

The clinical relevance of ferritin concentration in the cerebrospinal fluid

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SUMMARY By means of a new technique (Particle Counting Immunoassay), we have determined the level of ferritin in 470 samples of cerebrospinal fluid of patients with various neurological disorders. The median value obtained in a control group was 2.3 ng/ml with an upper limit at 5.5 ng/ml. The concentrations in the serum and cerebrospinal fluid were independent, but that in cerebrospinal fluid correlated with its total protein content. High values of ferritin were found in infectious meningoencephalitis, in vascular diseases of the central nervous system, and, unexpectedly, in several cases of dementia without obvious vascular pathology.

Ferritin is the major iron storage protein present in most tissues but particularly abundant in the liver, spleen and bone marrow. This protein with a molecular weight of 450 000 is composed of 24 identical subunits forming a nearly spherical hollow shell with a central core containing up to 4000 atoms of iron. Most tissues, when exposed to iron start producing ferritin, for example with haemoglobin resorption after vascular damage. Its serum concentration is now considered as the best index of the iron needs of the organism. However, inflammatory reactions, liver disorders, tissue necrosis and various forms of cancer can raise the ferritin concentrations in the serum in the absence of any iron overload.

Because of the wide tissue distribution of ferritin, we thought that its determination in the cerebrospinal fluid (CSF) of patients might help in the diagnosis of various neurological disorders and perhaps give insight into their pathogenesis. A recent publication indicates the potential role of ferritin assay in the diagnosis of cerebral infarction or bleeding.¹

Material and methods

PATIENTS

Lumbar CSF from patients of the Department of Neurology, Cliniques Saint-Luc, Brussels was obtained for routine analysis. After centrifugation, aliquots of 0.5 to 2 ml were kept frozen at -20°C . Blood was collected on the same day. Total proteins were determined in CSF by

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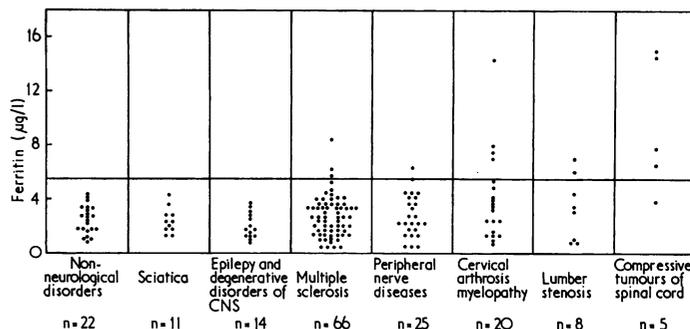
the method of Meulemans² and analysed by agar gel electrophoresis.³ Ferritin was assayed in 470 samples, and the clinical records of these patients were then reviewed. Cases whose diagnosis was doubtful were excluded from the study. Ferritin was also determined in 157 consecutive samples of serum.

Method

The levels of ferritin in the CSF and serum were determined by the Technicon PACIA (Particle Counting Immuno-Assay) system (Technicon International Division, Geneva, Switzerland). This fully automated technique is based on agglutination of latex. Particles coated with antibodies are agglutinated by the antigen to be determined.⁴ The use of $\text{F}(\text{ab})_2$ fragments rather than whole antibody molecules prevents non-specific agglutination or inhibition of agglutination⁵ by proteins interacting with the Fc region of IgG. Minor interferences by protein-protein interactions are avoided by working at high ionic strength.⁵ Agglutination is measured by counting the residual unagglutinated particles in a conventional optical cell counter, adjusted electronically to ignore aggregates.

Preparation of latex A rabbit was immunised by multiple site intradermal injections of 0.1 mg of ferritin in 1 ml of complete Freund's adjuvant at two-week intervals. The IgG of the antiserum was isolated after precipitation by half-saturated ammonium sulfate by DEAE-cellulose chromatography in 0.1 mol/l TRIS-HCl, pH 8.5. After concentration IgG was digested using pepsin at an enzyme/substrate ratio of 1/50 (Sigma Chemical Co., St-Louis, Mo, USA) in 0.1 M acetate buffer, pH 4.5, for 24 h at 37°C . The reaction was stopped by raising the pH to 7-8 with solid TRIS; the $\text{F}(\text{ab})_2$ fraction was purified on an Ultrogel Aca 4-4 column (2.5×100 cm) (LKB, Bromma, Sweden) in 1 M NaCl buffered by 0.07 M phosphate buffer, pH 7.2. The $\text{F}(\text{ab})_2$ fraction was then

Fig 1 Concentrations of ferritin in CSF of patients with or without neurological disorders.



concentrated and dialysed against physiological saline. Polystyrene particles of 0.8 μ diameter (100 g/l; Estapor K 150, batch No 314, Rhône-Poulenc, Courbevoie, France) was a gift from Dr JC Daniel. F(ab')₂ fragments were coupled to latex as previously described.⁷ To 50 μ l (100 g/l) carbodiimide-activated latex was added 75 μ g F(ab')₂. Such a preparation was sufficient for 300 assays, and was stored at 4°C after lyophilisation in small aliquots. These reagents are now commercially available from Technicon International Division, Geneva, Switzerland.

