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Diagnostic accuracy of cerebrospinal fluid cytochemical analysis to optimize the use of the FilmArray Meningitis/Encephalitis panel in immunocompetent and immunocompromised patients

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Abstract

Background:

Infectious meningoencephalitis is a medical emergency that requires prompt diagnosis and timely treatment to reduce its high mortality and neurological sequelae. The diagnostic approach combines clinical findings, cerebrospinal fluid (CSF) analysis, and microbiological tests. The emergence of molecular tests such as the FilmArray Meningitis/Encephalitis (FAME) panel has improved pathogen detection; however, its indiscriminate use presents challenges regarding cost-effectiveness and diagnostic value. This study aimed to explore the discriminative capacity of various CSF cytochemical parameters including absolute and differential leukocyte count, CSF-to-serum glucose ratio, and protein concentration for predicting abnormalities in FAME, considered the reference test, and to determine optimal diagnostic thresholds that correlate with FAME positivity in immunocompetent and immunocompromised patients, in order to establish criteria to guide test indication and increase its pretest diagnostic value.

Methods:

A retrospective observational diagnostic accuracy study was conducted in adults with suspected acute meningoencephalitis who underwent FAME testing at two hospitals in Medellín (2019–2023). CSF cytochemical parameters were evaluated as the index test, using FAME as the reference standard.

Results:

A total of 533 medical records were reviewed, with 478 patients included. The median age was 51 years; 264 (55%) were male. Of the patients, 353 (73.8%) were immunocompetent and 125 (26.1%) were immunosuppressed. Overall FAME positivity was 14.8%, identifying bacteria, viruses, and yeasts in 53.5%, 35.2%, and 11.2% of positive molecular results, respectively. Culture positivity was 9%, with 100% concordance with FAME for *Streptococcus pneumoniae*, *Cryptococcus neoformans*, *Listeria monocytogenes*, and *Neisseria meningitidis*. Patients with a positive FAME had significantly higher median CSF leukocyte counts (189 cells/mm³ vs. 3 cells/mm³), protein levels (167 mg/dL vs. 47 mg/dL), and lower glucose levels (31 mg/dL vs. 62 mg/dL) compared to FAME-negative patients. ROC curve analysis showed that CSF protein concentration had the best diagnostic performance (AUC 0.793, 95% CI 0.71–0.85), while opening pressure had the lowest discriminative value (AUC 0.666, 95% CI 0.55–0.73). A total of 287 (60%) molecular tests were performed on CSF samples without pleocytosis (< 10 leukocytes/mm³), yielding a positivity rate of 5.5%. When protein concentration (< 50 mg/dL) was added as a criterion, 192 (40.1%) samples met the combined criteria, with an overall positivity rate of only 2.6%. Only one clinically relevant positive result (0.5%) was found in samples with both < 10 leukocytes/mm³ and < 50 mg/dL protein. Restricting molecular testing to samples with pleocytosis or elevated protein (> 10 leukocytes/mm³ or > 50 mg/dL) would increase diagnostic yield by 55.4% and reduce unnecessary tests by 40.1%.

Conclusions:

An association was identified between abnormal CSF cytochemical parameters and FAME positivity, with CSF protein concentration showing the best diagnostic performance. Applying a combined threshold of > 10 leukocytes/mm³ or > 50 mg/dL protein as a clinical decision rule for molecular testing could improve diagnostic yield by 55.4% while reducing unnecessary testing by 40.1%.

Introduction

Infectious meningoencephalitis is a life-threatening condition associated with high rates of morbidity and mortality[1,2]. Given the severity of the infection, rapid diagnostic evaluation and early initiation of empirical antimicrobial therapy are required to improve patient outcomes[3].

The diagnostic process is based on a combination of clinical, microbiological, and radiological elements. [1,2] A large number of pathogens, including viruses, bacteria, fungi, and to a lesser extent parasites, can cause neuroinfection.[4] However, etiological workup presents a challenge due to the low diagnostic yield of microbiological studies, which require multiple tests to be performed in parallel. Moreover, most of them are performed on cerebrospinal fluid (CSF), a sample with some limitations and not always readily available. [4,5]

The development of molecular tests based on multiplex PCR panels allows the simultaneous identification of various microorganisms in a short period of time and with a small sample volume, enabling timely de-escalation or discontinuation of antimicrobial therapies in patients with suspected neuroinfection.[2] The FilmArray® Meningitis/Encephalitis panel (BioFire Diagnostics, LLC, Salt Lake City, UT, USA) can simultaneously detect 14 microorganisms: 7 viruses (*cytomegalovirus (CMV), enterovirus (EV), herpes simplex virus 1 (HSV-1), HSV-2, human herpesvirus 6 (HHV-6), human parechovirus (HPeV), varicella-zoster virus (VZV)*), 6 bacteria (*Escherichia coli K1, Haemophilus influenzae, Listeria*

monocytogenes, Neisseria meningitidis, Streptococcus agalactiae, Streptococcus pneumoniae), and 1 yeast (*Cryptococcus neoformans, Cryptococcus gattii*).[4–6]

