

QUANTIFICATION OF CEREBROSPINAL FLUID FERRITIN AS A BIOMARKER FOR CNS MALIGNANT INFILTRATION

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Abstract – Several markers have been studied for their ability to make the CNS infiltration diagnosis earlier and more precise; previous studies showed that CSF ferritin concentrations were higher in patients with malignant invasion of CNS. The objective was to determine the importance of CSF ferritin as a biomarker for the diagnosis of CNS neoplastic infiltration. This study is based on 93 CSF samples, divided into five groups: malignant cells present (n13); malignant cells not present (n26); inflammatory neurological diseases (n16); neurocysticercosis (n20); acute bacterial meningitis (n18). CSF ferritin values were determined by micro particle enzyme immunoassay. CSF ferritin level (mean±SD) in the group with neoplastic cells in the CSF was 42.8±49.7 ng/mL, higher than in the other groups (p<0.0001). We conclude that CSF ferritin with the cut off 20 ng/mL could be an adjuvant biomarker to the diagnosis of CNS malignant infiltration.

KEY WORDS: cerebrospinal fluid, ferritin, central nervous system, malignant cells, CNS neoplasm, CNS tumors, bacterial meningitis.

Quantificação de ferritina no líquido cefalorraquidiano como biomarcador para o diagnóstico de infiltração maligna no SNC

Resumo – Diversos marcadores foram estudados com a finalidade de avaliar sua capacidade de diagnosticar a infiltração neoplásica no SNC precocemente e de forma mais precisa. Estudos anteriores mostraram que as concentrações de ferritina no LCR eram mais elevadas nos pacientes com infiltração neoplásica no SNC. O objetivo foi determinar a importância da ferritina no LCR como biomarcador para o diagnóstico de infiltração neoplásica no SNC. Este estudo é baseado em 93 amostras do LCR, divididas em cinco grupos: células malignas presentes (n13); células malignas ausentes (n26); doenças neurológicas inflamatórias (n16); neurocisticercose (n20); meningites bacterianas agudas (n18). Os valores de ferritina no LCR foram determinados por ELISA de microparticulas. O nível de ferritina no LCR (média±desvio padrão) no grupo com células neoplásicas no LCR foi 42,8±49,7 ng/mL, mais elevado do que nos outros grupos (p<0.0001). Concluímos que a ferritina no LCR com cut off de 20 ng/mL pode ser um biomarcador para o diagnóstico de infiltração maligna no SNC.

PALAVRAS-CHAVE: líquido cefalorraquidiano, sistema nervoso central, células malignas, neoplasias do SNC, tumores do SNC, meningites bacterianas.

The cerebrospinal fluid (CSF) biochemical and cellular characteristics are important adjunct biomarkers for the diagnosis of the central nervous system (CNS) malignant infiltrations. Any type of malignant disease can spread to the leptomeninges^{1,2}.

There is previous evidence that increased serum ferritin levels may be used as an indicator of malignant disease³⁻⁶. Ferritin is present at very low concentrations in normal CSF. Previous studies showed that CSF ferritin concentrations were higher in patients with malignant inva-

sion on the CNS than in patients without CNS involvement⁷⁻⁹. High values of ferritin were also found in non-malignant diseases, such as infectious meningoencephalitis, CNS vascular diseases, and dementia¹⁰.

The hypothesis of CNS involvement by malignant neoplasms must be done in a patient with known malignant neoplasms and neurological symptoms¹¹. Because of the difficulty in early diagnosis of CNS involvement in malignant diseases, several markers have been studied for their ability to make the CNS infiltration diagnosis earlier and more precise¹²⁻¹⁵.

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The objective of this study was to determine the importance of CSF ferritin as a biomarker for the diagnosis of CNS malignant infiltration. In order for this, we studied the levels of CSF ferritin in CSF samples with the presence of malignant cells and comparing them with the levels in samples with no malignant cells, inflammatory CNS disease, infectious diseases chronic and acute.

