Accuracy of cerebrospinal fluid ferritin for purulent meningitis

Pedro Celiny Ramos Garcia ,[1] Andrea Lucia Machado Barcelos,[2] Cristian Tedesco Tonial ,[1] Humberto Holmer Fiori,[1] Paulo Roberto Einloft,[1] Caroline Abud Drumond Costa,[1] Janete L Portela,[2] Francisco Bruno,[1] Ricardo Garcia Branco[3]

[1]Postgraduate Program in Pediatrics and Child Health, Pontificia Universidade Catolica do Rio Grande do Sul, Porto Alegre, Brazil
[2]Universidade Federal de Santa Maria, Santa Maria, Brazil
[3]Pediatric Intensive Care Unit, Sidra Medical and Research Center, Doha, Qatar

Correspondence to
Dr Cristian Tedesco Tonial, PUCRS, Porto Alegre 90820090, Brazil; cristiantonial@gmail.com
Published Online First
28 February 2020

ABSTRACT

Objective To evaluate the use of cerebrospinal fluid (CSF) ferritin levels in the diagnosis of purulent meningitis (PM).

Method We studied 81 children between 28 days and 12 years of age who presented with clinical suspicion of meningitis to the emergency department. CSF ferritin levels were measured and compared between diagnostic groups (PM, aseptic meningitis (AM) and no meningitis).

Results The median age was 24 (IQR 8–69) months. There were 32 patients with AM (39%), 23 with PM (28%) and 26 with no meningitis (32%). Median CSF ferritin was 4.2 ng/mL (IQR 3.0–6.5), 52.9 ng/mL (IQR 30.7–103 ng/mL) and 2.4 ng/mL (IQR 2–4), respectively. CSF ferritin was higher in children with PM compared with AM ($p<0.001$) or no meningitis ($p<0.001$). There was no difference between AM and no meningitis.

Conclusion CSF ferritin may be a useful biomarker to discriminate PM in children with clinical symptoms of this disease.

What is already known on this topic?

- Purulent meningitis is an important cause of mortality. Diagnosis through cerebrospinal fluid (CSF) may initially be difficult.
- The presumptive diagnosis by CSF analysis is limited and the result may be inconclusive in some cases.
- Ferritin is a promising biomarker increasingly studied in acute infectious diseases.

What this study adds?

- Ferritin levels are elevated in the CSF of children with purulent meningitis.
- CSF ferritin has a good discrimination for the diagnosis of purulent meningitis and may be useful as a screening or adjunctive test for meningitis.

INTRODUCTION

Purulent meningitis (PM) is an important cause of child morbidity and mortality. Early initiation of treatment is critical, but establishing a definitive diagnosis is difficult.[1 2] The gold standard test for the diagnosis of PM is bacterial culture of cerebrospinal fluid (CSF). However, sensitivity is usually low and it requires at least 24–48 hours for definitive results. Presumptive diagnosis is made using CSF analysis (cell count and differential, glucose and protein, and Gram stains), but accuracy of these tests is limited.[3 4] CSF is often inconclusive (or appears normal) in the more fulminant presentations of bacterial infections, as well as in more fragile population (eg, children with immunosuppression). Moreover, in underdeveloped or remote areas CSF analysis can take hours or days to be performed. A number of studies have evaluated performance of classic and new CSF markers of meningitis, but diagnosis accuracy for such devastating illness remains a concern for paediatricians.[3–5]

CSF ferritin has been studied as a possible meningitis biomarker that can be rapidly and inexpensively performed.[6–8] Ferritin is an acute phase reactant that acutely rises in serum in response to infection. If ferritin could be used as a diagnostic test for meningitis, commercially available point-of-care testing could be used for rapid diagnosis in remote areas.[9] In this study we aimed to evaluate CSF ferritin as a biomarker for the diagnosis of PM and tested performance of the cut-off ferritin values of commercially available point-of-care tests.

MATERIALS AND METHODS

We performed a cross-sectional study in two hospitals in Brazil, Hospital São Lucas da PUCRS and Hospital Universitário de Santa Maria, from 2005 to 2015. Inclusion criteria were children aged 28 days to 12 years admitted to emergency department with clinical suspicion of acute meningitis. Exclusion criteria were traumatic lumbar punctures (lumbar punctures with CSF containing more than 400 red cells x 10^6/L), malignancy, intracranial bleed, Guillain-Barré syndrome and mycobacterial or fungal infection, and previous use of antibiotics. We also excluded patients with screening for other sources of infection, with borderline cellular values in the CSF who were treated with antibiotics but had no clinical evolution compatible with meningitis.