The use of the FilmArray Meningitis/Encephalitis panel (FAME) has been shown in several studies to reduce hospital length of stay, promote rational antibiotic use, and decrease care-associated costs.[4,7–9] However, the test has certain limitations, as noted in a post-market systematic review that estimated its overall sensitivity at 90% (95% CI, 86–93%) and specificity at 97% (95% CI, 94–99%), with variable values when stratified by specific microorganism, which means it is not exempt from false positives or negatives.[10]

The availability of FAME, combined with the simplicity and speed of its results, has facilitated the possibility of excessive and inappropriate use of the test, primarily in the context of non-infectious pathologies such as headaches or cerebrovascular accidents, and in some cases of subacute chronic meningitis. There is also an inherent risk of false positives associated with the use of a multiplex detection panel, which may identify pathogenic agents not necessarily relevant in immunocompetent adults. [6]

In the literature, with the aim of reducing unnecessary requests for molecular testing, the Reller criteria were established, originally designed to identify cases in which the HSV-1 PCR test in CSF could be omitted. These criteria include a leukocyte count > 5/mm³, protein levels > 50 mg/dL, age < 2 years, and a history of immunosuppression. The absence of all four criteria would indicate that molecular testing is not necessary, without the risk of missing patients with a positive result. [11]

Subsequently, the Reller criteria were extrapolated not only to patients with suspected HSV encephalitis but also to all pathogens that can cause meningoencephalitis and are identified by FAME, in order to create clinical decision rules and improve appropriate utilization of the molecular test.[6] Other cutoff points with solid evidence were evaluated to support the reduction of FAME requests in patients with CSF leukocyte counts < 10/mm³.[6,12] Finally, given the possibility of bacterial meningitis with a low leukocyte count,[13] other authors considered protein count in requesting the test.[14]

Based on the foregoing, we designed a study with the objective of exploring the discriminative capacity of the various CSF cytochemical parameters—including absolute and differential leukocyte count, CSF glucose/capillary blood glucose ratio, and protein concentration—for predicting abnormalities in FAME, considered the reference test. Additionally, the study proposes to determine optimal diagnostic thresholds that correlate with FAME positivity in immunocompetent and immunocompromised patients, in order to establish criteria to guide test indication and increase its pretest diagnostic value. Given the exploratory nature of this study, no formal a priori hypotheses with predefined quantitative thresholds were established; instead, optimal cutoff points were derived from the data using ROC curve analysis.

Materials and Methods

A retrospective diagnostic accuracy study was conducted at two high-complexity hospitals in Medellín between January 2019 and August 2023. Both institutions are tertiary-care university-affiliated hospitals serving a mixed population of immunocompetent and immunocompromised patients, including oncology, transplant, and HIV/AIDS programs. Eligible patients were identified through systematic review of medical records of all adult patients admitted with clinical suspicion of acute neuroinfection during the study period, selecting those in whom both FAME testing and cytochemical analysis had been performed on the same CSF sample. All patients meeting eligibility criteria during the study period were included consecutively; no random selection or convenience sampling was applied.

Patients older than 18 years admitted to the emergency department or inpatient services with clinical suspicion of acute neuroinfection (meningitis or encephalitis), for whom both CSF cytochemical and FAME results were available, were included. Patients with suspected chronic meningitis, incomplete medical record data, or more than 2 molecular studies on CSF were excluded.

The following information was obtained from the medical records: (i) demographic data such as age, sex, presence of underlying medical conditions, and immunosuppression status defined as history of HIV/AIDS (regardless of CD4 count or treatment), history of bone marrow or solid organ transplantation, use of chemotherapy or another immunosuppressive agent (i.e., prednisone > 20 mg/day, mycophenolate, cyclosporine), or history of immunodeficiency; (ii) CSF cytochemical study including cellularity, protein levels, and glucose; (iii) FAME result on CSF; (iv) other microbiological studies on CSF such as Gram stain and culture; (v) serum markers such as C-reactive protein (CRP). CSF cytochemical analysis was performed as the index test on the same sample used for FAME testing. Total and differential leukocyte counts were determined by automated cell counting. Protein concentration was measured by benzethonium chloride, and glucose concentration by standard enzymatic method. Opening pressure was measured manually with a manometer at the time of lumbar puncture. All analyses were performed in the clinical laboratories of each institution as part of routine care. The index test and reference standard were performed simultaneously on the same CSF sample, with no time interval between them. No clinical interventions occurred between the two tests. The FilmArray Meningitis/Encephalitis panel (BioFire Diagnostics, LLC, Salt Lake City, UT, USA) was used as the reference standard. Testing was performed on 200 µL of CSF by the clinical microbiology laboratory at each institution, with results available within approximately one hour. The panel simultaneously detects 14 pathogens, including 6 bacteria, 7 viruses, and 1 yeast. Results were reported as positive or negative for

