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Mycobacterium Tuberculosis, Herpes Simplex Virus Type 1 and *Enterovirus* in Clear Cerebrospinal Fluid Meningitis at Brazzaville University Hospital

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ABSTRACT

Introduction: Clear cerebrospinal fluid (CSF) meningitis represents a major public health problem in resource-limited countries. It is characterized by CSF pleocytosis with a macroscopically clear appearance, and its etiologies are mainly viral, tuberculous, or undetermined.

Objective: This study aimed to analyze clear CSF samples from a cytological and biochemical perspective, identify the main pathogens, and assess the diagnostic yield of examinations performed at the Brazzaville University Hospital laboratory.

Methods: An analytical cross-sectional study with prospective data collection was conducted from April 1 to September 30, 2021, including all clear CSF samples received at the bacteriology-virology-immunology laboratory of CHU-B. Analyses included cytology, biochemistry, and PCR detection of *Mycobacterium tuberculosis*, HSV-1, and *Enterovirus*.

Results: The frequency of clear CSF meningitis was 48.6%. Patients under 5 years accounted for 67.9% of cases, with a slight male predominance (sex ratio 1.03). Cytology showed a mean of 245 leukocytes/mm^3, with lymphocytic predominance. Protein levels were elevated (2.02 g/L) in all cases, while hypoglycorrhachia was observed only in tuberculous meningitis. The pathogens identified were mainly *Enterovirus* (20.3%), followed by *Mycobacterium tuberculosis* (5.8%) and HSV-1 (11.6%). A large proportion of cases (63.8%) remained of undetermined etiology. Diagnostic yield was higher among infants (<1 year, 53.6%) and young adults (20–40 years, 61.5%).

Conclusion: Clear CSF meningitis is frequent in Brazzaville, predominantly affecting young children. Cytological and biochemical CSF profiles were consistent with the identified etiologies. Improving laboratory capacity, particularly through systematic integration of multiplex film array nucleic acid amplification tests, is essential to reduce the number of undetermined cases and strengthen diagnostic and therapeutic management.

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Introduction

Meningitis is a severe condition characterized by inflammation of the meninges, usually associated with cerebrospinal fluid (CSF) pleocytosis. When the involvement extends to the brain parenchyma, it may progress to meningoencephalitis [1]. This disease remains a major global public health issue, affecting all age groups. In 2017, nearly five million new cases of meningitis were reported, resulting in approximately 290,000 deaths [2]. More recently, the Global Burden of Disease 2019 study confirmed that meningitis continues to be a significant cause of morbidity and mortality, particularly in sub-Saharan Africa [3-9].

Meningitis may be of infectious or non-infectious origin. Among infectious forms, purulent meningitis and clear CSF meningitis are distinguished. The latter mainly includes viral, tuberculous, fungal, and parasitic meningitis. In industrialized countries, the annual incidence of meningitis is estimated at 3.97 per 100,000 inhabitants, with 2.73 per 100,000 attributed to viral meningitis. The main viral agents involved are *Enterovirus*, Herpes simplex

virus, and Varicella-zoster virus [3]. In North Africa, the prevalence of clear CSF meningitis ranges between 55% and 61.9%, with viral causes predominating, followed by *Mycobacterium tuberculosis* [4,5]. Parasitic and fungal etiologies have also been reported [6,7]. More recently, studies have highlighted the emergence of opportunistic causes linked to immunosuppression, particularly among patients living with HIV [8-10].

Clinically, manifestations are often common to all clear CSF meningitis, making CSF analysis indispensable for diagnosis. In resource-limited countries, cytological and biochemical CSF examination remains the essential first step in etiological diagnosis [7]. The introduction of new techniques, such as antigen detection and multiplex PCR, has improved diagnostic speed and sensitivity, but their accessibility remains limited in many African settings [8-11]. Recent studies emphasize the need to integrate these tools into reference laboratories to strengthen surveillance and patient management [12].

In Congo, data on clear CSF meningitis are scarce and fragmented. This lack limits understanding of its epidemiological and biological profile, as well as evaluation of the diagnostic yield of available tests.

The objective of this study was to analyze clear CSF samples from cytological and biochemical perspectives, identify the main pathogens, and assess the diagnostic yield of examinations performed at the Brazzaville University Hospital laboratory.