METHOD

All CSF samples were collected for clinical purposes by lumbar puncture. The Ethical Committee of the Hospital de Clínicas of Universidade Federal do Paraná approved this investigation.

The current study was based on 93 CSF samples, divided in five groups:

Group 1

Malignant cells present (n 13). Malignant disease with CNS involvement, diagnosed on the basis of presence of malignant cells in CSF. CSF samples from patients with clinical suspicion of malignant CNS infiltration and from patients that underwent prophylactic intrathecal chemotherapy, mainly from hematology, bone marrow transplantation (BMT), neurology and neurosurgery services. The diagnoses were: spinal cord tumor (1); lung carcinoma (1); breast carcinoma (1); melanoma (1); chronic myeloid leukemia (5); acute linfoide leukemia (2); linphoma (2).

Group 2

Malignant cells not present (n 26). CSF samples from patients with clinical suspicion of malignant CNS infiltration and from patients that underwent prophylactic intrathecal chemotherapy, mainly from hematology, BMT, neurology and neurosurgery services without malignant cells detected in CSF. The diagnoses were: acute linfoide leukemia (23); lymphoma (1); Burkitt lymphoma (1); astrocitoma (1)

Group 3

Inflammatory neurological diseases (n 16), including Behçet disease (8); pseudotumor (2); Guillain-Barré syndrome (1); poli-neuritis (1); Vogt Koyanagui Harada disease (2); neurosarcoidosis (1); mitochondriopathy (1).

Group 4

Neurocysticercosis (n 20). CSF samples from patients with clinical suspicion of neurocysticercosis (NC), neuroradiologic characteristics of NC and positive CSF anti-cysticercosis ELISA.

Group 5

Acute bacterial meningitis (n 18). CSF samples from patients with clinical suspicion of acute bacterial meningitis and CSF with increase of WBC number, predominium of neutrophils and low glucose.

CSF cytology and biochemistry characteristics in all five groups are indicated at Table 1. CSF ferritin values were determined by micro particle enzyme immunoassay (MEIA) AxSYM ABBOTT in undiluted CSF samples, it was required 200 µL of CSF sample. The analytic sensitivity of the AxSYM ferritin assay was 1.0 ng/mL.

CSF total protein (TP) was quantified by turbidimetric method of sulphosalicylic acid and CSF glucose was quantified by enzymatic method. CSF total cell count was assessed by a Fuchs Rosenthal chamber. For differential cell count and to detect the presence of malignant cells, CSF samples were concentrated by Cytospin. CSF samples were protein enriched with albumin. The slides were stained by May Grünwald-Giensa technique and analyzed by, at least, two trained researchers (SMA and EN). CSF samples with more than 50 RBC/mm³ were excluded.

Statistical analysis

The continuous variables were compared using non-parametric test. To compare all the five groups were used the Kruskal-Wallis test. The groups were compared two by two by the Mann-Whitney test. A p value ≤0.05 was considered significant. The results were presented as mean±standard deviation (SD).

To evaluate the operational characteristics of CSF ferritin quantification was used the following formulas¹⁶: sensitivity $[\text{TrP}/(\text{TrP} + \text{FN})] \times 100$; specificity $[\text{TN}/(\text{TN} + \text{FP})] \times 100$; positive predictive value $[\text{TrP}/(\text{TrP} + \text{FP})] \times 100$; negative predictive value $[\text{TN}/(\text{TN} + \text{FN})] \times 100$; detection rate $\text{TrP}/\text{total tested}$; Youden index $[(\text{sensitivity} + \text{specificity}) - 1]$; error ratio $(\text{FP} + \text{FN})/\text{TrP}$; combined error $(\text{FP} + \text{FN})/\text{total tested}$. (TrP, true positive; TN, true negative; FP, false positive; FN, false negative).

Table 1. CSF cytology and biochemistry values (mean±SD).

