Primary Amebic Meningoencephalitis (PAM): Retrospective Validation of a Clinical Diagnostic Algorithm Using Confirmed and Comparator Cases

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Introduction

Primary Amebic Meningoencephalitis (PAM) is a rare, rapidly fatal brain infection caused by **Naegleria *fowleri***, a free-living **amoeba**. Early symptoms mimic **bacterial** or **viral meningitis**, leading to frequent misdiagnosis. PAM progresses quickly, with **neurologic decline within 24–48 hours**. Survival depends on **early recognition** and **immediate treatment**, yet fewer than a dozen patients worldwide have survived confirmed infections. This study validates a **stepwise diagnostic algorithm** for PAM using retrospective case scenarios, assessing its **sensitivity** and **specificity** in distinguishing PAM from similar acute meningitis/encephalitis presentations.

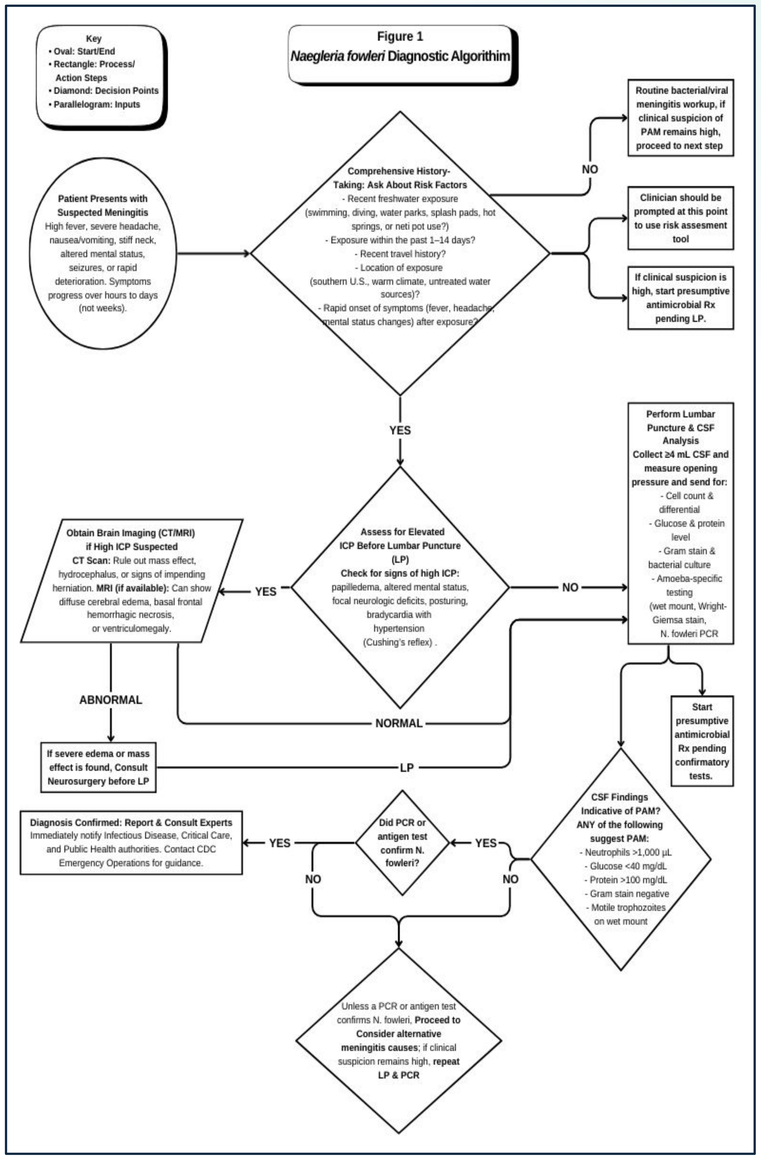
Methods

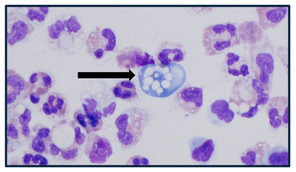
Diagnostic algorithm (Figure 1) is a stepwise workflow to triage suspected primary amebic meningoencephalitis, emphasizing early distinguishing clues and translating them into actions. **Table 1** converts each decision point into a scored criterion, progressing from history and symptoms to cerebrospinal fluid and microbiology and then to definitive evidence; totals stratify patients into **prespecified bands (≤8, 9 to 15, ≥16)** mapped to reassess, consider therapy while confirming, or treat immediately and notify public health. **Retrospective validation** used **13 de identified vignettes (5 primary amebic meningoencephalitis [PAM], 8 comparators)** scored by **10 blinded clinicians** with a point based rubric. **Sensitivity** and **specificity** at each threshold were estimated with two sided **95% exact binomial** (Clopper Pearson) intervals. **Discrimination** of the continuous score used the **receiver operating characteristic (ROC) curve** and **area under the curve (AUC)** with **DeLong 95% intervals**. **Interrater agreement** was quantified with the **intraclass correlation coefficient (ICC), two way random effects, absolute agreement, single measure**, with **95% intervals**.