Methodology

Study Design

This was an analytical cross-sectional study with prospective data collection. It was conducted at the Brazzaville University Hospital (CHU-B), the main referral center of the national health system of Congo, located in Moungali. The study period extended from April 1 to September 30, 2021 (6 months). Analyses were performed in the bacteriology-virology-immunology department of CHU-B, which has a real-time PCR unit.

Study Population

The target population consisted of all patients, regardless of age or sex, who underwent lumbar puncture and whose cerebrospinal fluid (CSF) presented a macroscopically clear appearance.

Inclusion Criteria: Clear CSF samples received at the laboratory within ≤ 2 hours, stored at −20 °C, with a volume ≥ 1000 µL.
Non-inclusion Criteria: Samples delivered beyond 2 hours or not respecting the cold chain.
Exclusion Criteria: CSF volume < 1000 µL.

A flow diagram (Figure 1) was used to illustrate the inclusion and exclusion procedure.

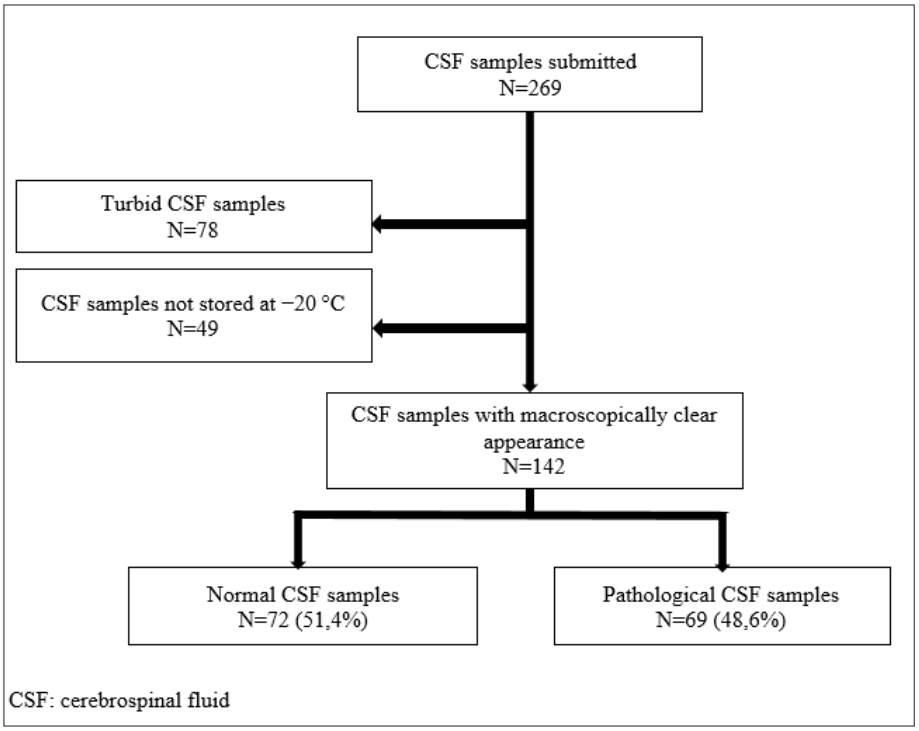

Figure 1: Flow Diagram of CSF Sample Selection

Sampling

Sampling was exhaustive, including all clear CSF samples meeting the inclusion criteria and received during the study period. The final sample size corresponded to the number of specimens included after applying the criteria.

Data Collection Procedure

Data were collected using a standardized form, based on the examination request forms accompanying the samples.

Variables Studied

Epidemiological: Age, Sex, Origin of CSF.

Biological: Macroscopic Appearance, Leukocyte Count and Differential, Protein Concentration, Glucose Concentration, Identified Pathogens.

Biological Analyses

Macroscopy

The macroscopic appearance of CSF was confirmed visually in daylight. Only samples with a clear appearance were retained for the study.

Cytology

Leukocyte quantification was performed using the Nageotte counting chamber, allowing precise enumeration per mm^3. Qualitative analysis was carried out after centrifugation of CSF, spreading of the pellet on slides, fixation, and staining with methylene blue. The leukocyte differential was determined by counting 100 leukocytes, distinguishing neutrophils and lymphocytes.