Group	With malignant cells	Without malignant cells	CNS inflammatory disease	Neurocysticercosis	Acute bacterial meningitis	p*
N	13	26	16	20	18	
Leuco/mm ³	320±825	1.2±1.4	1.8±2	22±42	7822±13080	0.001
RBC/mm ³	13±24	7.5±16.2	14±22	1.2±3.5	23±19	0.005
TP mg/dL	262±675	34±36	29±15	56±54	724±1512	0.018
Glucose mg/dL	50±25	57±8	68±33	57±18	20±23	

*P calculated excluding group 5; Leuco, leucocytes; RBC, red blood cells; TP, total protein.

RESULTS

CSF ferritin levels (mean±SD) in the group with malignant cells present in the CSF was 42.8±49.7 ng/mL; in the group without malignant cells in the CSF was 13.0±16.9 ng/mL; in the group with CNS inflammatory disease was 6.4±3.6 ng/mL; in the group with neurocysticercosis was 12.6±15.8 ng/mL and in the group with acute bacterial meningitis was 241.7±234.1 ng/mL. Figure 1 shows CSF ferritin levels (mean±SD) in the five groups studied.

Comparing all the five groups with the Kruskal-Wallis nonparametric test there was a statically significant difference between the five groups (p<0.0001). Comparing the group with presence of malignant cells in the CSF with each other group, the CSF ferritin was higher than in the group without malignant cells in CSF, CNS inflammatory disease and neurocysticercosis (p=0.02; 0.002; 0.02 respectively).

The CSF ferritin of the group without malignant cells in the CSF was not statistically different from the CSF ferritin of the groups with CNS inflammatory diseases or neurocysticercosis but was different from the CSF ferritin in the group with acute bacterial meningitis (p=0.37, 0.89, <0.0001 respectively). The CSF ferritin of the group with CNS inflammatory diseases and the group of neurocysticercosis was not different (p=0.53). The CSF ferritin in the group with acute meningitis was greater than the ferritin from the other groups (p<0.001).

The CSF ferritin in the group with more than 50% of malignant cells in the CSF was higher than the group with less than 50% of malignant cells in the CSF, 57.6±61.8 (median 26.9) and 36.2±47.5 (median 12.4) respectively (Fig 2). Although there was no statistic difference (p=0.18), probably because the low number of cases in each group (5 and 6 respectively).

The operational characteristics of CSF ferritin to detect CSF malignant infiltration with different cut-offs are showed at Table 2.

The best cut-off for CSF ferritin is 20 ng/mL, although

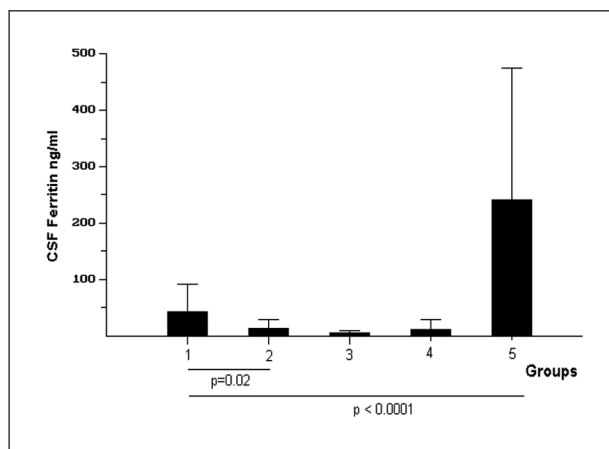


Fig 1. CSF ferritin levels (ng/mL). Values are mean and standard deviation.

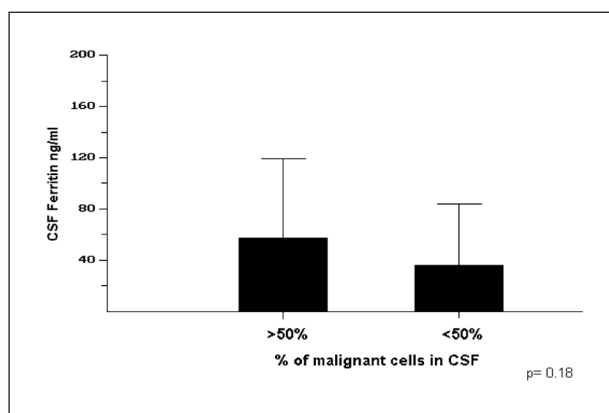


Fig 2. CSF ferritin levels and number of malignant cells in the CSF.

an increased CSF ferritin does not indicate the diagnosis of CNS neoplastic infiltration. Low CSF (less than 20 ng/mL) ferritin could be indicative of lesser chance of having CNS malignant infiltration, because the negative predictive value is higher with this CSF ferritin cut-off.