Patients were classified into three groups according to their diagnosis: PM, aseptic meningitis (AM) and no meningitis. The classification as PM was: identification of a bacterium in the CSF and/or presence of leucocyte count >500 x 10^6/L with predominance of neutrophils, protein >100 mg/dL

To cite: Garcia PCR, Barcelos ALM, Tonial CT, *et al*. *Arch Dis Child* 2021;**106**:286–290.

Table 1 General characteristics of patients in three groups

Variable	Groups			P value
	Aseptic (n=32)	Purulent (n=23)	No meningitis (n=26)	
Male sex, n (%)	25 (78.1)	12 (52.2)	17 (65.4)	0.130
Age (months)	24 (8–11)[a]	30 (8–63)[a]	10 (4–62)[a]	0.213
Weight (kg)	15.0 (8.6–31.5)[a]	12 (8.2–25)[a]	9.3 (7.0–19.3)[a]	0.172
Time from onset (days)	1 (0–3)[a]	1 (0–2)[a]	1 (0–2)[a]	0.76
Hospitalisation time (days)	3 (2–5)[a]	13 (10–14)[b]	1 (1–3)[a]	<0.01
Identification of virus/bacteria	17 (53.1)	16 (69.6)	–	0.70
Death, n	0	1	0	–
Blood ferritin (ng/mL)	87.3 (50–157)[a]	289.8 (79–500)[a]	50 (38–224)[a]	0.46
Blood leucocytes (cells x $10^{9/L}$)	10.615 (8.020–14.710)[a]	17.820 (8.110–27.630)[a]	12.280 (7.830–17.020)[a]	0.32
Blood glucose (mg/dL)	93 (83–108)[a]	103 (91–144)[a]	85 (82–93)[a]	0.06
Haematocrit (%)	32.7 (30.7–37.2)[a]	30.5 (26.5–34)[a]	32.9 (29–35.6)[a]	0.56
Haemoglobin (g/L)	111 (101–128)[a]	103 (90–118)[a]	110 (97–122)[a]	0.52
Platelets (x10^9/L)	275 (220–384)[a]	249 (150–372)[a]	292 (205–342)[a]	0.76
C-reactive protein (mg/dL)	0.9 (0.5–2.2)[a]	2.1 (0.0–15.7)[a]	3.1 (0.3–5.5)[a]	0.79

Categorical variables were expressed in percentages and compared using the χ^2 test and Fisher's exact test. The medians were expressed with the respective IQR and compared using the Kruskal-Wallis test. Same letters (a,b) indicate no significant difference between the groups according to the Dunn's post hoc test (p<0.05).

and glucose <40 mg/dL. AM was defined as: no detection of bacteria in CSF, negative blood culture, confirmation of viral aetiology by PCR and/or leucocyte count between 5 and 100 leukocytes x 10^6/ L with predominance of lymphocytes and monocytes, protein <100 mg/dL and glucose >40 mg/dL in CSF. In addition, there was a need for compatible clinical evolution without the use of antibiotics. No meningitis was defined as normal CSF tests and favourable clinical evolution, without need for antibiotics.

CSF ferritin levels were determined by chemiluminescence (IMMULITE 2000). Cytology, biochemical and bacteriological tests were performed, along with culture. CSF samples were assessed for direct detection of viral nucleic acid using PCR for herpes simplex I/II, varicella zoster virus and reverse transcriptase PCR for enterovirus as per protocol from Fundação Oswaldo Cruz (full methodology, primers and performance are available at https://www.arca.fiocruz.br/handle/icict/28040).

Categorical variables were expressed as percentages and compared using the Pearson χ^2 test or Fisher's exact test. Continuous variables were expressed as medians and IQR and compared using the Mann-Whitney tests. The predefined cut-off values of 20 and 30 ng/mL were used to calculate the hypothetical sensitivity and specificity of commercially available point-of-care tests.

The data were analysed in the program Statistical Package for Social Sciences V.17.0. A significance level of 5% was used for statistical decision criteria. Kruskal-Wallis test was used to compare the three groups. Informed consent was obtained from the parents or legal guardians to participate in the study.

