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Cerebrospinal Fluid Lactate as a Differential Biomarker for Bacterial and Viral Meningitis

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Abstract

Meningitis is a critical public health problem demanding immediate diagnosis and effective treatment due to high mortality rates. Cerebrospinal Fluid (CSF) lactate concentration is a promising test to distinguish bacterial from viral meningitis. This study aimed to assess the performance and usefulness of CSF lactate as a biomarker to differentiate between bacterial and viral meningitis, and to determine its optimal level to differentiate between them. This prospective study included 50 patients, presented to Abbassia Fever Hospital with clinical findings consistent with meningitis. Patients were divided into two groups: Group1 included 30 patients with bacterial meningitis. Group 2 included 20 patients with viral meningitis. CSF lactate and other conventional CSF parameters were recorded. For CSF culture, *Streptococcus pneumoniae* was identified in 53.3% of the bacterial meningitis group. The polymerase chain reaction (PCR) indicated that *S. pneumoniae* was present in 26/50 (52%) and 3/50 (6%) patients were PCR negative. Among bacterial meningitis patients, *S. pneumoniae* was the most pervasive organism 26/30 (86.7%). The mean CSF lactate level was 9.3 mmol/l ±5.0 (2.2-17.6). There was a statistically significant strong agreement (Kappa=0.957) between types of meningitis diagnosed by PCR, culture, and CSF lactate at cutoff level of 7.2 mmol/L. This cutoff value was the best to differentiate between bacterial and viral meningitis. The validity of CSF lactate as a differentiating tool showed sensitivity, specificity, positive predictive value, and negative predictive value of 93.3%, 100%, 100%, and 90.9%, respectively. In conclusion, CSF lactate could be a valuable, sensitive, specific, and rapid marker for identifying the most dangerous bacterial causes of CNS infection, especially *S. pneumoniae*. CSF lactate can be routinely used as an early biochemical warning marker and a useful point-of-care test. CSF lactate at cutoff level of >7.2 mmol/L can accurately detect *S. pneumoniae*, the most prevalent organism in Egypt.

Keywords: Meningitis; Lactate; CSF culture; CSF markers; *Streptococcus pneumoniae*.

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Introduction

Meningitis is a serious public health problem demanding early diagnosis, effective treatment, prevention, and control due to significant mortality rates. Any delay in starting the appropriate remedy might worsen the prognosis.[1] Unlike bacterial meningitis which

results in considerable morbidity and fatality rates even with advancements in antimicrobial therapy, aseptic meningitis is essentially requiring only supportive care.[2] Although the vaccines against prevailing pathogens are available, bacterial meningitis continues to be a health problem with long-term sequelae in children and adults, especially in low-income countries.[3]

In the past, most of the cases of acute meningitis were caused by *Haemophilus influenzae type b, Neisseria meningitidis, and Streptococcus pneumoniae*. But after the introduction of *H. influenzae type b* vaccines, the incidence of *H. influenzae type b* disease significantly reduced by 94%.[4] In Egypt, it was found that *S. pneumoniae* was recently described as the leading cause of bacterial meningitis.[5]

Differentiating acute bacterial and viral meningitis is not always easy. Though culture is the gold standard for diagnosis, the results are only available after several days. Rapid diagnosis carried out by assessment of conventional markers in cerebrospinal fluid (CSF) including proteins, glucose, leucocytes count, gram-staining, and ratio of CSF/serum glucose, was proposed as an effective method for differentiating acute viral meningitis from bacterial meningitis.[6] However, sometimes meningitis presents with atypical CSF manifestations, the symptoms and laboratory assays are often similar and overlapping, and cultures may not always be positive or available for early diagnosis. All these issues might pose a hindrance in front of the rapid diagnostic methods for diagnosing and differentiating bacterial and viral meningitis.[7]

CSF lactate concentration is a beneficial test that can distinguish bacterial from viral meningitis.[8] It results from anaerobic metabolism and its level rises in any situation associated with a lowering in oxygen supply to the brain with no correlation with the serum lactate level.[9] CSF lactate in bacterial meningitis comes from several sources. Bacterial pathogens generate varying quantities of lactate, representing 10% of the total CSF lactate.[10] Bacterial meningitis is associated with many pathological changes that cause cerebral ischemia and thus glycolysis via anaerobic metabolism. In addition, cytokines can lead to anaerobic metabolism and thus boost CSF lactate production by either flooding the brain interfering with the tissue oxygen uptake or directing the neutrophils into the subarachnoid space resulting in glycolysis.[11] Despite this, there is a debate among the investigators, some think that the elevated CSF lactate level is a non-specific finding and occurs in several diseases such as meningitis, hypoxic cerebral injury, subarachnoid hemorrhage, and head injury.[8] However, others believe that a CSF lactate level of 3.0 mmol/l or greater has been considered superior to the other CSF tests to diagnose and differentiate between bacterial and viral meningitis[1.] CSF lactate, at cut off 35 mg/dl (3.8 mmol/l) showed the best sensitivity for distinguishing between bacterial and viral meningitis.[8] This study aimed to assess the performance and discuss the usefulness of CSF lactate as a biomarker to differentiate between bacterial meningitis and viral meningitis in adult population, as well as to determine an optimal CSF lactate level that can be significant for the differentiation between the various types of meningitis.

