc} positive appendix patient is approximately 15-fold lower than the maximum level we have observed in vCJD brain[3] (fig 1D). Based upon these findings we estimate that biopsy tonsil and appendix, contain levels of PrP^{Sc} of about 4% and about 0.5%, respectively, of that found in the brain of the same vCJD patient (see legend to fig 1).

Importantly, we were able to correlate the detection of PrP^{Sc} by western blotting in vCJD appendix with the detection of abnormal prion protein staining by immunohistochemistry. Abnormal prion protein deposits were clearly observed on sections from the PrP^{Sc} positive vCJD appendix (fig 1E), while prion protein immunoreactivity was unremarkable on sections from the PrP^{Sc} negative vCJD appendix or on sections of appendix from the sporadic CJD or inherited prion disease cases (data not shown).

Discussion

Our findings, together with our previously reported inability to detect PrP^{Sc} in two other vCJD appendixes,[3] indicate that appendix does not reliably report vCJD infection even at the end stage of the disease. This observation must be considered when estimating the possible prevalence of vCJD based upon the analysis of archival appendicectomy tissues.[5] Although only a minority of appendixes in vCJD may contain detectable levels of PrP^{Sc}, surgical instruments used for appendicectomy should remain a cause of concern for potential iatrogenic transmission of vCJD prions.

S Joiner, J Linehan, S Brandner, J D F Wadsworth, J Collinge
MRC Prion Unit and Department of Neurodegenerative Disease, Institute of Neurology, University College, Queen Square, London WC1N 3BG, UK

Competing interests: none declared

Correspondence to: Professor John Collinge; j.collinge@prion.ucl.ac.uk

References

1 **Collinge J**. Variant Creutzfeldt-Jakob disease. *Lancet* 1999;**354**:317–23.
2 **Hill AF**, Butterworth RJ, Joiner S, *et al*. Investigation of variant Creutzfeldt-Jakob disease and other human prion diseases with tonsil biopsy samples. *Lancet* 1999;**353**:183–9.
3 **Wadsworth JDF**, Joiner S, Hill AF, *et al*. Tissue distribution of protease resistant prion protein in variant CJD using a highly sensitive immuno-blotting assay. *Lancet* 2001;**358**:171–80
4 **Hilton DA**, Fathers E, Edwards P, *et al*. Prion immunoreactivity in the appendix before clinical onset of new variant Creutzfeldt-Jakob disease. *Lancet* 1998;**352**:703–4.
5 **Ironside JW**, Hilton DA, Ghani A, *et al*. Retrospecive study of prion-protein accumulation in tonsil and appendix tissues. *Lancet* 2001;**355**:1693–9.

Cytokine profiles in HIV seropositive patients with tuberculous meningitis

The immunological response in pulmonary and pleural tuberculosis has been extensively studied. However, the response in tuberculous meningitis has not been well documented.[1] In pulmonary disease, exposure to tuberculous antigens results in a T cell and natural killer cellular response, elaborating various cytokines, mainly of T helper type 1 (Th1) origin. Stimulated macrophages elaborate tumour necrosis factor (TNF) α, interleukin (IL) 12, and IL 1, promoting further recruitment and activation of macrophages and lymphocytes.

TNF α correlates with disease severity and may contribute to tissue necrosis; however, TNFα has also contributed to survival in mouse studies.[2] Transforming growth factor β (Th3 cytokine) suppresses macrophage activation. IL 2 may be beneficial in promoting an immune response in HIV seropositive patients. Th1 and Th2 cytokine responses have been observed in cerebrospinal fluid (CSF) of HIV seronegative patients with tuberculous meningitis.[3 4]. Whether the response is similar in HIV seropositive patients with tuberculous meningitis is unknown.

We studied the cytokine response and its correlation with disease severity in HIV seropositive and HIV seronegative patients with tuberculous meningitis.

Tuberculous meningitis was diagnosed on clinical and CSF examination after exclusion of viral, acute bacterial, and other causes of aseptic meningitis. Disease severity was assessed according to the Medical Research Council stages 1 to 3. HIV ELISA was done on all patients. CSF samples were subjected to microscopy, culture, protein and glucose analysis, Venereal Disease Research Laboratory test, fluorescent treponemal antibody analysis, cryptococcal antigen analysis, viral studies, cysticercus ELISA, CD4 counts, and determination of concentrations of adenosine deaminase (ADA), CSF IgG, and albumin.