RESULTS

We enrolled 91 children with suspected acute meningitis, of whom 10 were excluded (two tumour, three traumatic tap, five unclear diagnosis). From the 81 children included, 23 were PM, 32 were AM and 26 were no meningitis. The characteristics of the children in each group are detailed in table 1.

In the PM group, Gram staining or CSF culture was positive in 16 patients (69.6%), with *Neisseria meningitidis* (n=7) and *Streptococcus pneumoniae* (n=5) being the most common pathogens. In the AM group, an aetiological agent was identified in 17 (54%) patients (enterovirus n=14 and herpesvirus n=3).

CSF ferritin levels were significantly different between groups (AM median 4.1 (3.0–6.5) ng/mL, PM median 52.9 (30.7–103) ng/mL and no meningitis median 2.4 (2–4) ng/mL, p<0.001). CSF analyses according to groups are shown in table 2. When comparing children in the PM group with the remaining 58 children with non-PM (AM and no meningitis) there was a significant difference in CSF ferritin levels (52.9 (30.7–103) ng/mL vs 3.9 (2.0–5.1) ng/mL, p<0.001). Similar results were obtained when we analysed only children with positive cultures or PCR tests (figure 1). CSF ferritin had a strong discriminatory capacity for PM, with area under the receiver

Table 2 Cerebrospinal fluid analyses according to groups

Variable	Groups			P value
	Aseptic (n=32)	Purulent (n=23)	No meningitis (n=26)	
CSF ferritin (ng/mL)	4.2 (3.0–6.5)[a]	52.9 (32–103)[b]	2.4 (2–4)[a]	<0.01
CSF leucocytes (cells x 10^6 /L)	111 (22–215)[a]	1197 (400–2390)[b]	3 (1–4)[c]	<0.01
CSF neutrophils (cells x10^6/L)	27 (12–37)[a]	83 (70–85)[b]	0 (0–0)[c]	<0.01
CSF lymphocytes (cells x10^6/L)	60 (48–76)[a]	16 (11–25)[b]	0 (0–0)[c]	<0.01
CSF protein (mg/dL)	47 (38–68)[a]	145 (127–240)[b]	27 (23–33)[c]	<0.01
CSF glucose (mg/dL)	58 (49–68)[a]	22 (15–30)[b]	60 (54–64)[a]	<0.01

Medians are expressed with the respective IQR and compared using the Kruskal-Wallis test. Same letters (a,b,c) indicate no significant difference between the groups according to the Dunn's post hoc test (p<0.05).
CSF, cerebrospinal fluid.

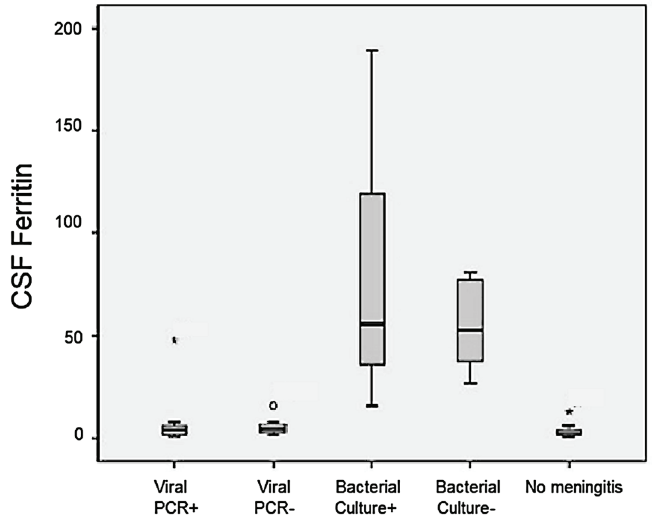

Figure 1 CSF ferritin levels (median IQR) according to clinical diagnosis. CSF ferritin was significantly higher in children with purulent meningitis (culture+ and culture–) when compared with children with aseptic meningitis (PCR positive or PCR negative) or no meningitis. CSF ferritin was not different between aseptic meningitis and no meningitis groups (p=0.092). CSF, cerebrospinal fluid.

operating characteristic (ROC) curve of 0.993 (95% CI 0.973 to 1.000) (figure 2).

There were nine children with CSF leucocytes between 100 and 500 cells x 10^6/L. Of these patients, four were excluded for having an unclear diagnosis (ie, received a full course of antibiotics despite clinical doubts of a bacterial infection), one had a culture-positive bacterial infection with high ferritin (44.6 ng/mL) and four had viral infections with positive PCR and appropriate clinical evolution presenting with low ferritin levels (7.7, 7.06, 9.47 and 3.6 ng/mL).