Subjects and Methods

This prospective study included 50 patients, presented to the Emergency Department of the Abbassia Fever Hospital (Cairo, Egypt) with clinical findings consistent with meningitis (e.g. fever, headache, vomiting, nuchal rigidity, and impaired consciousness). This study was conducted during the period from October 2019 to February 2022.

After the initial clinical assessment initial, laboratory tests were done. For these, we collected blood samples for performing blood cultures simultaneously with CSF samples. We categorized the patients according to baseline investigations and lumbar puncture (CSF analysis and culture) into two groups. Group 1 included 30 patients with bacterial meningitis. While Group 2 included 20 patients with viral meningitis.

The inclusion criteria of patients in the study included the following for Group 1 (bacterial

meningitis), the criteria comprised patients positive CSF or blood culture, positive gram-staining, or CSF total leucocytic count (TLC) > 1,000/mm^3 with any of the following: CSF neutrophil > 60%, CSF glucose <40% of random blood sugar (RBS), a ratio of CSF glucose to RBS of ≤ 0.4 and CSF protein: 50 - 1000 mg/dL.

Group 2 (viral meningitis) the criteria comprised patients with any of the following: negative CSF and blood culture with positive polymerase chain reaction (PCR), or CSF total leucocytic count (<1,000/mm^3) with any of the following: predominant lymphocytic pleocytosis, CSF glucose > 60% of RBS and CSF protein: 50 – 250 mg/dL[1].

The exclusion criteria comprised patients with any of the following: critical illness, recent neurosurgical intervention, trauma, any non-meningitis focus of infection and those who had received antibiotics before hospitalization.

Ethical considerations

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Ain Shams University. (Ethical approval number: FMASU MD 297/2019, September 2019). An informed written consent was obtained from each patient before enrollment in the study.

Study procedures

Lumbar puncture was done for all 50 patients after the initial clinical assessment, and CSF samples were obtained under complete aseptic precautions [4] . The total leukocytic count, and RBS results were obtained from the hospital records of the patient. Blood samples were collected for performing blood cultures using BD BACTEC™ blood culture bottles (Becton Dickinson, Sparks, MD, USA).

All the CSF samples were subjected to physical examination (appearance), biochemical analysis, cytological examination, culture (conventional and after enrichment by inoculation on BD BACTEC™ pediatric blood culture bottles (Becton Dickinson, Sparks, MD, USA), and multiplex PCR to detect the most common bacterial meningitis, *N. meningitidis*, *S. pneumoniae* and *H. influenzae* and viral pathogens (*Herpes simplex virus*, *Epstein barrvirus* and *human Enterovirus*).

Blood Culture

BD BACTEC™ blood culture bottles (Becton Dickinson, Sparks, MD, USA) were inoculated with 8-10 ml of blood for each patient. Blood samples were collected under complete aseptic conditions. We incubated the blood culture bottles in the BACTEC 9050 series instrument (Becton Dickinson, Sparks, MD, USA) for seven days maximum, according to the manufacturer's instructions. When a bottle was signaled positive by BACTEC instrument, a gram-stained film and subculture onto blood agar, MacConkey agar, and chocolate agar were done. The agar plates were incubated in the appropriate atmospheric conditions at 37 °C with 5 – 10% CO2 for 18-24 hours. The culture plates were examined for any evidence of growth, and in case of absent growth, they were re-incubated and re-examined daily for 72 hours before reporting them negative. We identified any retrieved organism by culture morphology, gram-stained films, biochemical reactions.[4]

Gram-positive bacteria were identified via testing the hemolytic activity on blood agar and further identification using different biochemical tests as catalase reaction, culture on bile esculin, in addition to different differentiating antibiotic discs as optochin and bacitracin. For gram-negative bacteria, identification was conducted by biochemical tests such as oxidase, triple sugar iron, motility indole ornithine, citrate, lysine iron arginine, and urease tests. All media were supplied by (Oxoid, Uk).[12]

Physical examination of the CSF

CSF samples were examined for the presence of turbidity. Normally, CSF is perfectly clear, and transparent. Pathologically, it may be turbid/cloudy.