each target by the laboratory, and final clinical interpretation was performed by the treating physician or infectious disease specialist in the context of each patient's clinical presentation. A FAME result was considered positive at the patient level if at least one of the 14 target pathogens was detected, regardless of the number of targets identified. No additional criteria were applied to define reference standard positivity. The FAME panel was selected as the reference standard rather than CSF culture given its superior sensitivity for detecting a broader range of pathogens, including viruses not detectable by culture, and its ability to provide rapid results within a clinically actionable timeframe. Although FAME has an overall sensitivity of 90% (95% CI 86–93%) and specificity of 97% (95% CI 94–99%) and is therefore not error-free, it represents the best available reference standard in this clinical setting for simultaneously identifying the full spectrum of pathogens causing acute meningoencephalitis. CSF culture results were reported in parallel as a complementary reference. Cutoff points for CSF leukocyte count (< 5 and < 10 cells/mm³) and protein concentration (< 50 mg/dL) were selected a priori based on previously published criteria, including the Reller criteria and subsequent studies evaluating FAME utilization. The combined threshold of < 10 leukocytes/mm³ and < 50 mg/dL protein as a decision rule for restricting FAME testing was prespecified as the primary criterion of interest. Additional cutoff combinations were explored post hoc using ROC curve analysis to identify optimal discriminative thresholds for each individual CSF parameter. All exploratory analyses are identified as such in the results. As this was a retrospective study, no blinding procedures were applied during data extraction. In routine clinical practice, CSF cytochemical analysis and FAME testing were performed simultaneously on the same sample, and results of both tests were available concurrently in the medical record. Consequently, treating clinicians had access to both results at the same time. For the purposes of this study, CSF cytochemical parameters were evaluated as predictors of FAME positivity; however, since data were extracted retrospectively from medical records where both results were simultaneously available, the potential for incorporation bias cannot be excluded and is acknowledged as a study limitation. No invalid or indeterminate FAME results were encountered during the study period. Patients with incomplete CSF cytochemical data were excluded from the analysis, as specified in the exclusion criteria. Three patients were excluded due to incomplete medical record data. Among included patients, opening pressure and serum glucose were the variables with the highest frequency of missing data, as these are not routinely measured in all patients undergoing lumbar puncture. These variables were included in analyses only for patients in whom data were available, and no imputation of missing values was performed. The number of patients with available data for each variable is reported in the corresponding results. As both the index test and reference standard were performed on CSF samples obtained during lumbar puncture as part of routine clinical care, no additional procedures were performed for the purposes of this study. Accordingly, no study-related adverse events were recorded.

Cases with a positive FAME and ≤ 10 leukocytes were reviewed in detail by a specialist in infectious diseases, and the clinical relevance of the results was assessed through documentation in the medical record. Results were considered clinically significant when pathogen detection led to appropriate changes in management, according to the authors' judgment. Demographic data and CSF parameters were reported as medians with interquartile ranges (IQR).

The project was reviewed, submitted, and approved by the ethics committees of both institutions, as well as by the university ethics committee of Universidad Pontificia Bolivariana. The investigators adhered to the Helsinki Declaration, 2024 version, for research involving human subjects. As this was a no-risk study, informed consent from participants was not required.

Statistical Analysis

For patient description, absolute and relative frequencies were used for categorical variables, and median with interquartile range (IQR) for continuous variables. For the comparison of dichotomous variables between patients with and without immunosuppression, the Chi-squared test or Fisher's exact test was used as appropriate. For continuous variables, the Mann–Whitney U test was applied. A p-value of < 0.05 was considered statistically significant.

ROC curves were used to estimate CSF values of leukocytes, neutrophils, proteins, and opening pressure. Statistical analysis was performed using SPSS (Chicago, IL), version 25. Subgroup analyses stratified by immune status (immunocompetent vs. immunocompromised) were prespecified in the study design, given the known differences in CSF inflammatory response and pathogen distribution between these populations. No additional post hoc subgroup analyses were performed. Given the retrospective design, no sample size calculation was performed prior to the study. All eligible patients identified during the study period (January 2019 – August 2023) were included, resulting in a total sample of 478 patients. The precision of diagnostic accuracy estimates is reflected in the 95% confidence intervals reported for each measure.

Results

Over the 4-year period, a total of 533 FAME tests were performed on CSF. Fifty-one patients with a diagnosis of chronic meningitis, 3 patients with incomplete medical record data, and 1 patient under 18 years of age were excluded, resulting in a total of 478 patients included in the analysis. The patients included in the study are described in Fig. 1.

Demographic data and CSF characteristics are reported in Table 1. The median age was 51 years; 264 (55%) were male. There was a total of 353 (73.8%) immunocompetent patients and 125 (26.1%) categorized as immunosuppressed, of whom the main cause was HIV/AIDS in 47.2% (59/125), followed by steroid use in 36.8% (46/125), and finally chemotherapy use in 17.6% (22/125). The mean values of CSF indices (leukocytes, proteins, and glucose), as well as C-reactive protein values, are also reported in Table 1.

Table 1
demographic characteristics and CSF indices of study patients.

Characteristic	**Total sample (478)**
Age in years (Median and IQR)	51 (34–65)
% male	264 (55%)
Patients with immunosuppression. No. (%)	125 (26.1)
• HIV/AIDS	59
• Steroid use	46
• Chemotherapy use	22
• Solid organ transplantation	11
• Hematopoietic transplantation	3
• Primary immunodeficiency	1
• Other types of immunodeficiency	3 (2.4%)
Median CSF indices (Median and IQR)	
• Leukocytes (cells/mm³)	5 (1– 44.5)
• Proteins (mg/dL)	52 (34–108.9)
• Glucose (mg/dL)	59 (48–74)
• C-reactive protein (mg/dL)	4.2 (1–12.7)
IQR: interquartile range	

Overall FAME positivity was found in 14.8% (71/478) of samples, with a higher proportion of positive studies in immunocompromised patients (18.4%) compared to immunocompetent patients (13.5%). Regarding detected microorganisms, bacteria were the most frequently identified, followed by viruses and yeasts at 53.5%, 35.2%, and 11.2%, respectively. In immunocompetent patients, the microorganisms primarily identified were bacteria in 70.8% (34/48) of positive tests, while in immunocompromised patients, viral etiology predominated in 47.8% (11/23) and yeast identification accounted for 34.8% (8/23) of cases. Complete individual isolates stratified by immunocompetence status are reported in Table 2.

