antinuclear antibodies were normal or negative. Sixty eight patients with other neurological diseases and 43 normal subjects were tested as controls. Serum samples obtained during the past year were assayed "blind" for antibodies to ganglioside GM1, GD1b, GD1a, and asialic-GM1 by enzyme linked immunoabsorbent assay (ELISA) according to a modification of the method described by Nobile-Oracio *et al.*[3] Briefly, serum was added to microwells coated with 1% bovine serum albumin (BSA) or ganglioside. Reaction products were visualised with *o*-pheylenediamine as substrate and optical density was read spectrophotometrically in an ELISA reader (Titertek Multiskan plus) at 490 nm. Serum samples were tested in duplicate or three occasions, with a positive sample (a gift from Dr Nobile-Orazio) tested as control in each plate. Results were calculated as the absorbance obtained from the well coated with ganglioside minus the absorbance from the BSA coated well. Samples were considered positive when this difference exceeded 0·1. Antibody titres were considered increased when they were higher than 3 SD from the mean of the results from the 43 normal controls. High titres were confirmed by high performance thin layer chromatography according to the method described by Ilyas *et al.*[4] Positive samples were also studied by immunostaining after absorption in nitrocellulose by a method developed in our laboratory.

Antiganglioside antibodies were found in four patients with toxic oil syndrome. Three (25%) had raised titres of IgM antibodies to ganglioside GD1a (1:800 to 1:400) and one to asialic-GM1 (1:200). Low titres (lower than 3 SD of the controls) of IgM antibodies against GM1, GD1b, and Asialic-GM1 were detected in the GD1a positive patients. Two of these patients had positive titres of IgG anti-GD1a antibodies. In 111 controls tested, two cases of positive IgM antibodies to GD1a were found, one patient with optical neuritis and one with spinal haemorrhage. Reactivity to GD1a was not detected in 43 controls. Anti-GD1a IgM antibodies were significantly higher in patients with toxic oil syndrome than in control groups (ANOVA $F_{2\ 121} = 5{\cdot}71$; $P = 0{\cdot}004$). There was no association between the pattern of clinical signs including proximal *v* distal, motor *v* sensorimotor, and CNS involvement and the positivity of anti-GD1a antibodies. Reactivity showed no significant relation with severity of disease at presentation (Kruskall-Wallis test $\chi = 5{\cdot}02$; $P = 0{\cdot}1$), course (Mann-Whitney U test; $P = 0{\cdot}6$), or age (Spearman coefficient $-0{\cdot}19$; $P = 0{\cdot}53$). Neurophysiological studies showed axonal neuropathy in 10 patients and axonal demyelination in the other two. Nerve conduction blocks were not seen, although a specific search for blocks with proximal evaluation was not carried out.

In this sample of patients with toxic oil syndrome the main finding was the presence of high titres of anti-GD1a antibodies in three. These had no relation with the clinical features evaluated in this small sample. Diverse immunological disorders have been reported in patients and animal models of toxic oil syndrome, such as increased serum IgE or IgM, positivity of antinuclear antibodies, and of several tissue specific antibodies against glomerular basement membrane or collagen, but there are no previous reports of antiganglioside antibody reactivity in this syndrome. Data from the medical literature point to a chronic T lymphocyte activation in toxic oil and eosinophilia-myalgia syndromes. Chronic inflammation could facilitate antigen exposure that induces an immune response to neural ganglioside. Although increased titres of antiganglioside antibodies are associated with lower motor neuron diseases and predominantly motor neuropathy,[5] their role is not understood. The relevance of this reactivity in our patients is also not clear, but in our opinion, the finding provides new insight about the pathogenesis of toxic oil syndrome, suggesting an immune mechanism implicating antiganglioside antibodies, and expands the range of diseases with antiganglioside antibodies.

C IÑIGUEZ
Department of Neurology, Hospital Clínico, Zaragoza, Spain
A JIMÉNEZ-ESCRIG
J GOBERNADO
Department of Neurology
G DE BLAS
Department of Neurophysiology
M NOCITO
M L DEL VILLAR
P GONZALEZ-PORQUÉ
Department of Immunology, Hospital Ramón y Cajal, University of Alcala, Madrid, Spain

Correspondence to: Dr Jiménez Escrig, Servicio de Neurología, Hospital Ramón y Cajal, Carret de Colmenar Km 9, 28034 Madrid, Spain.