Biochemical analysis of CSF

The CSF glucose and protein results were collected from the hospital records of the patients. CSF glucose concentration's normal value is 40 - 70 mg/dl (2.2-3.9 mmol/L), approximately 60% of the RBS level. The CSF

protein concentration's normal value is 15-48 mg/dl.[4]

We measured the CSF lactate by the Enzymatic Colorimetric method (Lactate oxidase/ Peroxidase) (MG; Science and Technology Center) by a manual photometer (5010) (ROBERT RIELE Gmbh & CO KG, Germany). The absorbance of the CSF lactate was measured by an end point assay at a wavelength of 546 nm, according to the manufacturer's instructions. Regarding the CSF lactate level, the expected values in Adult CSF are 10 - 22 mg/dL (1.1 - 2.4 mmol/L), and in Neonates 10 – 60 mg/dL (1.1 - 6.7 mmol/L).

Cytological examinations

We manually measured the CSF total leukocytic count by microscopic examination of a well-mixed, uncentrifuged fluid in a slide counting chamber (hemocytometer).[4] To examine the type of cells, we made a smear and stained it with Leishman stain. Moreover, to ensure that it correlates well with the cell count, neutrophils and lymphocytes were counted.

Gram stain

For each positive bacterial culture, a slide was stained by Gram stain to identify the causative bacteria organism according to the UK standards for staining procedures.[13] The Gram stain was examined immediately and observed for the presence of pus cells and bacteria.

Culture of CSF samples

From each participant, a CSF sample was centrifuged for 20 min at 3000 xg, and the sediment was cultured on conventional media such as chocolate agar, blood agar, and MacConkey agar (Oxoid, UK) for diagnosing aerobic bacteria. The agar plates were incubated in the appropriate atmospheric conditions at 37 °C with 5 – 10% CO2 for 18-24 hours. We examined the culture plates for any evidence of growth, and in case of absent growth, they were re-incubated and re-examined daily for 72 hours before reporting them negative. We identified any retrieved organism by culture morphology, gram-stained films, biochemical reactions.[12]

In parallel with agar culture on solid media, we inoculated 1-3 ml of each sample into a BD BACTEC™ Peds Plus™ /F Culture bottle (Becton Dickinson, Sparks, MD, USA) supplemented with resins. We incubated the blood culture bottles in the BACTEC 9050 series instrument (Becton Dickinson, Sparks, MD, USA) for seven days maximum, according to the manufacturer's instructions. When a bottle was signaled positive by BACTEC 9050, a gram-stained film and subculture onto blood agar, MacConkey agar, and chocolate agar were done. All plates were processed as described above in the CSF culture.

Multiplex Polymerase Chain Reaction (PCR)

PCR was done for the detection of bacterial pathogens *N. meningitidis*, *S. pneumoniae* and *H. influenzae* and viral pathogens (*Herpes simplex virus*, *Epstein barrvirus* and human *Enterovirus*) in the 50 CSF collected samples.

DNA extraction, amplification, and detection

For extraction of *S. pneumoniae*, *H. influenzae*, and *N. meningitidis* DNA from CSF samples, we used commercially available kits (QIA amp DNA Mini Kit, Qiagen, USA), according to the manufacturer's instructions. As for the simultaneous purification of viral DNA and RNA, we used commercial kits (QIAamp MinElute Virus Spin Kit, Qiagen, USA) according to the manufacturer's instructions.

For DNA amplification and detection, we used two real-time (RT) PCR Detection Kits (VIASURE®, CerTes tBiotec, S.L. San Mateo de Gallego, Zaragoza, Spain), one for the detection of the bacteria pathogens and the other for the viral pathogen in CSF specimens from symptomatic patients. For the RT-PCR methos, we used a thermal cycler instrument (CFX96TM Deep Well IVD Real-Time PCR Detection System, Bio-Rad, USA). Each run for each assay included a positive and negative control.

We programmed the thermocycler with the following conditions: one cycle for initial denaturation at 95 °C for 2 minutes, followed by 45 cycles each of denaturation at 95ºC for 10 seconds, and annealing/extension at 60°C for 50 seconds. Fluorogenic data were collected during the extension step.

The samples were analyzed and interpreted according to the manufacturer's instructions[14]. First, we validated the reaction by checking the negative control well, the positive control well and then the internal control signal. A sample was considered positive if the cycle of threshold (Ct) value was less than 40. On the other hand, the sample was negative if it showed no amplification signal in the detection system with positive internal control.

Statistical analysis

Data were tabulated and statistically analyzed using SPSS, version 20 (SPSS Inc., Chicago, IL). Quantitative data were described as mean and standard deviation (minimum – maximum) and median (Interquartile Range) (IQR). Independent sample Mann Whitney test and Kruskal Wallis test were used for comparing quantitative variables between groups. Qualitative data were expressed as frequencies (n) and percentage (%). The Fisher exact test and chi square test were used to test the association between qualitative variables. Pearson correlation coefficient was used to correlate between quantitative variables (A correlation coefficient greater than zero indicates a positive relationship (0.1 -0.2 none agreement, 0.21 – 0.39 minimal agreement, 0.41 – 0.59 weak agreement, 0.6 – 0.79 moderate agreement, 0.8 – 0.9 strong agreement, >0.9 perfect agreement) while a value less than zero signifies a negative relationship, and A value of zero indicates no relationship between the two variables being compared). Receiver operating characteristics (ROC) curve analysis was used to detect the cutoff point for CSF lactate level. The Kappa agreement test was used to assess the agreement between PCR findings and CSF lactate at cutoff point findings. A *p*-value ≤ 0.05 was considered statistically significant.