CSF culture was performed in 92% (440/478) of patients, with positivity in 9% (40/440) of samples. The main isolated agent was *Streptococcus pneumoniae*, identified in 13 cases, followed by *Cryptococcus neoformans* in 6 samples, *Klebsiella pneumoniae* and *Staphylococcus epidermidis* in 4 cases each, *Listeria monocytogenes and Staphylococcus warneri* in 2 cases each, and one case each of *Haemophilus influenzae, Enterococcus faecium, Neisseria meningitidis, Paenibacillus glucanolyticus, Staphylococcus hominis, Streptococcus vestibularis, Streptococcus equi, Streptococcus salivarius*, and *Pseudomonas aeruginosa.*

FAME was positive in 55% (22/40) of patients with positive cultures. When stratified by microorganism, 100% concordance was observed for cultures of *Streptococcus pneumoniae, Cryptococcus neoformans, Listeria monocytogenes*, and *Neisseria meningitidis*. Additionally, Gram staining was performed on 225 (47%) samples, with positivity for some type of microorganism in 9.8% (22/225) of the stains performed. Finally, there was positivity in 2.3% (6/254) of PCR tests performed for *Mycobacterium tuberculosis*. Microbiological results are presented in Table 2.

Table 2
results of microbiological and molecular tests in study patients stratified by immunocompetence status.

Characteristic	Immunocompetent patient (353)	Immunocompromised patient (125)	Total 478 patients
FAME positivity. No. (%).	48 (13.5)	23 (18.4)	71 (14.8)
Bacteria	34 (70.8)	4 (17.3)	38 (53.5)
• *Streptococcus pneumoniae*	18	1	19 (26.7)
• *Haemophilus influenzae*	5	0	5 (7)
• *Neisseria meningitidis*	5	0	5 (7)
• *Group B Streptococcus*	4	0	4 (5.6)
• *Listeria monocytogenes*	2	3	5 (7)
• *E. coli*	0	0	0
Viral	14 (29.2)	11 (47.8)	25 (35.2)
• *VZV*	8	5	13 (18.3)
• *HSV 1*	3	0	3 (4.2)
• *HSV 2*	2	0	2 (2.8)
• *HHV-6*	1	1	2 (2.8)
• *CMV*	0	3	3 (4.2)
• *Enterovirus*	0	2	2 (2.8)
• *Parechovirus*	0	0	0
Yeast	0 (0)	8 (34.8)	8 (11.2)
• *Cryptococcus neoformans/gattii*	0	8	8 (11.2)
CSF cultures performed. No. (%)	324 (91.7)	116 (92.8)	440 (92)
• Microorganism growth in CSF culture. No. (%).	28 (8.6)	12 (10.3)	40 (9)
Gram staining performed on CSF. No. (%)	172 (48.7)	53 (42.4)	225 (47)
• Abnormality on CSF Gram stain. No. (%)	16 (9.3)	6 (11.3)	22 (9.8)
PCR for Mycobacterium tuberculosis performed. No. (%)	159 (45)	95 (76)	254 (53.1)
• Abnormality in PCR for *Mycobacterium tuberculosis*. No. (%)	2 (1.2)	4 (4.2)	6 (2.3)

Patients with a positive FAME compared to those with a negative FAME exhibited, on average, higher median CSF index values: leukocyte count of 189 cells/mm³ vs. 3 cells/mm³, proteinorrhachia of 167 mg/dL vs. 47 mg/dL, and lower glycorrhachia with a median of 31 mg/dL vs. 62 mg/dL. A statistically significant difference was observed between both groups in all three CSF analysis indices (cellularity, proteins, and glucose), as well as in opening pressure values, CRP levels, identification of microorganisms on Gram stain, and culture positivity, with a higher proportion of abnormality in patients with positive FAME. Complete comparative microbiological results are presented in Table 3.

Table 3
demographic data, CSF cytochemical indices, and CSF microbiological studies in patients with positive and negative FAME.

Characteristic	Patients with positive FAME 71 (14.8%)	Patients with negative FAME 407 (85.2%)	p value
Age in years (Median and IQR)	56 (41–66.5)	50 (34–65)	0.07
% male	33 (46)	230 (56.5)	0.11
Immunosuppressed patients. No. (%).	23 (32.3)	102 (25)	0.19
Infectious etiology as primary diagnosis. No. (%).	70 (98.5)	350 (85.9)	0.00
Median CSF indices (Median and IQR)			
• Leukocytes (cells/mm³)	189 (14–1941.2)	3 (1–17.2)	0.027
• Proteins (mg/dL)	167 (77–544)	47 (33–86)	0.00
• Glucose (mg/dL)	31 (10–53.5)	62 (52–76)	0.00
Opening pressure (mmH_2O)	26 (18–38.2)	19 (12–27)	0.00
CSF Gram staining performed	44 (62)	181 (44.4)	0.00
• Abnormality on CSF Gram stain. No. (%).	19 (43.1)	3 (1.6)	< 0.001
CSF culture performed	67 (94.3)	373 (91.6)	0.43
• Microorganism growth in CSF culture. No. (%).	24 (35.8)	16 (4.2)	0.00
Serum C-reactive protein (mg/dL)	5.5 (0.8–21)	4 (1–12)	0.00
IQR: interquartile range			