Table 2. Operational characteristics of CSF ferritin with different cut-offs.

CSF Ferritin	>5 ng/mL	>20 ng/mL	>40 ng/mL	>60 ng/mL	>80 ng/mL
TrP (N)	11	6	5	3	2
Sensibility (%)	84.6	76.9	38.5	23.1	15.4
Specificity (%)	22.2	52.2	67.8	71.1	75.6
PPV (%)	13.6	18.9	14.7	10.3	8.3
NPV (%)	90.9	94	88.4	86.5	86.1
Detection rate (%)	10.7	5.8	4.8	2.9	1.9
Error ratio (%)	6.6	7.2	7.4	12	16.5
Combined error (%)	69.9	41.7	35.9	34.9	32.0
Efficiency (%)	32	55.3	64.1	65.1	68
Youden index	0.07	0.30	0.06	0	0

PPV, positive predictive value; NPV, negative predictive value; TrP, true positive.

DISCUSSION

The early diagnosis of CNS involvement in malignant diseases is difficult and traditionally based on clinical evidence and the presence of malignant cells in CSF¹⁷⁻²².

Methods with more sensibility and specificity than the cellular morphology are necessary to correctly identify malignant cells in the CSF^{23,24}. Although CSF cytology is useful, malignant cells are not detected in as many as one third of patients who have compelling clinical or radiographic evidence of neoplastic meningitis. Novel assays are being tested that may enhance the early identification of malignant cells in CSF. Currently, the diagnosis occurs after the onset of neurologic manifestations and heralds a rapidly fatal course for most patients²¹. Immunocytochemistry techniques, immunophenotyping and biochemical or immunologic markers can help in this diagnosis^{13-15,22-24}. The analysis of CSF biochemical and cellular characteristics, although not specific for the diagnosis of malignant involvement of CNS, are important¹ and can help for the diagnosis of CNS malignant disease when associated with other clinical or biomarker characteristics.

In this study CSF ferritin levels were analyzed as a biochemical marker for the diagnosis of CNS involvement in malignant diseases. CSF ferritin levels were higher at the group with CNS malignant involvement proved by the presence of malignant cells in CSF, than in the group with inflammatory CNS diseases. The number of erythrocytes (RBC) in CSF correlates with CSF ferritin levels^{9,10,25}, because of this we excluded the CSF samples with more than 50 RBC/mm³.

Ferritin is found in concentrations of up to 10 ng/mL in normal CSF, other studies report lower levels as 2.3 ng/mL with an upper limit at 5.5 ng/mL¹⁰. In this study the CSF ferritin median levels at the group without neoplastic cells was 13.0±16.9 ng/mL.

The best cut-off of CSF ferritin for the diagnosis of CNS involvement in malignant diseases is 20 ng/mL. With this cut-off sensibility and specificity have almost similar values and the Youden index is higher than with other values.

Lower CSF ferritin values (less than 20 ng/mL) could indicate that there is less chance of having a CNS malignant infiltration, because the negative predictive value is high with this CSF ferritin cut-off. Higher cut-off values sensibility decreases although specificity increases and Youden index is zero, this means that the test is no better than chance. The Youden index completely ignores the effect of prevalence on the test situation.

Although an increased CSF ferritin does not indicate the diagnosis of CNS malignant neoplastic infiltration, with low ferritin levels CNS malignant infiltrations seems less probable.