Results

This prospective study included 50 patients suffering from signs and symptoms of meningitis. These patients were classified into two groups: Group1 included 30 patients with bacterial meningitis. Their age ranged from 2-70 years with 27±20.9 years (mean± standard deviation). Group 2 included 20 patients with viral meningitis. Their age ranged from 2-75 years with 34 ±23.9. There was no statistically significant difference between the age of patients with bacterial and viral meningitis.

At the time of sample collection, 17/50 (34%) patients were on antibiotic treatment before confirming the diagnosis, while 33/50 (66%) patients did not use any antibiotics. The outcome of the studied patients was as follows; 30/50 (60%) discharged, 13/50 (26%) died, and 7/50 (14%) referred to another hospital.

Correlation studies

Blood samples showed no growth in 94% of patients' blood cultures. There was no difference between bacterial and viral meningitis in blood sample findings including TLC, RBS, and blood culture.

The CSF findings among the studied patients are shown in Table 1. CSF culture (conventional and after enrichment) showed growth in 34% and 40% of cases, respectively. Conventional culture, after enrichment, gram stain cultures, and appearance of the CSF were statistically significantly different between patients with bacterial and viral meningitis.

Table 1. Distribution of CSF findings among total patients with meningitis and those with Bacterial and viral meningitis.

		Total patients with meningitis		Bacterial meningitis		Viral meningitis		*p* value
		N	%	N	%	N	%	
CSF (Conventional culture)	No growth	33	66.0%	13	43.3%	20	100%	<0.001
	S. pneumoniae	14	28.0%	14	46.7%	0	0.0%	
	E. coli	1	2.0%	1	3.3%	0	0.0%	
	Salmonella spp.	1	2.0%	1	3.3%	0	0.0%	
	Proteus spp.	1	2.0%	1	3.3%	0	0.0%	

Table 1. Continued.

		Total patients with meningitis		Bacterial meningitis		Viral meningitis		*p* value
		N	%	N	%	N	%	
CSF culture (After enrichment)	No growth	30	60.0%	10	33.3%	20	100%	<0.001
	S. pneumoniae	16	32.0%	16	53.3%	0	0.0%	
	E. coli	1	2.0%	1	3.3%	0	0.0%	
	Salmonella spp.	1	2.0%	1	3.3%	0	0.0%	
	Proteus spp.	1	2.0%	1	3.3%	0	0.0%	
	H. influenzae	1	2.0%	1	3.3%	0	0.0%	
Direct Gram	No organism	32	64.0%	12	40.0%	20	100%	<0.001
	Gram positive diplococci	15	30.0%	15	50.0%	0	0.0%	
	Gram negative bacilli	3	6.0%	3	10.0%	0	0.0%	
CSF Appearance	Turbid	32	64.0%	28	93.3%	4	20.0%	<0.001
	Clear	10	20.0%	0	0.0%	10	50.0%	
	Bloody	5	10.0%	1	3.3%	4	20.0%	
	S. turbid	3	6.0%	1	3.3%	2	10.0%	

*$P \leq 0.05$ is significant.

Regarding the PCR findings among all studied patients, only 3/50 (6%) patients displayed negative results. *S. pneumoniae* was the most prevalent organism 26/50 (52%), followed by *herpes simplex virus* 8/50 (16%) and *Epstein barr virus* 8/50 (16%), *human Enterovirus* 4/50 (8%), and *H. influenzae* 1/50 (2.0%). It is worth noting that the CSF conventional culture failed to recover 12/26 (46.15 %) of *S. pneumoniae* isolates and the single *H. influenzae* isolate. As for the distribution of PCR findings between bacterial and viral meningitis, there was a statistically significant difference (Table 2). There was a significantly higher CSF lactate levels in bacterial meningitis (positive culture or PCR) than in viral meningitis (Table 3, Figure 1).

Table 2. Distribution of PCR findings among patients with bacterial and viral meningitis.

PCR	Bacterial meningitis patients (n=30)		Viral meningitis patients (n=20)		*p* value
	N	%	N	%	
Negative	3	10.0%	0	0.00%	<0.001
S. pneumoniae	26	86.7%	0	0.00%	
Herpes simplex virus	0	0.0%	8	40.00%	
Epstein barrvirus	0	0.0%	8	40.00%	
Human Enterovirus	0	0.0%	4	20.00%	
H. influenzae	1	3.3%	0	0.00%	
Negative	3	10.0%	0	0.0%	<0.001
Bacterial	27	90.0%	0	0.0%	
Viral	0	0.0%	20	100.0%	

*$P \leq 0.05$ is significant.