Calibration curve Ferritin was purified from human liver. The calibration curve used a pool of normal human sera (NHS) of 1000 blood donors, diluted 200 times in 0.1 M glycine buffer saline (GBS), pH 9.2, containing 50 mM EDTA. When necessary, the CSF samples were diluted in the same medium (NHS-GBS). A plot of peak heights or concentration of free particles against the log of antigen concentration forms a decreasing sigmoidal curve from 0.5 to 50 μ g/l. CSF was generally assayed undiluted, unless the ferritin level exceeded 20 μ g/l: samples were then diluted in NHS-GBS. Sera were assayed after a tenfold dilution in a normal rabbit serum diluted itself 10 times in GBS.

Precision The intra-assay and interassay precisions were studied on serum samples at three concentrations of ferritin, the assays being repeated either 20 times on the same day or once each day for 20 days. Maximal intra-assay and interassay coefficients of variation were 4% and 7.3% respectively.

Analytical recovery Ferritin was assayed in 26 samples of CSF diluted with one volume of NHS-GBS containing 4.8 ng/ml of ferritin. The results were then compared to those obtained in samples diluted with NHS-GBS without ferritin. The analytical recovery was 97.1% with an SD of 13%.

Results

CONCENTRATION OF FERRITIN IN SERUM

The ferritin concentrations in sera of 157 patients with various neurological disorders ranged from 10 μ g/l to 500 μ g/l, with one exception; one patient with malignant testicular tumour and epidural metastases had a level of 1130 μ g/l. The mean was 141 μ g/l (SD = 115), a value somewhat higher than those reported by Jacobs and Worwood⁸ for 280 healthy

men (123 μ g/l; range: 10–580 μ g/l) and for 153 women (56 μ g/l; range: 10–400 μ g/l). No correlation was observed between the concentration of ferritin in the serum and CSF in 157 consecutive paired samples ($r = 0.074$).

NORMAL CONCENTRATION OF FERRITIN IN CSF (fig 1)

Twenty-two patients were taken as controls. They were devoid of clinical signs of neurological disorders. Their CSF were normal regarding total protein content (<400 mg/l), number of cells per mm³ (<5), and agar gel electrophoresis; electroencephalography and computed tomography were negative. The ferritin concentration in CSF ranged in these patients from 0.9 to 4.4 μ g/l (median: 2.3 μ g/l; SD: 1.6 μ g/l). The upper normal limit was set up at 5.5 μ g/l (median value + 2 SD). Similar values were observed in patients with sciatica (N = 11) and with either epilepsy or degenerative disorders of the central nervous system (CNS) (N = 14), including four cases of amyotrophic lateral sclerosis, one case of Parkinson's disease, one case of familial spastic paraparesia and one case of olivo-ponto-cerebellar degeneration. With only a few exceptions (three out of 66 cases) multiple sclerosis patients as well as those with peripheral nerve diseases (N = 25) had normal values. Slightly increased levels were found in patients with compression of the spinal cord including a few cases of cervical arthrosis myelopathy (four out of 20), lumbar stenosis (two out of eight) and compressive tumours (four out of five).

HIGH CONCENTRATIONS OF FERRITIN IN CSF (fig 2)

The highest levels of ferritin were found in infectious diseases of the CNS, in vascular disorders and in several cases of dementia. A significant correlation was found between the levels of ferritin in CSF and the total protein content (N = 194, $R = 0.33$, $P < 0.001$) (fig 3).