In this study CSF ferritin measurement seems to be not useful in the differential diagnosis of malignant CNS involvement and CNS inflammation because in both conditions CSF ferritin levels are increased confirming the results of previous studies^{8,9}. Also, CSF ferritin was higher in the group with bacterial meningitis than the other groups, in accordance with previous papers¹⁰. Ferritin metabolism and blood brain barrier, could explain the great increase on the group with acute bacterial meningitis. This could be related to the blood brain barrier disruption that is present in acute bacterial meningitis, probably with a leakage of ferritin from blood to CSF. Although for some authors CSF ferritin can be considered to be derived almost exclusively from sources within the CNS itself, as even in cases of severely impaired blood CSF barrier function, the amount of ferritin present in CSF by far exceeds the amount explicable by its molecular size²⁵. The association of CNS malignant involvement and bacterial meningitis could occur because immunosuppressions by chemotherapy, corticosteroids, BMT or by the malignant process itself. In these cases CSF ferritin could not help as a marker of malignant neoplastic infiltrations, because it is increased in both cases.

Ferritin is a macromolecule with molecular weight of 450kD, occurring in at least 20 isoforms. Each ferritin molecule is thought to consist of a spherical protein shell made up of 24 subunits with a variable amount of iron as a core of ferric oxide-phosphate. Ferritin that is not combined with iron is called apoferritin. Ferritin is the main intracellular iron storage protein, keeping it in a soluble and non-toxic form. Inside the ferritin shell, iron ions form crystallites together with phosphate and ions. Each ferritin complex can store about 4500 iron (Fe³⁺) ions. It has been demonstrated that the ferritin molecule, when fully saturated, may consist of over 20% iron by weight²⁶. It serves as iron storage proteins in liver, spleen and bone marrow. While the serum ferritin concentration reflects the iron load of the organism, information on the origin and relevance of CSF ferritin is scarce. Ferritin is slightly elevated in CNS inflammations, stroke and tumor infiltration and exceeding serum values after subarachnoid hemorrhage. There was no correlation between CSF and serum ferritin concentration and no correlation between CSF/ serum ferritin ratio and the albumin ratio²⁵. The concentrations in the serum and CSF were independent, but that in CSF correlated with its total protein content¹⁰. The immunoblot after isoelectric focusing showed 6 to 10 ferritin bands at an IP 5.0 to 6.0, corresponding to the properties of basic storage ferritins. Very high ferritin content was observed in macrophages after subarachnoid hemorrhage, and moderately high content in tumor cells; leukocytes were weakly ferritin positive on membrane surfaces.

The cellular ferritin content, but not the cell count correlates to CSF ferritin concentration in the corresponding diseases²⁷. A macromolecule such as ferritin can cross the blood-CSF barrier only in traces. Whereas an intact blood-CSF barrier can be expected to enable the passage of only 0.1% of a 450 kD protein. The ferritin found in CSF is predominantly derived from sources within the CNS itself. Apart from cerebral parenchyma, at least in cases with pleocytosis, CSF cells may account for ferritin production. Degradation of erythrocytes by macrophages after subarachnoid hemorrhage yields high amounts of surplus iron, which must be stored as non-toxic basic ferritin. High concentrations are released into CSF, which facilitate the discrimination of genuine hemorrhage and traumatic spinal puncture²⁵.

In malignant process possible mechanisms responsible for increased serum ferritin levels are chronic inflammation secondary to the malignant process and increased secretion of ferritin by the malignant cells^{3,4,28,29}. The higher concentration of CSF ferritin in the group with >50% of neoplastic cells described in the present study are in accordance with the hypothesis of ferritin production by the neoplastic cells.

We conclude that CSF ferritin with the cut-off of 20 ng/mL could be an important CSF biomarker adding to the diagnosis of CNS malignant infiltration. In contrast to many other CSF proteins, the interpretation of CSF ferritin concentrations does not require the taking into account of plasma ferritin or the permeability of blood-CSF barrier²⁵. With the present study no final conclusion can be made regarding the behavior of CSF ferritin in relation to the time of onset of infiltration.

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