Table 3. Distribution of CSF Lactate levels among patients with bacterial (positive culture or PCR) and viral meningitis.

	Bacterial meningitis		Viral meningitis		*p* value
	Mean ± SD (min – max)	Median (IQR)	Mean ± SD (min – max)	Median (IQR)	
CSF Lactate (mmol/l)	12.5 ±3.8 (2.5-17.6)	13.2 (10.6-15.3)	4.4 ±1.6 (2.2-7.2)	4.4 (3.1-5.8)	< 0.001

*$P \leq 0.05$ is significant.

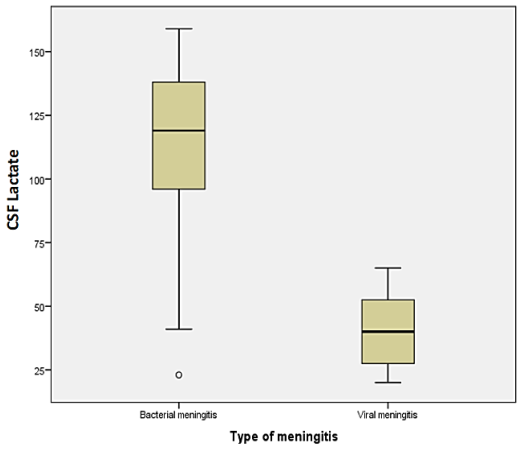

Figure 1. Distribution of CSF lactate levels in bacterial and viral meningitis.

There was no statistically significant correlation between CSF lactate levels and blood sample findings (TLC and RBS). However, there was a statistically significant moderate positive correlation between CSF lactate levels with CSF cytological findings Polymorphonuclear cells (PMN) (r=0.7) and a moderate negative correlation with cytological findings (lymphocytes) (r= -0.7). Also, there was a statistically significant positive weak correlation between CSF lactate levels with CSF protein (r= 0.3) and a weak negative correlation with CSF glucose (r= -0.4) and CSF glucose/serum blood glucose ratio (r= -0.4) (Table 4).

Table 4. Correlation of CSF Lactate levels with blood sample findings (TLC and RBS) and with CSF Findings among patients with meningitis.

		CSF Lactate
blood sample findings		
TLC	r	0.098
	p value	NS
RBS	r	-0.162
	p	NS
CSF Findings		
CSF Count	r	0.276
	p	0.052
PMN	r	0.747
	P	0.00*
Lymphocytes	r	-0.747
	p	0.00*
Glucose	r	-0.406
	p	0.003*
Ratio	r	-0.444
	P	0.001*
Protein	r	0.376
	p	0.007*

**(r)Pearson correlation test was used. P* > 0.05 is not significant (NS).

The CSF lactate cutoff point of 65.50 mg/dl (7.2 mmol/l) was found to be the best point to differentiate between bacterial and viral meningitis. Patients with CSF lactate levels less than or equal to 7.2 mmol/l were infected with viral meningitis and patients with more than 7.2 mmol/l had bacterial meningitis. The validity of CSF lactate with an area under the ROC curve of 0.956 for differentiating between bacterial and viral meningitis showed sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of 93.3%, 100%, 100%, and 90.9%, respectively (Table 5, Figure 2). Table 6 shows that there was a statistically significant strong agreement (Kappa= 0.957) between the types of meningitis and cutoff CSF lactate level of 7.2 mmol/L.

Table 5. Area under the receiver operating characteristics (ROC) curve for CSF lactate for differentiating between bacterial and viral meningitis.

Area Under the Curve	*p* value	95% Confidence Interval	
		Lower Bound	Upper Bound
0.956	<0.001	0.893	1.000

*$P \leq 0.05$ is significant.

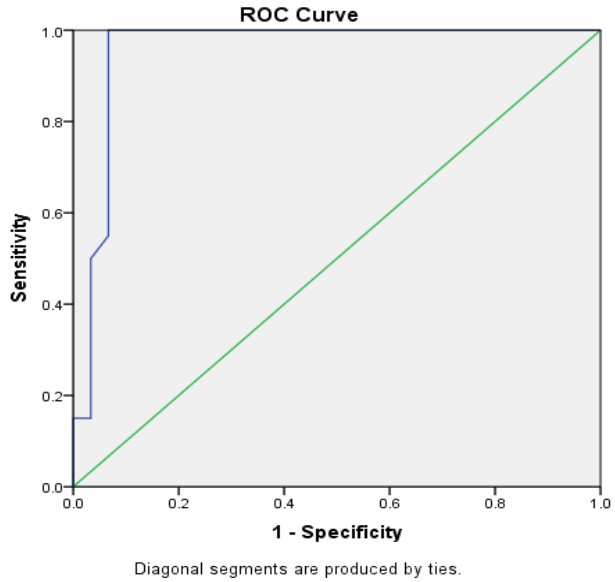

Figure 2. Receiver operating characteristics curve for CSF lactate for differentiating between bacterial and viral meningitis.