Biochemistry

Protein (proteinorachia) and glucose (glycorachia) concentrations were measured in partner private laboratories using standard automated spectrophotometers and dedicated reagent kits. Proteins were assayed by colorimetric methods (biuret or equivalent), and glucose by enzymatic methods using glucose oxidase/peroxidase. Results were recorded from patients' laboratory reports.

Nucleic Acid Extraction

- DNA (*Mycobacterium tuberculosis*, HSV-1): Extraction was performed either with Chelex-100® resin (Bio-Rad) for mycobacteria or with the commercial DNA NORGEN BIOTEK CORP® kit for HSV-1, following the manufacturer's protocols.

- RNA (*Enterovirus*): Extraction was performed manually using the RNA NORGEN BIOTEK CORP® kit, with steps of lysis, column binding, successive washes, and final elution. Extracts were checked by nanodrop quantification.

Bacteriology Detection

Detection of *Mycobacterium tuberculosis* was performed by real-time PCR targeting the IS6110 sequence. Amplification was carried out on the MiniOpticon (Bio-Rad), with curve analysis using CFX-Manager software. Specific primers and probes were supplied by Omunis (Clapiers, France).

Virology Detection

Nucleic acid amplification was performed by real time PCR on the Fast 7500 Dx real-time PCR system (Applied Biosystems®) using TaqMan® technology.

Then HSV-1 detection was performed using the RealStar® HSV PCR Kit 1.0 and RealStar® *Enterovirus* RT-PCR Kit 1.0 (Altona Diagnostics®), targeting the UL29 region (129 bp). Fluorescent probes FAM™ and JOE™ allowed detection of viral DNA and internal control, respectively. *Enterovirus* viral RNA was converted into cDNA by reverse transcriptase, then amplified and detected using specific fluorescent probes.

Results were automatically interpreted by the software associated with the PCR system, based on amplification curves and internal controls provided by the kits.

Operational Definitions

Pleocytosis: >10 leukocytes/mm^3.
Pathological proteinorachia: >0.40 g/L.
Low glycorrhachia: <0.5 g/L.
Clear CSF meningitis: pleocytosis associated with proteinorachia >0.40 g/L, with macroscopically clear CSF.

Statistical Analysis

Data were entered into CsPro 7.2, exported to Excel 2016, and analyzed with SPSS 25.
Qualitative variables were expressed as counts and percentages. Quantitative variables were presented as means ± standard deviation.
Proportion comparisons used Pearson's Chi^2 test or Fisher's exact test when counts <5.
The significance threshold was set at $p < 0.05$.
Diagnostic yield was calculated by relating the number of pathological CSF samples to the total number of samples received, expressed as a percentage according to age and origin.

Ethical Considerations

The study was approved by the Faculty of Health Sciences, the Brazzaville University Hospital, and the Research Ethics Committee in Health Sciences (IRSSA). Patient anonymity was ensured by assigning an identification number to each sample. Digital data were password-protected. No conflict of interest was declared.

Results

Frequency of Clear CSF Meningitis

The frequency of clear CSF meningitis (CCSM) at the bacteriology-virology-immunology laboratory of CHU-B was 48.6%.

Sociodemographic Characteristics

Table 1 shows the distribution of patients with clear CSF meningitis according to age, sex, and origin of samples. It highlights the predominance of infants under one year of age and the balanced sex ratio, with most samples originating from CHU-B. The mean age was 9.41 ± 15.90 years (median: 1.21), and the sex ratio was 1.03 (Male/Female).

Table 1: Sociodemographic Characteristics of Patients with Clear CSF Meningitis (n = 142)

Variables	Categories	n	%
Age (years)	[0 – 1]	69	48.6
	[1 – 5]	27	19.0
	[5 – 10]	11	7.7
	[10 – 20]	11	7.7
	[20 – 40]	13	9.2
	[40 – 60]	9	6.3
	> 60	2	1.4
Sex	Female	70	49.3
	Male	72	50.7
Origin of samples	CHU B	114	80.3
	Blanche Gomes Hospital	22	15.5
	TRH (Talangaï) *	6	4.2

*TRH: Talangaï Reference Hospital

Macroscopic Characteristics of CSF

All included samples presented a macroscopically clear appearance.