Viral infections of the CNS The three highest values (133, 84 and 36 μ g/l) were in patients with herpetic encephalitis, a disease characterised by

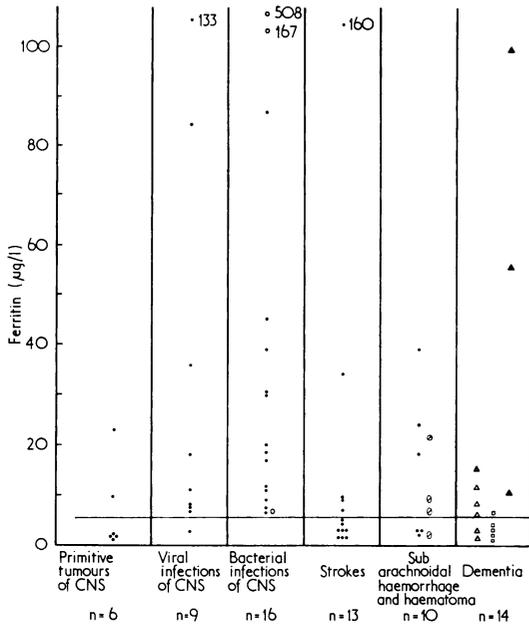


Fig 2 Concentrations of ferritin in CSF of patients with various neurological disorders; ○ = tuberculous meningitis; □ = haematoma; △ = presenile dementia; ◻ = multi-infarct dementia; ▲ = normal pressure hydrocephalus.

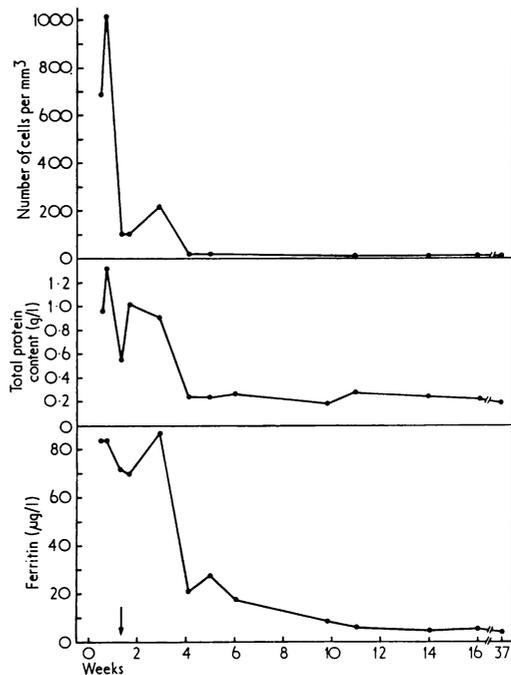


Fig 4 CSF analysis in a longitudinal study of a patient with herpetic encephalitis. The arrow indicates the appearance of herpetic antibodies in the CSF. The cell count and the total protein content returned to normal after four weeks, whereas the ferritin concentration remained elevated up to the tenth week.

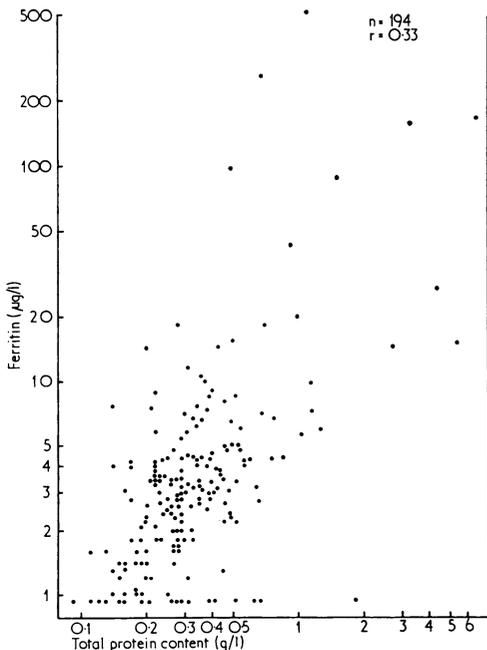


Fig 3 Correlation between the concentration of ferritin and total proteins in CSF of patients with various neurological disorders.

haemorrhagic necrosis particularly in temporal areas. The concentration of ferritin in one case of herpetic encephalitis followed over a nine-month period was closely related to other signs of infection such as the number of cells and total protein content in CSF (fig 4). A high level (18 µg/l) was also found in one case of arbovirus encephalitis. Four slightly elevated values (6.7 to 11 µg/l) and one normal were observed in patients with viral meningitis. Two cases of subacute sclerosing panencephalitis and two cases of Creutzfeldt-Jakob disease gave normal values (results not shown).

Bacterial infections of the CNS All cases of this group (N=16) had high CSF concentrations of ferritin which declined with successful treatment. The results ranged from 6.7 to 508 µg/l. Of three cases of tuberculous meningitis, two had very high levels, 161 and 508 µg/l respectively. The latter value decreased to 11 µg/l after 6 months' continuous treatment. The third patient with tuberculous meningitis was already treated three weeks before the first sampling; the ferritin level was 6.7 µg/l and

returned to normal values (2.6 $\mu\text{g/l}$ after two weeks and 0.8 $\mu\text{g/l}$ after one month).