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Dose standardisation of botulinum toxin

Several studies have shown that botulinum toxin A is the treatment of choice in blepharospasm and hemifacial spasm. There is no dose standardisation, however, between the two commercial preparations available—namely, Botox (Allergan, 100 U/vial), and Dysport (Speywood, 500 U/vial); 1 U or μg of one is not equivalent to 1 U or μg of the other. Quinn and Hallet[1] drew attention to the need for a comparative study. Schantz and Johnson[2] replied but in basic biological terms. Brin and Blitzer[3] suspected that 1 U of Botox is roughly equivalent to 4 to 5 U of Dysport, but there was a much wider range in their accompanying table of "standard injection doses" of the two products. Marsden[4] also suggested that 1 U of Botox is roughly equivalent to between 4 and 5 U of Dysport, but pointed out that the exact equivalence should be resolved as soon as possible. Pickett and Hambleton[5] suggested an equivalence of 1:3, based on "clinical observations", but gave no details and the same authors[6] recently found a similar equivalence using a mouse essay. We now wish to report a study of the clinical equivalence between the two preparations of botulinum toxin.

We selected 74 patients, 37 with idiopathic blepharospasm and 37 with hemifacial spasm. All patients had responded favourably and repeatedly to Dysport for at least 12 months (table). We injected increasing doses of Botox until a satisfactory response lasting as long as with Dysport was obtained (patients serving as their own controls). The injection technique was otherwise unchanged. Botox (like Dysport) is presented as a powder in a vial to be dissolved. We always injected the same volume with each toxin; we obtained a higher dose of Botox by using less diluent. Our initial dilution with 4 ml saline gave a satisfactory response in less than 50% of patients. Dilutions with 3 ml, 2·5 ml, and 2·0 ml all produced a similar poor response rate (<50%). Finally, when we used 1·5 ml saline, we obtained a satisfactory response in all patients lasting as long as with Dysport (table) without side effects such as ptosis or diplopia. The total number of patient injection episodes was 59 for blepharospasm and 52 for hemifacial spasm; some patients were injected more than once, but a three month interval between injections was always respected.

The final dilution of 100 U of Botox with 1·5 ml saline gives a concentration of 67 U/ml; 500 U of Dysport is dissolved in 2·5 ml saline to give a 200 U/ml concentration. Hence, we arrived at a clinical equivalence of 67 U Botox equal to 200 U Dysport—that is, a ratio of 1:3. We think that this will be a useful guideline, particularly when clinicians attempt to change from one toxin to the other.

MARIE-HÉLÈNE MARION
4 rue Léon Delhomme, 75015 Paris
MICHAEL SHEEHY
King's College Hospital and Queen Mary's Hospital for Children, London
SOPHIE SANGLA
Hôpital Delafontaine, 93205 Cedex 1 Saint Denis, France
SOPHIE SOULAYROL
Hôpital Ste Marguerite, 13009 Marseille, France

Equivalence of Dysport and Botox in treatment of blepharospasm and hemifacial spasm

	No of patients	*Sex (n)*	*Mean (range) age at onset (y)*	*Mean (range) duration (y)*	*Mean dose of Dysport (Dysport U)*	*Mean effective dose of Botox (Botox U)*
Hemifacial spasm	37	M (14) F (23)	54·6 (32–89)	8·16 (3–22)	85	32
Blepharospasm	37	M (7) F (30)	54·7 (33–80)	8·34 (1·5–24)	223	77

Correspondence to: Dr M H Marion.

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Changes of diffuse neurofibrillary tangles with calcification (DNTC) in a woman without evidence of dementia

Kosaka has proposed the term "diffuse neurofibrillary tangles with calcification" (DNTC) for a form of presenile dementia characterised by cortical dementia, neurofibrillary tangles, and neuropil threads, but lacking plaques in the cerebral cortex, and coexisting with Fahr's type calcification and temporal or temporofrontal atrophy with neuronal loss and astrocytosis.[1] We have recently encountered a similar pathological change, however, in a woman with no history of dementia. As a result, five other cases of idiopathic intracerebral calcification were reviewed, specifically to determine whether neurofibrillary tangles were present.

The recent case involved a 64 year old woman whose sudden death was attributed to ischaemic heart disease. Her brain, which weighed 1300 g, had calcified masses up to 2 cm in diameter in the cerebellum, with further patches of calcification in the cerebral hemispheres. Moderate generalised cerebral atrophy particularly affected the temporal lobes, and the ventricles seemed mildly dilated.

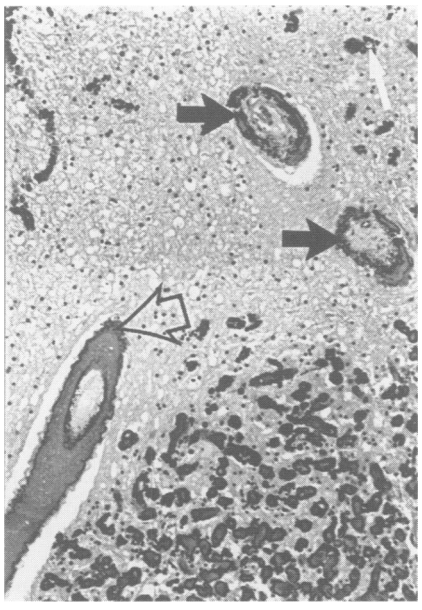

Figure 1 Calcification in the cerebellum within the media (open black arrow) and adventitia (solid black arrows) of arterioles, and around capillaries (white arrow). Also note calcospherules, bottom right (haematoxylin and eosin × 60).