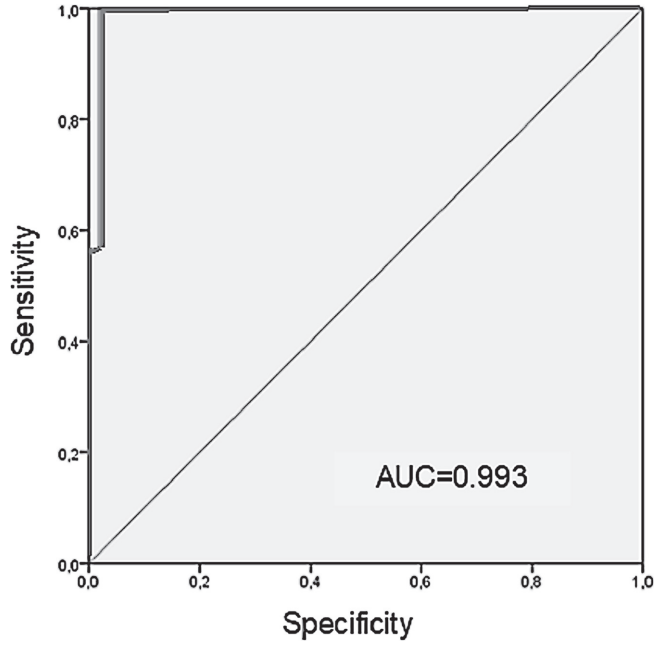

Figure 2 Performance of cerebrospinal fluid (CSF) ferritin in the discrimination of children with or without purulent meningitis. The area under the receiver operating characteristic (ROC) curve (AUC) is 0.993 (95% CI 0.973 to 1.000).

When analysing the predefined cut-off value of 20 ng/mL to evaluate the possibility of using a commercially available point-of-care test, we found a sensitivity of 100% and a specificity of 98.3% for the diagnosis of PM in our population, while a cut-off value of 30 ng/mL had a sensitivity of 95.7% and a specificity of 98.3% for the diagnosis of PM.

DISCUSSION

Our study demonstrated that CSF ferritin is elevated in children with PM, and measurement of CSF ferritin had a good performance as a diagnostic test for PM in children with suspected meningitis in the acute care setting. It also suggested that the use of a point-of-care test with a ferritin cut-off of 20 ng/mL could have an excellent performance as a screening test for PM.

The incidence of PM has decreased significantly over the last years with the widespread introduction of effective vaccines against *Haemophilus influenzae* type b and *S. pneumonia*. CSF pleocytosis is now most commonly observed in children with AM rather than PM.[1 2] Despite these changes, accuracy of rapid diagnostic methods remains low, and a large number of children are exposed to unnecessary prolonged antibiotic treatment and hospital admissions.[2] Several biomarkers have been studied in an attempt to improve diagnosis of PM but have not translated into clinical practice due to poor performance or lack of feasibility. Clinical decision rules have improved sensitivity of diagnosis but specificity remains very low.[5] In our study, CSF ferritin had an excellent discrimination capacity for diagnosis of PM. Ferritin was previously described as a biomarker for the diagnosis of meningitis by Kim and colleagues.[6] In their study, CSF ferritin was significantly higher in children with PM (n=26) when compared with children with AM (n=76). The authors suggested a CSF ferritin level of 15.6 ng/mL as the ideal cut-off point for the diagnosis of PM. In our previous pilot study, we found similar results.[7] More recently, Sanaei Dashti and colleagues[8] assessed CSF ferritin for diagnosis of meningitis in 50 children (12 PM and 38 AM). The authors also reported higher values of CSF ferritin among children with PM in comparison with AM, however, ferritin had a poor diagnostic performance when the chosen cut-off value of 35.5 ng/mL was used. Two aspects influenced this result, first the focus of the study looking at a high specificity biomarker, hence a higher cut-off was used; second, the study had less strict diagnostic and exclusion criteria when compared with our study, in special lack of information about use of antibiotics. It is important to highlight that, outside that acute care setting, CSF ferritin has a significantly worse performance in diagnosing meningitis.[10]

Our study went on to evaluate possible cut-off values for the use of point-of-care ferritin tests. Basic CSF analysis in remote or poor resource settings can be difficult. Results of CSF cytology and biochemistry, when available, can take several hours and be subject to time restrictions. Use of a point-of-care test would provide invaluable information to clinicians in this difficult situation. Commercially available ferritin point-of-care tests use cut-off values of 20 or 30 ng/dL. In our study, using a cut-off value of 20 ng/dL would yield sensitivity of 100%, suggesting it could be a very useful tool for screening of PM in remote and resource poor areas. We are currently performing an accuracy assessment of point-of-care ferritin kit to measure CSF ferritin, and planning a prospective evaluation of the use of point-of-care ferritin as an adjunctive diagnostic tool for PM.