Cytological and Biochemical Characteristics of Pathological CSF (n = 69)

Table 2 summarizes the main cytological and biochemical characteristics of pathological CSF. It highlights a constant lymphocytic predominance, elevated protein levels in all cases, and hypoglycorrhachia specific to tuberculous meningitis.

Table 2: Cytological and Biochemical Characteristics of Pathological CSF (n = 69)

Parameter	Mean ± SD	Median	Range	Particularities
Leukocyte count (cells/mm³)	245.16 ± 45.00	8	0 – 1000	Lymphocytic predominance (100%)
Glycorrhachia (g/L)	0.54 ± 0.19	0.55	0.22 – 1.32	Hypoglycorrhachia observed only in tuberculous cases
Proteinorachia (g/L)	2.02 ± 1.51	1.71	0.23 – 6.52	Elevated protein levels in all cases

Etiology and CSF Characteristics (n = 69)

Table 3 presents the distribution of identified etiologies and the cytological and biochemical characteristics of pathological CSF. It highlights the predominance of viral and tuberculous profiles, as well as the high proportion of cases with undetermined etiology.

Table 3: Etiology and Cytological/Biochemical Characteristics of Pathological CSF (n = 69)

Etiology	n (%)	Cytology Mean ± SD (cells/mm³)	Range	Median (Q1–Q3)	Glycorrhachia Mean ± SD (g/L)	Range	Median (Q1–Q3)	Proteinorachia Mean ± SD (g/L)	Range	Median (Q1–Q3)
HSV 1	17 (24.6)	352.50 ± 419.87	19–1000	157 (34–852.75)	0.61 ± 0.08	0.48–0.72	0.61 (0.58–0.69)	1.51 ± 0.69	0.25–2.28	1.71 (1.02–1.99)
Enterovirus	4 (5.8)	206.64 ± 379.30	10–1000	19 (10.75–206)	0.57 ± 0.14	0.34–0.95	0.55 (0.50–0.64)	1.40 ± 0.35	0.35–3.05	1.26 (0.81–1.99)
M. tuberculosis	4 (5.8)	434.75 ± 383.62	158–1000	290.5 (180.25–833.5)	0.38 ± 0.12	0.22–0.50	0.39 (0.25–0.49)	3.63 ± 0.95	2.59–4.54	3.69 (2.71–4.49)
Co infection (EV + MTB)	1 (1.4)	—	—	—	—	—	—	—	—	—
Undetermined	44 (63.8)	237.82 (mean)	11–1000	—	0.55 ± 0.22	—	—	2.14 ± 1.61	—	—

Diagnostic Yield

This table presents the diagnostic yield of clear CSF meningitis according to hospital structure and age groups. It highlights better performance among infants and young adults, as well as variability depending on the origin of samples.

Table 4: Diagnostic Yield of Clear CSF Meningitis According to Hospital Structure and Age (n = 142)

Variables	Categories	n	Pathological cases (%)
Structure	CHU B	114	58 (50.9)
	Blanche Gomes	22	10 (45.5)
	TRH (Talangaï)*	6	1 (16.7)
Age group (years)	[0 – 1]	69	37 (53.6)
	[1 – 5]	27	11 (40.7)
	[5 – 10]	11	1 (9.1)
	[10 – 20]	11	5 (45.5)
	[20 – 40]	13	8 (61.5)
	[40 – 60]	9	5 (55.6)
	> 60	2	2 (100.0)

*TRH: Talangaï Reference Hospital

Associated Factors

Table 5 presents the results of statistical analysis of factors associated with the occurrence of CCSM. No significant correlation was observed for hospital structure, sex, or age group.

Table 5: Analysis of Factors Associated with the Occurrence of Clear CSF Meningitis (CCSM)

Factor studied	Valeur de p	Corrélation significative
Hospital structure	0.302	No
Sex	0.097	No
Age group	0.062	No

Discussion

Clear cerebrospinal fluid (CSF) meningitis remains a frequent condition in resource-limited countries and represents a major public health challenge. The prevalence observed in our study (48.6%) falls within the range reported by several African and international studies, where it varies between 45% and 62% [6]. Recent surveillance data from WHO in the African meningitis belt confirm that clear CSF forms account for a substantial proportion of cases, particularly in pediatric settings [12].