For cytokine assays, CSF was centrifuged at 3000 *g*, and supernatant was aliquoted and stored at –70°C. TNF α, interferon (IFN) γ, and IL 10 concentrations were measured by ELISA kits (Genzyme Diagnostics, Cambridge, Massachusetts, USA) with detection limits of 3 pg/ml, 3 pg/ml, and 5 pg/ml, respectively.

Data were summarised as medians and ranges. Non-parametric Wilcoxon rank sum tests were used to compare HIV seropositive groups with HIV seronegative groups, tuberculous meningitis severity groups, and groups derived according to the blood brain barrier index for cytokine concentrations. Spearman's rank correlation was used to derive correlations of cytokine concentration, ADA concentrations, and CD4 counts in CSF.

There were 27 patients: 18 (67%) women and 9 (33%) men. Seventeen were HIV seropositive and 10 HIV seronegative. The average interval between onset of symptoms and the first clinical assessment was 17 days (range 5–90 days) in 18 patients where this was recorded. The mean (SD) age was 26.8 (11.6) years. There was one patient aged 10 and one aged 60, and the rest were between 25 and 40. The cytokine concentrations were not analysed according to age, as this would make the categories too small and of little value. The IgG index was calculated for 23 patients. There was no significant difference between the HIV seropositive and HIV seronegative groups for ADA ($p = 0.4$) and CD4 counts ($p = 0.19$) in CSF and cytokine concentrations (table 1).

Ten patients (37%) were classified as having grade 1 tuberculous meningitis. Sixteen (59%) had grade 2 and one (4%) grade 3, which for analysis was considered to be grade 2. Table 1 summarises the cytokine concentrations for patients in stages 1 and 2.

Patients with stage 2 disease had significantly stronger Th1 responses. There was no difference in the IL 10 concentrations. The two patients with stage 2 disease who died had very high IFN γ concentrations, both greater than 2048 pg/ml.

IL 10 concentrations were moderately positively correlated with IFN γ concentrations ($r = 0.53$). The correlation coefficients were –0.18 for IFN γ, –0.33 for TNF α, and –0.34 for IL 10. Correlation coefficients between ADA and cytokine concentrations were 0.34 for IFN γ, 0.47 for TNF α, and 0.22 for IL 10. Cytokine concentrations correlated poorly with CD4 counts in CSF.

It is postulated that in HIV infection a predominant Th2 response accounts for extrapulmonary disease.[5] This study does not favour a predominance of either Th1 or Th2 in the CSF. It is possible that a Th0 response, which is a non-differentiated response seen early on in immune activation, was seen in our patients, as they were examined untreated and relatively early in the disease. Other investigators have also documented this phenomenon.[3] The positive correlation between IFN γ and IL 10 suggests that these were produced concurrently. This may reflect a control mechanism regulating Th1 and Th2 responses.

There was no difference in cytokine and ADA concentrations and CD4 counts between HIV seropositive and HIV seronegative patients. It is known that the clinical response to antituberculous treatment in both groups is similar.[5] Perhaps this similarity correlates with similar immune responses in both groups. The size of each group is small and a type 1 statistical error has to be considered.

Table 1 Differences between HIV seropositive and HIV seronegative groups and tuberculous meningitis severity

	HIV positive		HIV negative			Stage 1		Stage 2		
Cytokine	Median	Range	Median	Range	p Value	Median	Range	Median	Range	p Value
IFN γ (pg/ml)	569.9	16.0–2048	890.6	0–2048	0.9	184.5	0–1771.0	1000.0	16.0–2048	0.03
TNF α (pg/ml)	1.6	0–67.5	9.8	0–309.3	0.11	0.65	0–19.2	9.8	0–309.3	0.008
IL 10 (pg/ml)	24.6	0–127.9	17.3	0–296.3	0.9	3.68	0–53.0	27.4	0–296.4	0.97

IFN, interferon; Il, interleukin; TNF, tumour necrosis factor.

Further studies to confirm our findings would be of value.