Table 6. The agreement between types of meningitis and cutoff CSF lactate level (7.2 mmol/L).

		Type of meningitis				Kappa agreement	*p* value
		Bacterial meningitis		Viral meningitis			
		N	%	N	%		
CSF Lactate (Cutoff level 7.2 mmol/L)	Bacterial meningitis	28	93.3%	0	0.0%	0.918	<0.001
	Viral meningitis	2	6.7%	20	100%		

$P \leq 0.05$ is significant.

We also evaluated the diagnostic performance of CSF lactate cutoff according to the guideline (3.8 mmol/l). It showed a sensitivity, specificity, PPV, and NPV of 96.7%, 45.0%, 100%, and 90.0%, respectively, with a statistically significant weak agreement (Kappa= 0.483) between the guideline cutoff value of 3.8 mmol/l and the cutoff CSF lactate level of 7.2 mmol/L (Table 7).

Table 7. The agreement between cutoff CSF lactate level (7.2 mmol/L) and the UK joint specialist societies guideline cutoff (3.8 mmol/l).

		CSF Lactate (cutoff level 7.2 mmol/L)				Kappa	*p* value
		Bacterial meningitis		Viral meningitis			
		Count	%	Count	%		
CSF Lactate (cutoff level 3.8 mmol/L)	Bacterial meningitis	28	100.0%	12	54.5%	0.483	<0.001
	Viral meningitis	0	0.0%	10	45.5%		

*Cohen's kappa agreement test was used. $P \leq 0.05$ is significant.

Discussion

This study aimed to assess the performance and discuss the usefulness of CSF lactate as a biomarker to differentiate between bacterial meningitis and viral meningitis. It included 50 patients suffering from signs and symptoms of meningitis. CSF culture is the gold standard method for diagnosis, as cultured bacteria are sources of data for antibiotic susceptibility and complete subtyping. In the present work, only 17/50 (34%) of the studied CSF specimens gave positive results by the conventional agar culture method versus 20/50 (40%) by the Bactec CSF culture.

In the current work, the rate of positive CSF culture was near to the results reported by previous studies.[5-15] They reported CSF culture positivity rates of 36%, 34.5%, 34.7%, respectively. However, our rates were lower than the rates obtained by Welinder-Olsson et al., 2007,[15] (52.7%) and by Zeighami et al. 2021 (46%).[16]

In this study, the lower rate of organisms' isolation by conventional culture may be because 34% of our patients were on antibiotic therapy at the time of sample collection, negative culture suggests that viruses, parasites, and other bacterial agents might be causing meningitis. [16] Furthermore, because of the drawbacks of the culture as suboptimal storage and transportation conditions and culture practice, the culture was negative for *S. pneumonia*e and potentially other fastidious pathogens.

In the present study, it was noted that all the positive bacterial cultures (conventional or after enrichment) recovered a single bacteria isolate. Among the bacterial meningitis which revealed positive cultures, *S. pneumonia*e was the most identified organism 16/30 (53.3%), followed by Gram negative bacilli (*E. coli, Proteus* spp. *and Salmonella* spp.) 3/30 (10%), and finally *H. Influenzae* 1/30 (3.3%). A study conducted in Egypt by Abdelkader et al., 2017, reported similar results. The authors found that *S. pneumoniae* was the most frequently isolated organism from CSF cultures (64.6%) of patients with bacterial meningitis.[5] A study by Fouad et al., 2014, reported that among the bacterial meningitis patients, the isolated organisms on the CSF bacterial cultures was *S. pneumoniae*, the most frequently isolated species (52%) while *N. meningitidis* in 22.2% and *H. influenzae* in 14.8% of their studied bacterial meningitis patients.[17]

The agreement between our findings and those of the previously mentioned studies in Egypt consolidates the conclusion of Shaban & Siam, 2009, in their review article that pneumococcal meningitis remains the leading cause of meningitis in Egypt as its incidence is constantly rising at the expense of meningococcal meningitis, which may reflect the increased use of polysaccharide meningococcal vaccines versus the

pneumococcal vaccines that still non-compulsory.[18]

In the current work, we performed PCR for the detection of bacterial pathogens (*N. meningitidis, S. pneumoniae and H. influenzae*) and viral pathogens (Herpes simplex virus, Epstein barr virus and Human Enterovirus). The distribution of PCR findings among the whole studied patients with meningitis demonstrated that only 3/50 (6%) of patients have negative PCR results. The reason for the negative PCR results can be because gram-negative pathogens were not part of the used target panel. This resulted in negative PCR versus positive CSF culture (agar or BACTEC) which showed growth of *E. coli, Salmonella* spp., and *Proteus* spp.