ROC curve analysis was performed to evaluate the performance of the various CSF cytochemical variables and opening pressure in predicting a positive FAME result. The area under the curve (AUC) values were as follows: CSF leukocytes 0.777 (95% CI 0.71–0.85), CSF neutrophils 0.779 (95% CI 0.70–0.84), CSF proteins 0.793 (95% CI 0.71–0.85), and opening pressure 0.666 (95% CI 0.55–0.73). Among these variables, CSF protein concentration showed the best diagnostic performance with the highest AUC value (0.793), while opening pressure had the lowest value (0.666) with the lowest discriminative capacity. Results are presented in Fig. 2.

Of the 478 total molecular studies, 60% (287/478) were performed on CSF samples with cellularity < 10 leukocytes/mm³, with a positivity rate of 5.5% (16/287). Stratified by immune status, FAME was performed on samples without pleocytosis in 56.9% (201/353) of immunocompetent patients and 68% (86/125) of immunocompromised patients. Positivity in both groups was 2.9% (6/201) and 11.6% (10/86), respectively. Figure 1 represents the relationship between tests ordered and positive results according to immunocompetence status. The proportion of positive studies in samples with > 10 leukocytes/mm³ in CSF increases to 28.7% (55/191) in the overall population, 27.6% (42/152) for immunocompetent patients, and 21.3% (13/61) for samples from immunocompromised patients.

When protein concentration with a cutoff of < 50 mg/dL was added as an additional variable to the cellularity criterion (< 10 cells/mm³), 40.1% (192/478) of CSF samples showed neither pleocytosis nor elevated proteins, with a FAME positivity rate of 2.6% (5/192). Stratified by immune status, FAME was performed on samples without pleocytosis or proteinorrhachia in 40.2% (142/353) of immunocompetent patients and 40% (50/125) of immunocompromised patients, with positivity rates of 2.1% (3/142) and 4% (2/50), respectively. In contrast, in samples with pleocytosis or elevated proteins (> 10 cells/mm³ or > 50 mg/dL), overall FAME positivity was 23% (66/286), 21.3% (45/211) in immunocompetent patients, and 28% (21/75) in immunocompromised patients.

Table 4 presents the FAME tests performed at different cutoff points for CSF cellularity and protein variables, along with the proportion of test positivity stratified between immunocompetent and immunocompromised patients.

Table 4
number of FAME tests performed in patients with different pleocytosis and proteinorrhachia cutoff points with the number of positive results in the sample.

Characteristic	Immunocompetent patient (353) — 48 positive	Immunocompromised patient (125) — 23 positive	Total 478 patients
FAME performed in patients with CSF **< 5 cells/mm³. No. (%)**	157 (44.4)	71 (56.8)	228 (47.6)
• FAME positivity when CSF **< 5 cells/mm³. No. (%)**	5 (3.1)	7 (9.8)	12 (5.2)
FAME performed in patients with **CSF < 10 cells/mm³. No. (%).**	201 (56.9)	86 (68)	287 (60)
• FAME positivity when CSF < 10 cells/mm³. No. (%).	6 (2.9)	10 (11.6)	16 (5.5)
FAME performed when CSF **< 5 cells/mm³ and proteins < 50 mg/dL. No. (%).**	118 (33.4)	44 (35.2)	162 (33.8)
• FAME positivity when CSF **< 5 cells/mm³ and proteins < 50 mg/dL. No. (%)**	3 (2.5)	1 (2.2)	4 (2.4)
FAME performed when CSF **< 10 cells/mm³ and proteins < 50 mg/dL. No. (%)**	142 (40.2)	50 (40)	192 (40.1)
• FAME positivity when CSF **< 10 cells/mm³ and proteins < 50 mg/dL. No. (%)**	3 (2.1)	2 (4)	5 (2.6)

There was an increase in the proportion of positive FAME results in samples with > 5 leukocytes/mm³ up to 21.9% (43/196) and for > 10 leukocytes/mm³ to 28.7% (55/191), representing relative increases of 47.9% and 93.9%, respectively, compared to the overall positivity of 14.8% (71/478). When protein levels > 50 mg/dL were considered in addition to pleocytosis, an even greater increase in positivity rate was observed: 29.1% (54/185) for samples with > 5 leukocytes/mm³ and proteins > 50 mg/dL (relative increase of 96.6%) and 32.6% (51/156) for those with > 10 leukocytes/mm³ and proteins > 50 mg/dL (relative increase of 120.2%). Table 5 summarizes the proportion of positive studies, and the relative increase associated with different leukocyte and protein cutoff points in CSF.

Table 5
Proportion of positive FAME results for different CSF protein and cell count cutoff points, with the relative increase compared with the overall positivity in the study.