The median age of 1.21 years and the predominance of children under five years (67.9%) are consistent with international observations. In China, Hu et al. reported a median age of 1.75 years, while in Saudi Arabia Aldreweesh et al. found a frequency of 66% among children under four years [13-14]. Similarly, Ghuneim et al. in Palestine reported a frequency of 85.2% among children under five years [15]. These findings confirm the particular vulnerability of young children, related to the immaturity of the blood–brain barrier and the fragility of the immune system. The slight male predominance observed in our series (sex ratio 1.03) has also been reported in several multicenter studies, without a clear biological explanation, but probably linked to local demographic structure [16,17].

The cytological and biochemical characteristics of CSF observed in our study are consistent with those described in the literature. The mean leukocyte count of 245 cells/mm³, with lymphocytic predominance, aligns with profiles reported in viral and tuberculous meningitis [4]. Elevated protein levels (2.02 g/L) and reduced glucose levels (0.54 g/L) are comparable to results obtained in Morocco and the United States [18,19]. The distinction between viral profiles (moderate proteinorachia, normal glycorrhachia) and tuberculous profiles (high proteinorachia, low glycorrhachia) was clearly observed in our series, confirming the findings of Caudi et al. in France and Dollo et al. in Morocco [20,21].

From an etiological perspective, viruses were the most frequent agents, with *Enterovirus* (20.3%) and HSV-1 (11.6%), followed by *Mycobacterium tuberculosis* (5.8%). These results are consistent with international data, where *Enterovirus* remain the main agents of clear CSF meningitis, accounting for 30–45% of cases in Europe and Asia [3-22]. The proportion of HSV-1 meningitis is lower, generally between 8% and 13%, which is in line with our findings [23]. In Africa, tuberculous meningitis remains an important cause, especially in areas with high HIV prevalence [10]. The high proportion of undetermined etiologies (63.8%) in our study reflects local diagnostic limitations, related to the high cost and limited availability of multiplex PCR tests, a problem widely highlighted in recent studies on meningitis management in sub-Saharan Africa [11].

Diagnostic yield was higher among infants under one year (53.6%) and young adults aged 20–40 years (61.5%). These results are consistent with recent WHO observations [12-24], which show that clinical suspicion is stronger in these age groups, favoring lumbar puncture. The lower yield in other age groups underscores the importance of better clinical information on laboratory request forms and broader access to biochemical and molecular tests.

Ultimately, our results confirm that clear CSF meningitis is a frequent condition in Brazzaville, mainly affecting young children. The cytological and biochemical CSF profiles are consistent with those described in recent literature. Viruses (*Enterovirus*, HSV-1) and *Mycobacterium tuberculosis* were the main identified etiologies, but a large proportion of cases remained undetermined, reflecting local diagnostic limitations. Improving patient management requires the integration of modern molecular techniques, standardization of biochemical analyses, and better clinical data collection, in line with WHO's global meningitis strategy [12].

This study has certain limitations, including the absence of advanced diagnostic techniques (multiplex PCR, antigen tests), the sometimes reduced volume of CSF samples, and incomplete collection of clinical data. In addition, the relatively short duration (six months) did not allow assessment of seasonal or multi-year variations. These constraints explain the high proportion of undetermined cases and highlight the need to strengthen diagnostic capacity and data quality in future research. Despite these limitations, this study provides novel data on clear CSF meningitis in Brazzaville and constitutes a solid basis for improving epidemiological surveillance and strengthening diagnostic capacity [25].

Conclusion

Clear CSF meningitis is a frequent condition in our setting, mainly affecting infants and young adults. Cytological and biochemical CSF analysis showed elevated protein levels in all cases, while hypoglycorrhachia was specific to tuberculous meningitis. The identified agents were mainly *Enterovirus*, followed by *Mycobacterium tuberculosis* and HSV-1, but a significant proportion of cases remained of undetermined etiology, reflecting current diagnostic limitations. Improving laboratory capacity, particularly through systematic integration of nucleic acid amplification tests (multiplex syndromic PCR, FilmArray®), appears essential to reduce undetermined cases and strengthen diagnostic and therapeutic management. Beyond these findings, this study provides novel data for Brazzaville and constitutes a solid basis for initiating national epidemiological surveillance.

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