Among our patients suffering from bacterial meningitis, PCR showed that *S. pneumoniae* was the most prevalent organism 26/30 (86.7%), and *H. influenzae* the least common 1/30 (3.3%). Whilst the isolated pathogens among those with viral meningitis were the *Herpes simplex* virus 8/20 (40%) and *Epstein barr* virus 8/20 (40%), followed by Human Enterovirus 4/20 (20%). These results agreed with those previously reported in the Egyptian study by Afifi et al., 2007.[19] The authors investigated purulent, culture-negative CSF specimens withdrawn from patients who met the criteria for case definition of bacterial meningitis using PCR. They reported *S. pneumoniae* as the most common etiologic agent of bacterial meningitis (46.6%). Moreover, Khater & Elabd, 2016, investigated the common bacterial pathogens causing meningitis in culture-negative CSF samples using real-time PCR. They reported that *S. pneumoniae* was the most detected bacterial pathogen representing 90% of the cases.[20] Başpınar et al., 2017, found that the most observed pathogen in their study by PCR was *S. pneumoniae* (97.05%), followed by *N. meningitidis* (2.95%). The authors could not isolate *H. influenza.*[21]

CSF culture is the gold standard for the diagnosis of bacterial meningitis according to WHO and the Centers for Disease Control and Prevention (CDC).[4] But there is diminished sensitivity of the CSF culture in the patients who received antibiotics before the lumber puncture and the 72-h test period hinders clinicians from reaching a prompt diagnosis and starting the treatment in the ideal period. The routine use of PCR-based molecular methods, although it is more expensive than culture, can avoid any delay in diagnosis and treatment in patients with suspicious bacterial meningitis with a turnaround time of hours compared to days in culture. This method, which is highly sensitive and specific, can also indicate the microorganisms in the CSF in patients who have culture-negative specimens and patients need rapid administration of antibiotics before the lumber puncture.[22]

According to previous studies, conducted in other countries, the frequency of *S. pneumoniae* was 31.7–73.8% in all meningitis patients. This variation in *S. pneumoniae* frequency may be because of the performance of pneumococcal vaccination programs in some countries, patients' age, diagnostic approaches used, type of specimens.[23]

Regarding viral meningitis, McGill et al.,2018, showed that human *Enterovirus* were the most frequent viruses, accounting for 55% of all viral meningitis cases and being the single most common cause, accounting for 20% of all meningitis. *Herpes simplex virus* represented (44%) of all viral meningitis cases and *Epstein barrvirus* (1%) [24].

Mathew et al., 2021, found that human *Enterovirus* was the most frequently detected virus, comprising 68.7% of cases, followed by *Epstein barrvirus* (7.5%), *adenovirus* (6.8%), and *cytomegalovirus* (4.5%). Other viral meningitis agents were also reported, with lower frequencies, including *varicella-zoster virus* (4.8%), *Parechovirus* (4.1%), and *Herpes simplex virus-1* (1.6%), and *Herpes simplex virus-2* (1.9%).[25] *de Ponfilly et al., 2021,* showed lower values than these observed in our study, where infections due to *human Enterovirus* and *Herpes simplex virus-2* accounted for 12.3% and 4.8%, respectively of all studied patient [26].

Previous studies showed that the etiology of viral meningitis differs from one geographic area to another. For instance, *Herpes simplex virus* was ranked second in adolescents and adults in developed countries like France, England, Spain, and the USA.[27] Whilst *Epstein barr virus*-type 1 was prevalent in Western

Europe, *Epstein barrvirus*-type 2 predominated in central Africa, Papua New Guinea, and Alaska. On the other hand, *Varicella zoster virus* and *Parechovirus* were prevailed worldwide.[28]

In the present study, there was a remarkable difference between CSF culture (conventional culture) and PCR findings regarding the detection of *S. pneumoniae and H. influenzae*, where 13 samples gave no growth (conventional culture) versus positive results by PCR. Out of the 47 positive PCR specimens, 14 (28%) samples were positive by conventional culture yielding growth of *S. pneumoniae.* Afifi et al., 2007. reported comparable results. They obtained low rates of positive CSF cultures in suspected cases of bacterial meningitis compared to PCR. The authors suggested that this might be because most patients in Egypt receive antimicrobial agents that are readily available as over-the-counter medications even before clinical evaluation.[19]

These findings are also in agreement with those reported by other researchers.[18, 22,23, 24] They stated that although real-time PCR is more expensive than culture, it provides more sensitive detection with a turnaround time of hours compared to days and can provide a diagnosis in culture-negative specimens, which often occurs due to the need for rapid administration of antibiotics.