The significantly greater TNF α and IFN γ concentrations in the severe group of tuberculous meningitis is confirmed by other studies[6] and suggests that disease severity results mainly from the immune response rather than the organism itself.

The lack of correlation between CD4 and cytokine concentrations may be explained by the fact that there are other sources of cytokines in the CSF, namely macrophages and natural killer cells. Concentrations of ADA, which are derived from lymphocytes, are consistent with other reports, where they were correlated with cytokine concentrations.

There was no correlation between the IgG index and cytokine concentrations, suggesting that the blood brain barrier did not significantly influence concentrations. Unfortunately, corresponding serum concentrations were not available. This would have been valuable. This is the first study correlating CSF cytokine responses to severity of tuberculous meningitis and comparing HIV positive with HIV negative groups. Further studies should be done to confirm these findings, perhaps to define their relevance to complications and to explore the possibility of IL 2 treatment in HIV positive patients.

Acknowledgements

This study was sponsored by the Glaxo TB initiative.

VB Patel, AI Bhigjee, PLA Bill
Division of Neurology, Nelson R Mandela School of Medicine, University of Natal, Durban, South Africa

CA Connolly
Biostatistics, Medical Research Council, Durban, South Africa

Correspondence to: Dr VB Patel, Department of Neurology, Ward A3, Wentworth Hospital, Private Bag Jacobs, Durban 4026, South Africa; patelv@nu.ac.za

References

1 **Barnes PF**, Modlen RL, Milner J. *T-cell responses and cytokines in tuberculosis, pathogenesis, protection and control*. Washington: BR Bloom American Press, 1994:417–35.
2 **Flynn JL**, Goldstein MM, Chan J, *et al*. Tumor necrosis factor-alpha is required in the protective immune response against *Mycobacterium tuberculosis* in mice. *Immunity* 1995;**2**:561–72.
3 **Donald PR**, Schoeman JF, Beyers N, *et al*. Concentration of IFN-γ, TNF-α and IL1-β in CSF of children treated for TBM. *Clin Infect Dis* 1995;**21**:924–9.
4 **Havlir DV**, Barnes PF. Tuberculosis in patients with human immunodeficiency virus infection. *N Engl J Med* 1999;**340**:367–73.
5 **Berger JR**. Tuberculous meningitis. *Curr Opin Neurol Neurosurg* 1994;**7**:191–200.
6 **Thwaites G**, Chau TTH, Mai NTH, *et al*. Tuberculous meningitis. *J Neurol Neurosurg Psychiatry* 2000;**68**:289–99.

Festination as the leading symptom of late onset idiopathic aqueductal stenosis

Late onset idiopathic aqueductal stenosis (IAS) may become manifest clinically either by headaches or by hydrocephalic symptoms such as gait disturbance, urinary urge, and cognitive impairment.[1] Rarely, patients with IAS may also present with parkinsonism following repeated episodes of shunt failure.[2 3] Although the gait disorder of IAS has not been fully characterised, it shares similar features with that of normal pressure hydrocephalus of the elderly. Here, we report on two patients who presented with festination as the leading symptom of IAS.

Case histories

Case 1

A 59 year old man had a seven year history of gait disturbance. During the months before admission, he became more unsteady and he was not able to walk without assistance because of pronounced hastening of his steps. He fell frequently. For several months, no diagnosis was made and his gait disorder was considered possibly to be psychogenic. Only after imaging studies showed pronounced triventricular hydrocephalus was he referred for further evaluation and treatment. On admission, he also reported occasional nocturnal urinary urge and incontinence. The most remarkable finding of his physical examination was his gait disorder. He was able to walk without falling only when holding on to a handrail or to the wall. When he walked freely, his stride length became successively shorter and step height decreased, while his walking speed increased. He was unable to slow his walking speed or to stop abruptly. He then would bend his upper body forward and fall if he could not hold on to a wall or to an object. He could stand without support. On testing of propulsion and retropulsion he had mild postural instability but he recovered unaided. There was no gait ignition failure or freezing when passing through obstacles. Arm swing was preserved when walking. He took several extra steps on turning. There was mild bilateral bradykinesia of his upper extremities but no tremor or rigidity. Otherwise, the neurological examination was unremarkable. He scored 29 of 30 points on the mini-mental state examination. Magnetic resonance imaging including high resolution sagittal constructive interference in steady state (CISS) sequences showed aqueductal stenosis. Lumbar puncture was performed and 40 ml cerebrospinal fluid was drained. One day later, there was mild improvement of the gait disorder. Subsequently, the patient underwent endoscopic third ventriculostomy. The operative procedure and the postoperative course were unremarkable. Within a few days after surgery, further improvement of the gait disturbance was notable. At follow up four months after surgery, festinating gait had completely resolved and the patient could walk freely without assistance.