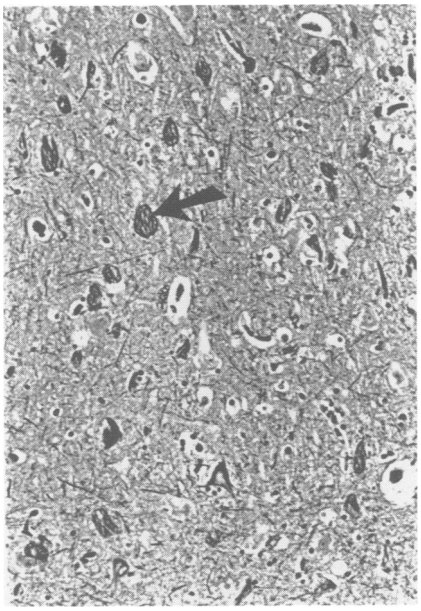

Figure 2 Neurofibrillary tangles in the hippocampus with an absence of plaques (a well formed tangle is arrowed; Hicks/Gallyas × 75).

Histological examination showed calcospherules and larger concretions, predominantly in the dentate nucleus, central cerebellar white matter, cerebellar cortical granular layer, and basal ganglia, often related to capillaries. In addition, there was continuous calcification in the media and adventitia of many small arteries and veins in these areas (fig 1). The brain stem was not affected. Spongiosis and gliosis accompanied pronounced neuronal loss in the atrophic temporal cortex, these features being most obvious within the superficial laminae. Many neurofibrillary tangles (fig 2), which were tau positive, were present, but there were virtually no plaques. Tangles, without plaques, were also noted in the hippocampus and parahippocampal cortex. Otherwise neurons seemed unaffected, even in areas of dense calcification.

Subsequent enquiry confirmed that there was no history of neurological impairment or relevant past illness. Specifically, there had been no evidence of dementia or movement disorder. This was corroborated by her apparent state of well being—she had lived alone and was well nourished and of neat appearance.

Review of archive material from the past 14 years yielded five further cases of Fahr's type calcification, two in males (aged 45 and 34 years) and three in females (aged 60, 30, and 6 years). All showed appreciable calcification of Fahr's type, but no evidence of neurofibrillary tangle formation. One patient had had psychosis and depression, but none had shown evidence of dementia during life.

Extensive calcification of the cerebellum and cerebrum has been recorded in patients with hypoparathyroidism and also as a result of high lead exposure,[2 3] but a review of the medical literature failed to find an association between the calcification and tangle formation or dementia in such cases. One paper did refer to unexplained intracranial calcification in patients also with dementia, and an association with hypothyroidism was proposed.[4] Extensive neuropathological examination of these cases was not, however, performed.

Diffuse neurofibrillary tangles with calcification (DNTC) seems to be a rare entity, largely confined to Japan, with only 16 cases recorded in the medical literature.[1] The macroscopic and microscopic features in our 64 year old patient very closely match the findings described in patients with the postulated dementing illness DNTC,[1 5] except that the brain was rather heavier than in recorded cases. The patient did not manifest dementia during life, however: nor was there any suggestion of the movement disorders that may accompany DNTC.[1 5] Therefore, it is proposed that the term diffuse neurofibrillary tangles with calcification (DNTC) encompasses a specific constellation of neuropathological changes, but is not necessarily associated with dementia.

N E I LANGLOIS
J H K GRIEVE
P V BEST
Department of Pathology, University Medical Buildings, Foresterhill, Aberdeen AB9 2ZD, UK

Correspondence to: Dr Langlois.

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Increased serum concentrations of protein S-100 after minor head injury: a biochemical serum marker with prognostic value?

Protein S-100 is a calcium binding protein, synthethised in astroglial cells in all parts of the CNS. High concentrations of protein S-100 in CSF have been found in patients with different neurological diseases or injuries.[1] Only very low concentrations of this protein are normally present in serum, whereas high serum concentrations indicate damage to glial cells and blood brain barrier dysfunction. To predict and prevent an eventual development of symptoms after concussion, there is a need for an early sensitive marker of brain damage in patients with negative radiological examination after minor head injury. Most patients with minor head injury have a good outcome, but in 15% to 50% symptoms develop after concussion.[2] After minor head injury CT has not shown an association between neuroradiological abnormalities and the development of symptoms, but on a microscopic level, there is evidence for organic brain lesions.[3]