Characteristic	Proportion of positive studies % (positive FAME / FAME performed)	Relative increase vs. overall positivity
Total sample (n = 478)	14.8% (71/478)	—
Proteins > 50 mg/dL	24.7% (62/251)	66.8%
CSF ≥ 5 leukocytes/mm³	21.9% (43/196)	47.9%
CSF ≥ 5 leukocytes/mm³ or proteins ≥ 50 mg/dL	18.5% (67/362)	25%
CSF ≥ 5 leukocytes/mm³ and proteins ≥ 50 mg/dL	29.1% (54/185)	96.6%
CSF ≥ 10 leukocytes/mm³	28.7% (55/191)	93.9%
CSF ≥ 10 leukocytes/mm³ or proteins ≥ 50 mg/dL	23% (66/286)	55.4%
CSF ≥ 10 leukocytes/mm³ and proteins ≥ 50 mg/dL	32.6% (51/156)	120.2%

Table 6 individually describes the five patients with positive FAME results in CSF samples with < 10 leukocytes/mm³ and < 50 mg/dL protein. Of these, two had some degree of immunosuppression. Three microorganisms of viral origin were identified *(HHV-6, VZV*, and *enterovirus*), one bacterial agent *(Haemophilus influenzae)*, and one yeast *(Cryptococcus neoformans)*.

Table 6
cases with positive FAME and CSF leukocyte count < 10 cells/mm³ and CSF protein < 50 mg/dL.

Case	Age	Immuno-suppression	CSF leukocyte count	CSF protein (mg/dL)	CSF glucose (mg/dL)	CSF Gram stain	Prior antibiotic use	FAME organism identified	Culture result	Clinically significant
1	21	No	0	23	50	Negative	No	HHV-6	Negative	Yes
2	40	No	0	28.78	88	Negative	No	Haemophilus influenzae	—	Limited utility
3	78	No	3	38.58	53	Negative	Yes	VZV	Negative	Limited utility
4	39	Yes	8	45.8	51	Negative	No	Enterovirus	Negative	Limited utility
5	23	Yes	0	10	72	Positive	No	Cryptococcus neoformans	Cryptococcus neoformans	Limited utility

Only one of the molecular identifications (Case 1) was considered clinically significant, having prompted a change in medical management and initiation of antibiotic treatment in an immunocompetent patient. In Case 5, corresponding to a patient with severe immunosuppression due to HIV, an India ink stain was positive with visualization of yeasts, along with positive results for *Cryptococcus neoformans* antigen and culture. Therefore, FAME positivity did not imply a change in therapeutic management and was considered of limited clinical utility.

Overall, in immunocompetent patients, the FAME result was considered clinically significant in 1.9% (4/201) of tests performed on CSF samples with < 10 leukocytes/mm³, in 1.9% (3/157) of those with < 5 leukocytes/mm³, and in 0.7% (1/142) of those performed on samples with < 10 leukocytes/mm³ and < 50 mg/dL protein.

When extending the analysis to immunosuppressed patients with molecular tests performed on samples with < 10 leukocytes/mm³ and < 50 mg/dL protein, no clinically significant cases were identified among the 50 FAME tests performed. Finally, considering the total population (immunocompetent and immunosuppressed) with CSF samples meeting both criteria (< 10 leukocytes/mm³ and < 50 mg/dL protein), clinically relevant positivity was observed in only 0.5% (1/192) of tests performed, corresponding to Case 1 in Table 6.

Discussion

The objective of our study was to evaluate the relationship between CSF cytochemical abnormalities and the positivity of the multiplex molecular test, with the aim of proposing recommendations for its use and reducing the number of unnecessary tests that may represent avoidable excess costs in the care of patients with neurological pathologies.

CSF composition showed significant differences between patients with positive and negative FAME results. In those with a positive molecular test, elevated protein and leukocyte levels were observed, along with a reduction in CSF glucose concentration—differences that reached statistical significance and are consistent with other cohorts that performed the same comparison.[14,15] An increase in the proportion of positive molecular studies up to 21.9% was observed when the test was restricted to samples with pleocytosis (> 5 leukocytes/mm³), representing a relative increase in diagnostic yield of 47.9%. Although this finding is relevant, it is lower than that reported in other previously described series,[16] though the absolute increase was similar at 7%. The discordant result is explained by a higher proportion of positive FAME results in our overall population (14.8%) compared to other studies.[14–16]

When evaluating which CSF cytochemical variable had the greatest discriminative capacity for predicting a positive molecular test result, ROC curve analysis identified protein concentration as the parameter with the highest AUC, followed by neutrophil count, both with superior performance compared to absolute leukocyte count and opening pressure. Taken together, these findings suggest an association between CSF cytochemical alterations and molecular test positivity, with protein concentration standing out as the marker with the greatest discriminative value. Furthermore, this parameter showed a substantial impact on the diagnostic yield of the test when combined with both pleocytosis cutoff points, doubling the proportion of positive results (from 14.8% to 32.6%) when FAME was performed on samples with > 10 leukocytes/mm³ and > 50 mg/dL protein.