Criterion	Value
Fever and headache	3
Altered mental status, disorientation, or seizures	2
Coma or respiratory failure within 48 hours	3
Meningeal signs such as neck stiffness, Kernig sign, or Brudzinski sign	1
Nasal exposure to warm freshwater or similar (freshwater swimming, splash pads, water sports, neti pot use, ablution)	3
Exposure in an endemic area or during warm months	1
Cerebrospinal fluid white blood cell count greater than 1000 per microliter with neutrophilic predominance	2
Cerebrospinal fluid protein greater than 200 milligrams per deciliter and glucose less than 40 milligrams per deciliter	2
Cerebrospinal fluid opening pressure greater than 250 millimeters of water	1
Gram stain and culture negative for bacteria	2
Multiplex panel negative for other pathogens	3
Motile trophozoites on wet mount (definitive evidence)	3
Polymerase chain reaction positive for Naegleria *fowleri* (definitive evidence)	3
Positive cytospin smear with Wright Giemsa or hematoxylin and eosin staining (definitive evidence)	2

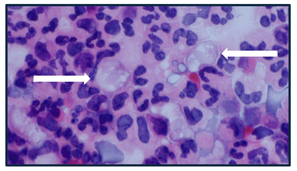
Table 1. PAM scoring rubric used by reviewers with criteria and corresponding point values.

Figure 1
***Naegleria fowleri* Diagnostic Algorithm**





Giemsa-stained CSF smear showing *Naegleria fowleri* trophozoite. Note the single nucleus with central karyosome and cytoplasmic vacuoles, characteristic of the trophozoite stage in PAM. *Image source: CDC / Dr. James Roberts*



H&E-stained CSF smear showing *Naegleria fowleri* trophozoites (arrows). Trophozoites appear amid inflammatory cells, aiding in the cytological diagnosis of PAM. *Image source: CDC / Dr. James Roberts*

Results

Statistical model and analysis: We used an **additive point-based risk-score classifier**. Discrimination was assessed with the **receiver operating characteristic (ROC)** curve and its **area under the curve (AUC)**. Agreement between reviewers was measured with the **intraclass correlation coefficient (ICC)**.

0–8 points: No **PAM**cases flagged; **specificity** = 1.00.

9–15 points: Sensitivity = 1.00; **specificity** = 0.85. Functions as a safety-net **threshold** for early intervention.

≥16 points: Sensitivity = 1.00; **specificity** = 1.00, achieving complete separation between **PAM** and comparator cases.

Reviewer agreement was excellent (**ICC** = 0.981). Score discrimination was complete (**AUC** = 1.00), and reviewer medians closely tracked baseline scores (Table 3). **False positives** at the 9-point **threshold** were concentrated in one noninfectious aseptic comparator case, reflecting the **algorithm's** design to flag aseptic presentations while awaiting **definitive testing**. No **false positives** occurred at the 16-point **threshold** (Table 2).

Operationalizing the Algorithm

A **risk score** runs alongside a **stepwise flowchart** to enforce sequence and safety: confirm **exposure risk**, assess rapid **neurological decline**, clear **lumbar puncture (LP)** with **neuroimaging (CT)**, and perform an immediate **wet mount**. In the **electronic health record (EHR)**, a **two-question gate** embedded in the head **CT** or **LP** order triggers the score: (1) ask about **warm freshwater** or **inadequately chlorinated water** exposure in the prior **1–14 days**, **non-sterile nasal rinsing**, **ritual ablution**, or **recent travel** that increases such exposure; if **yes or unsure**, (2) ask about **neurologic decline within 24–48 hours**. If both suggest risk, the **scoring sheet auto-totals** and links to **empiric therapy**, **CSF handling** for **wet mount** and **cytospin**, **Naegleria *fowleri* PCR**, **public health notification**, and **intracranial pressure management**.

Threshold or Band	Lens	Sensitivity (95% C.I.)	Specificity (95% C.I.)
Low: ≤ 8	Score only and Bias aware	1.00 (0.929-1.000)	0.85 (0.756-0.912)
Intermediate: 9-15	Score only and Bias aware	1.00 (0.929-1.000)	0.85 (0.756-0.912)
Intermediate: 9-15	Policy aware	1.00 (0.929-1.000)	1.00 (0.954-1.000)
High: ≥ 16	All lenses	1.00 (0.929-1.000)	1.00 (0.954-1.000)

Table 2. Interpretation bands and diagnostic accuracy by analytic lens.

Case	Baseline Total	Reviewer Median	Reviewer Minimum	Reviewer Maximum	Median - Baseline
1	26	26	24	28	0
2	26	26	23	26	0
3	5	5	5	5	0
4	8	8	8	11	0
5	5	5	2	7	0
6	23	24.5	20	26	1.5
7	5	5	3	10	0
8	4	4	2	4	0
9	5	7.5	3	8	2.5
10	24	22	17	22	-2.0
11	10	10	10	11	0
12	3	3	3	6	0
13	28	28	26	28	0

Table 3. Baseline template versus reviewer totals by case.

Discussion and Conclusions

PAM presents diagnostic challenges due to **nonspecific early symptoms** and resemblance to **bacterial meningitis**. The validated **algorithm** closes these gaps by combining **exposure history**, **rapid symptom progression**, and hallmark **CSF findings** into actionable thresholds. The **"Consider Therapy"** threshold maximizes sensitivity, while the **"Treat Now"** threshold provides a decisive trigger for immediate intervention.

EHR ntegration reduces burden and speeds action. Screening starts with a **single exposure question** embedded in **head CT** or **lumbar puncture** orders. If risk is identified, the **score auto-calculates** and links to **empiric therapy**, **specimen handling**, and **CDC** guidance, accelerating time to **suspicion**, **lumbar puncture**, and **definitive testing**. Sites without full EHR support can use a **printable checklist** that mirrors the same thresholds and actions.

This validated **scoring algorithm** enables earlier recognition of PAM during the **critical treatment window**. Its **high sensitivity and specificity**, together with practical **EHR integration**, make it well suited for **emergency settings**. **Prospective studies**, **multicenter validation**, and **public health integration** are recommended to further improve outcomes.