Strokes As spinal taps are not usually done after cerebral strokes the 13 cases whose CSF were available cannot be considered as representative of this group. The concentrations were particularly high in patients with extensive hemispheric lesions (N=4; range: 7 to 160 $\mu\text{g/l}$) with one reaching 160 $\mu\text{g/l}$ in a comatose patient, who died four months later with a large left parieto-occipital lesion. The concentration was normal in five of the six cases of ischaemic attack restricted to areas supplied by the anterior spinal and basilar arteries.

Haematoma and subarachnoid haemorrhage Three out of four cases of intra-parenchymatous haematoma proved by computed tomography had abnormally high concentration of CSF ferritin. The sample with the highest concentration (22.6 $\mu\text{g/l}$) was xanthochromic, the others were colourless. No cases of subarachnoid haemorrhage with grossly blood stained CSF were available. We had only six patients who were in hospital for further neuro-radiological investigations for suspected subarachnoid haemorrhage. In three cases, CSF was colourless and in the other three, xanthochromic or slightly haemorrhagic. Three samples had a high concentration but no correlation was observed between the hue of the CSF and its ferritin content: one colourless sample contained 18.5 $\mu\text{g/l}$, whereas another which was xanthochromic had a normal concentration.

Dementia All patients of this group had severe intellectual impairment. Cerebral atrophy or enlargement of ventricles or both were shown by either computed tomography or gas encephalography. Very high values were found in two cases of normal pressure hydrocephalus, whereas the concentrations tended to be normal in multi-infarct dementia. The values in presenile dementia were moderately elevated or normal.

Tumours of the CNS Ferritin was increased but to a moderate extent late in the course of the illness of two patients with primitive tumours of the CNS: an anaplastic hypophyseal adenoma after surgery and irradiation, and a malignant pineal tumour with meningeal dissemination

Miscellaneous In two cases of cerebellar atrophy of unknown origin, the ferritin concentration was raised to 9 and 9.5 $\mu\text{g/l}$. In a third case where alcoholism was obvious, the ferritin concentration was normal. In one case of acute transverse myelitis, high values were observed (27 and 155 $\mu\text{g/l}$).

Discussion

The present retrospective study aimed at seeing whether the concentration of ferritin in the CSF was

high in some neurological disorders. Values exceeding by ten to one hundred times the normal were observed in patients with viral and bacterial infections, strokes, subarachnoid haemorrhage, and normal pressure hydrocephalus. Except for normal pressure hydrocephalus, of which the pathogenesis is unknown, the common features of the other diseases are either inflammation, haemorrhage or tissue necrosis. Those processes could explain the presence of ferritin in the CSF; the resorption of haemoglobin induces the biosynthesis of ferritin; the protein can then be released by cellular excretion. Cellular death due to infections or vascular obstruction can also result in the release of ferritin, which is a normal constituent of brain cells.⁹ The recovery of iron from the cytochromes of dead cells could increase the ferritin content of the brain tissue. A significant source of ferritin might also be the macrophages involved in all inflammatory reactions. Macrophages may contain up to 50 ng of ferritin per million cells.

In one patient, normal pressure hydrocephalus occurred six months after cranial trauma suggesting also the possible role of necrosis or haemorrhage in the rise of the ferritin concentration in the CSF. However in all cases of dementia, no clear mechanism can be proposed to explain the high concentrations of ferritin. Two patients among the three with Alzheimer's disease had high concentrations of CSF ferritin. This has to be considered in the light of the histological findings of Goodman¹⁰ and Holgren and Sourander.¹¹ A characteristic feature was the presence of large amounts of an iron staining substance in cells which had undergone neurofibrillary degeneration, in the oligodendroglia and in the microglia within the argentophilic plaques.

Could the determination of ferritin concentration in the CSF be useful in the diagnosis of neurological disorders? The present results suggest that ferritin estimation in CSF could help to distinguish between the various forms of dementia, being in the normal range in the multi-infarct form, slightly elevated in some cases of pre-senile dementia and very high in two out of three cases of normal pressure hydrocephalus. However, a prospective study on a large number of patients is necessary to assess the possible clinical value of this test.

The longitudinal study of the patient with herpetic encephalitis, the significant correlation with the total protein content of the CSF as well as the work of Hällgren *et al*¹ suggest that the concentration of ferritin in the CSF could be a useful index of the intensity of the inflammatory reaction and the extent of either the tissue damage or haemorrhage.

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