With the aim of establishing clinical decision rules applicable to our setting for requesting the molecular panel, different cutoff points for pleocytosis and proteinorrhachia were evaluated, analyzing the clinically significant results that would have been missed had FAME not been performed under those criteria. It was found that restricting the molecular test to immunocompetent patients with < 10 leukocytes/mm³ would have meant missing four clinically significant findings, corresponding to 1.9% of the samples analyzed. This value does not show a relative reduction when the threshold is adjusted to < 5 leukocytes/mm³; however, a significant difference is observed when protein

concentration is added as a variable, establishing a limit of < 50 mg/dL in samples with < 10 leukocytes/mm³. With this criterion, only one clinically significant result was identified (0.7% of tests performed), which would have prevented ordering 142 studies.

Meningoencephalitis without CSF pleocytosis has been described in patients with bacterial meningitis, as well as in pediatric patient series and in immunosuppressed individuals.[5][13][16] In the latter group, it has been proposed that the absence of an inflammatory response is due to their limited capacity to generate an adequate immune reaction.[16] As a consequence, immunosuppressed patients have been excluded from articles evaluating clinical decision rules for requesting the molecular test.[6][14][16] However, although our study was consistent with previous findings in identifying positive FAME results in samples from immunocompromised patients without pleocytosis, with positivity rates of 9.8% and 11.6% for cutoff points of < 5 and < 10 leukocytes/mm³ respectively, when the additional criterion of proteinorrhachia < 50 mg/dL was incorporated, positivity was reduced to only two cases. In neither of them did the FAME result lead to a significant change in clinical management.

This finding raises the possibility of applying in our setting a combined cutoff of < 10 leukocytes/mm³ and proteins < 50 mg/dL, not only in immunocompetent patients but also in immunocompromised ones, as a criterion for restricting molecular test requests. This strategy would increase the overall proportion of positive results to 23%, by limiting its use to samples presenting at least one of these two criteria (> 10 leukocytes/mm³ or proteins > 50 mg/dL), with a relative increase in diagnostic yield of 55.4%. Furthermore, it would prevent 40.1% (192/478) of molecular tests from being performed unnecessarily.

As study limitations, the retrospective design stands out, with no confirmatory tests such as individual PCR performed, nor evaluation of the sensitivity of the molecular test, as this was not an objective of the study. Likewise, since data were obtained from medical records, complete information was not available for variables that could be useful, such as the ratio between CSF glucose and peripheral blood glucose or the opening pressure at lumbar puncture. Additionally, there is the inherent subjectivity in defining 'clinically significant' based on the authors' judgment regarding the management changes made by the treating physicians. Furthermore, no cost-effectiveness analysis was performed to precisely measure the financial impact of implementing pleocytosis as a diagnostic criterion. Finally, the impact of the ME panel on clinical outcomes, such as length of hospitalization, was not investigated.

In conclusion, an association was identified between alterations in the various CSF cytochemical parameters and FAME positivity, with CSF protein concentration standing out as the parameter with the best diagnostic performance. It is proposed that the application of clinical decision rules in immunocompetent patients, using as restriction criteria a leukocyte count < 10 cells/mm³ and a protein concentration < 50 mg/dL in CSF, could represent a viable strategy for reducing unnecessary molecular test requests and optimizing their diagnostic yield. Furthermore, the findings of this study open the possibility of extending this recommendation to immunosuppressed patients. These hypotheses should be validated through prospective studies analyzing different combinations of paraclinical variables and incorporating clinical components, taking as a reference, some diagnostic decision rules previously described in the literature.[14]

Declarations

Ethics approval and consent to participate

This study was reviewed and approved by the institutional ethics committees of Hospital Pablo Tobón Uribe, Clínica Universidad Bolivariana, and Universidad Pontificia Bolivariana. As this was classified as a no-risk retrospective study involving review of existing medical records, the requirement for informed consent from participants was waived by the ethics committees. All procedures were conducted in accordance with the ethical standards of the 2024 Declaration of Helsinki.

Consent for publication

Not applicable. This manuscript does not contain any individually identifiable data, images, or personal information from study participants.

Competing interests

The authors declare that they have no competing interests. No author has any financial or non-financial relationship with BioFire Diagnostics, LLC or any other entity with a commercial interest in the findings of this study.

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collection, analysis, interpretation of data, or the decision to submit the manuscript for publication.

Author Contribution

JFMR, LRA and AMC conceived and designed the study, collected the data, and wrote the main manuscript text. JHDG served as methodological advisor and supervised the study design and statistical analysis. JPVF, PGO and SLAM served as scientific advisors, contributing their expertise in infectious diseases, neurology, and clinical microbiology, respectively, to the interpretation of results. All authors reviewed and approved the final manuscript.

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Data Availability

The datasets generated and analysed during the current study are not publicly available due to institutional data protection policies and the confidential nature of patient clinical records, but are available from the corresponding author on reasonable request.