Case 2

An 81 year old woman who had previously been well presented with a one year history of weakness and unsteadiness of the legs along with several falls. At the time of admission she was unable to walk or transfer herself independently and had a persistent fear of falling. There had been some urgency of micturition and urinary incontinence, since on occasions she could not reach the toilet in time. The major neurological abnormality was a difficulty in maintaining the erect posture and even walking with the assistance of a Zimmer frame. There was a stooped, flexed posture and festinating gait with short steps. Neurological examination was unremarkable except for diminished light touch sensation in a glove and stocking distribution in the hands and feet. In particular there was no rigidity or tremor, paresis, or impairment of joint position sense. The Middlesex elderly assessment of mental state and Wechsler memory scales showed no significant abnormality. Magnetic resonance imaging showed a lateral and third ventricular hydrocephalus with small aqueduct and normal fourth ventricle. Serial lumbar punctures, which showed pressures from 10–14 cm H_2O, had no beneficial or adverse effects. Late onset IAS was diagnosed. The patient underwent ventriculoperitoneal shunting with a medium pressure valve and an antisiphon device. Postoperatively she made a slow but steady recovery. Two months after shunting her postural stability and balance had improved considerably. She no longer walked with a stooped posture and her speed of walking had improved significantly. She is now able to walk around the house with the assistance of a cane.

Discussion

Festination was the leading symptom of late onset IAS in both patients reported here. Lack of awareness of this association may cause diagnostic difficulties and may result in delayed treatment. This is important, in particular with regard to the observation that cerebrospinal fluid diversion either by third ventriculostomy or by shunting may result in pronounced amelioration or resolution of this peculiar gait disturbance. Hydrocephalic gait usually is characterised by a decreased walking speed, stride length, stride frequency, step height, and foot floor clearance.[4] The expression "magnetic gait" fairly well depicts its typical clinical features. The gait may also adopt a shuffling appearance resembling somewhat a parkinsonian gait disorder. It may be classified as both a middle level and a higher level gait disorder according to its clinical presentation and biomechanical evaluations.[5 6] Clinical aspects of hydrocephalic gait vary widely depending on the progression of the underlying condition. Gait ignition failure and freezing may occur in as many as 30–50% of patients with idiopathic normal pressure hydrocephalus.[7 8] Festination, however, is seen only rarely in hydrocephalic patients but when it occurs it is generally associated with more severe hydrocephalic symptoms and a clinical picture of parkinsonism. It is not fully understood how hydrocephalic disorders induce gait disturbance and parkinsonian symptoms.[9] The underlying pathomechanisms may include mechanical distension of fibres of the corticospinal tract and of dopaminergic pathways but also disturbed supraspinal control mechanisms of gait secondary to compromised autoregulation and blood flow in the periventricular and deep white matter. Festination, as well as freezing, cannot be explained by specific neurological abnormalities.[10] Festination is seen most frequently in more advanced stages of Parkinson's disease.[11] In patients with Parkinson's disease, there was no association between significant postural reflex abnormalities and festination but a significant association was found between festination and freezing as reported in the activities of daily living part of the unified Parkinson's disease rating scale. Festination is considered to be a clinical feature of a subcortical hypokinetic gait disorder. Festination in the presented cases of IAS may have been related more specifically to distension of the third ventricle. The anatomical substrates of festination have not been elucidated but it is most likely related to functional disturbance of diencephalic or brainstem locomotor centres. This assumption would also explain the reversibility of festination after third ventriculostomy and shunting. In summary, this is the first report of the occurrence of festination as the leading symptom of IAS. As shown, festination may be misinterpreted and be considered psychogenic in an elderly patient who otherwise is suffering only mild hydrocephalic symptoms.