Our study has some limitations that need to be addressed. First, patients with previous use of antibiotics or traumatic lumbar puncture were not included in our analysis. These are

known factors that reduce accuracy of CSF tests and may also significantly affect accuracy of CSF ferritin. Second, we only compared children with well-defined diagnoses. We chose this approach to validate the accuracy of CSF ferritin, however, its performance needs to be confirmed in children in the 'grey zone' where current diagnostic tests are unclear. Third, we only included children presenting to emergency department with clinical suspicion of acute meningitis. Children with more complex presentations (meningitis complicating a primary infection, meningococcal septic shock, children with haematologic disorders) could have different ferritin levels in CSF. Finally, we only identified bacterial and viral meningitis, and CSF ferritin analysis in other types of meningitis (eg, tuberculosis) needs to be further evaluated.

In conclusion, CSF ferritin showed a good performance for the diagnosis of PM in our population, and use of a point-of-care device with a cut-off value of 20 ng/mL may be an excellent adjunctive tool to diagnose PM in remote and deprived areas.

Twitter Pedro Celiny Ramos Garcia @Pedro Celiny Ramos Garcia

Contributors PCRG, ALMB, CTT and RB conceptualised and designed the study, and the data collection instruments, collected data, carried out the initial analyses, drafted the initial manuscript, and reviewed and revised the manuscript. HHF, CADC, JLP, PRE and FB conceptualised and designed the study, coordinated and supervised data collection, and critically reviewed the manuscript for important intellectual content. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The protocol was approved by the ethics committee of the main institution under registry number CAAE-17300913.6.1001.5336.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information. Deidentified data may be available from the corresponding author upon reasonable request and approval from participating institutions and local ethics committee.

ORCID iDs
Pedro Celiny Ramos Garcia http://orcid.org/0000-0002-1863-0727
Cristian Tedesco Tonial http://orcid.org/0000-0001-6643-2090

REFERENCES

1 Thigpen MC, Whitney CG, Messonnier NE, *et al*. Bacterial meningitis in the United States, 1998-2007. *N Engl J Med* 2011;364:2016–25.
2 Nigrovic LE, Fine AM, Monuteaux MC, *et al*. Trends in the management of viral meningitis at United States children's hospitals. *Pediatrics* 2013;131:670–6.
3 Brouwer MC, Thwaites GE, Tunkel AR, *et al*. Dilemmas in the diagnosis of acute community-acquired bacterial meningitis. *Lancet* 2012;380:1684–92.
4 Ray B, Rylance G. Question 1. normal CSF: does it exclude meningitis? *Arch Dis Child* 2009;94:988–91.
5 Dubos F, Korczowski B, Aygun DA, *et al*. Distinguishing between bacterial and aseptic meningitis in children: European comparison of two clinical decision rules. *Arch Dis Child* 2010;95:963–7.
6 Kim YO, Kang JS, Youm MH, *et al*. Diagnostic capability of CSF ferritin in children with meningitis. *Pediatr Neurol* 2003;28:271–6.
7 Branco RG, Pretto CG, Garcia PCR, *et al*. Csf ferritin as a marker for bacterial meningitis. *Pediatr Neurol* 2004;31:309.
8 Sanaei Dashti A, Alizadeh S, Karimi A, *et al*. Diagnostic value of lactate, procalcitonin, ferritin, serum-C-reactive protein, and other biomarkers in bacterial and viral meningitis: a cross-sectional study. *Medicine* 2017;96:e7637.
9 Lab P. "Self-Diagnosis Test: Iron-FER test". Available: https://primahometest.com/iron_fer_test [Accessed 19 Jul 2019].
10 Shokrollahi MR, Shabanzadeh K, Noorbakhsh S, *et al*. Diagnostic value of CRP, procalcitonin, and ferritin levels in cerebrospinal fluid of children with meningitis. *Cent Nerv Syst Agents Med Chem* 2018;18:58–62.