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Figures

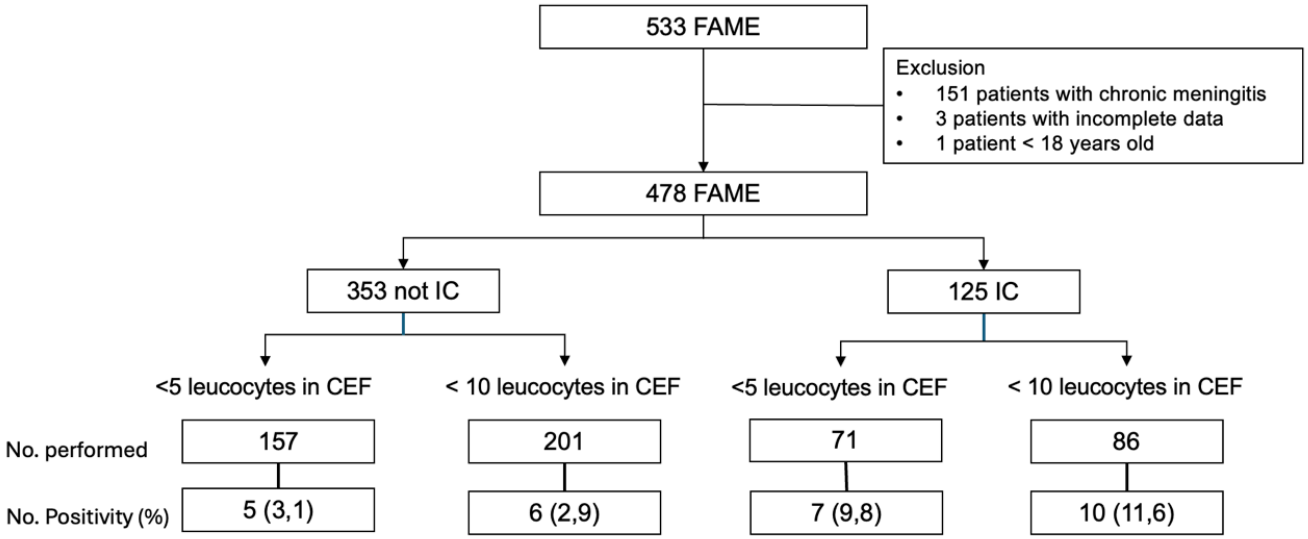


Figure 1

description of patients included in the study with FAME results stratified by immunocompetence status and CSF leukocyte count

IC: Immunocompromised. **CSF:** Cerebrospinal fluid. **FAME:** FilmArray Meningitis/Encephalitis

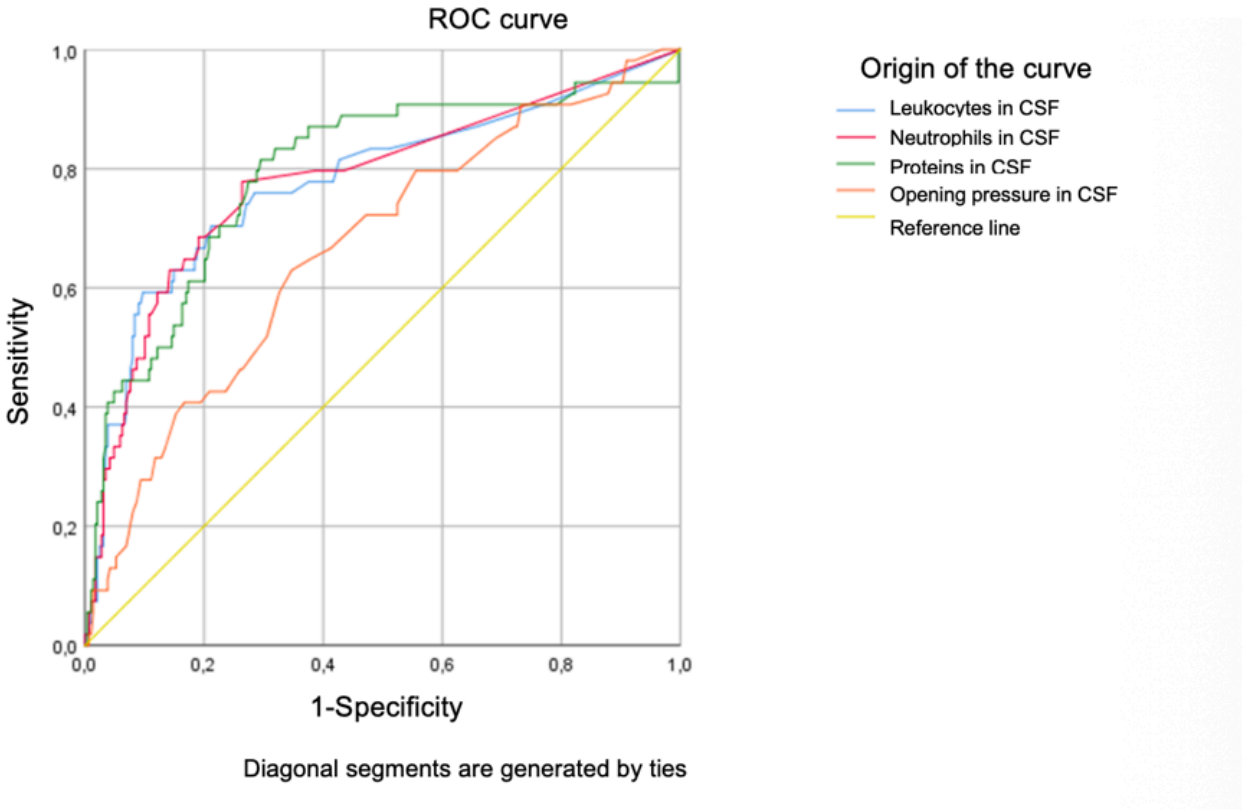


Figure 2

ROC curve for analysis of CSF cytochemical variables and opening pressure in predicting a positive FAME result.