In the current study, the cutoff point of CSF lactate was 65.50 mg/dl (7.2 mmol/L). This value represented the best point to differentiate between bacterial and viral meningitis as patients with CSF lactate levels less than or equal to 7.2 mmol/l were viral meningitis and more than (7.2 mmol/l) were bacterial meningitis. The validity of CSF lactate for differentiating between bacterial and viral meningitis showed a sensitivity, specificity, PPV, and NPV of 93.3%, 100%, 100%, and 90.9%, respectively, making CSF lactate a good single marker for differentiation and comparison between bacterial meningitis and viral meningitis with AUC for CSF lactate of 0.956.

The comparison between our cutoff point (7.2 mmol/L) and the UK joint specialist societies guideline[8] suggested cutoff (3.8 mmol/l) showed that the sensitivity, specificity, PPV, and NPV were 96.7%, 45.0%, 100%, and 90.0%, respectively. Such a low specificity is not suitable for accurately diagnosing the prevalent pathogens in the current study. So, in the present work, according to the local distribution of causative agents of meningitis the cutoff level of 7.2 mmol/L displayed the best specificity and hence can be implemented instead of that recommended by the guideline.

Our results were close to those observed by de Almeida et al., 2020*,*[29]. They reported a median CSF lactate level in acute bacterial meningitis of 9.0 mmol/l (IQR=5.70–12.89). Whereas, in viral meningitis, the CSF lactate median level was 2.4 mmol/l (IQR= 2.0–3.0 mmol/l). Similarly, Nazir et al., 2018*,* found that CSF lactate has high sensitivity and specificity in differentiating bacterial and viral meningitis but with a lower mean of 5.95 mmol/l (5.26 - 6.64 mmol/l) and 1.84 mmol/l (1.76 - 1.91 mmol/l), respectively. They reported a cutoff value of 3 mmol/L with an AUC of 0.979. They found that CSF lactate had a sensitivity, specificity, PPV, and NPV of 90%, 100%, 100%, and 96.3%, respectively. The accuracy, CSF lactate was 0.972, indicating an excellent overall accuracy in differentiating between bacterial and viral meningitis [1].

The study by Stephani et al., 2021, recorded a CSF lactate of > 3.5–4.2 mmol/l, and demonstrated its high reliability in predicting non-viral meningitis.[30] Also, *Griffiths et al., 2018,* found that CSF lactate concentration of >3.8 mmol/L could reliably discriminate between viral/aseptic meningitis and acute bacterial meningitis.[11]

Giulieri et al., 2015*,* showed that the median CSF lactate concentrations were 13 mmol/l in bacterial and 2.3 mmol/l in viral meningitis. The patients included in their study were 45 patients with microbiologically documented meningitis, 18 had bacterial meningitis (*Streptococcus pneumoniae*, n=11; *Neisseria meningitidis*, n=5; *Haemophilus influenzae*, n=1, and *Streptococcus agalactiae*, n=1).[6]

The Infectious Diseases Society of America (IDSA) guidelines recommends to measure CSF lactate for the diagnosis of post-neurosurgical bacterial meningitis and the initiation of empirical antibacterial therapy if CSF lactate level is ≥4 mmol/l.[30] A recent study validated

CSF lactate as a useful point-of-care test for rapid diagnosis of bacterial meningitis.[31]

Bosworth et al. observed a weak correlation between levels of white blood cell count (WBC) in CSF and both protein (R=0.4 p = 0.001) or glucose (R = 0.17 p = 0.001). But there was no significant correlation between the numbers of WBC and the number of red blood cells (RBC) with CSF lactate levels. CSF Lactate levels above 3.8 mmol/L showed high specificity for *N. meningitidis* and *S. pneumoniae* of 94% (CI 95% 93–94).[32] And that was greater than the previously described in published guidelines.[8]

Bosworth et al., 2019, mentioned that these organisms appeared to cause a marked rise in measured CSF lactate levels, with patients recorded with an average of 13.1 mmol/L *(N. meningitidis)* and 13 mmol/L (*S. pneumoniae).*[32] This range is similar to our values where the median was 13.2 mmol/l (IQR=10.6-15.3), *and S. pneumoniae* was the most prevalent organism (86.7%). They suggested that the measurement of CSF lactate is beneficial in identifying the most dangerous bacterial causes of CNS infection and should be considered for routine use as an early biochemical warning marker that would trigger a significant escalation of patient care. The data presented in their study provided evidence to support the use of elevated CSF lactate as a marker of meningitis caused by infection with *N. meningitidis* or *S. pneumoniae* and is a valuable aid to early diagnosis before specific microbiological or virological testing results become available.

In conclusion, CSF lactate exhibited high sensitivity and specificity in discriminating bacterial and viral meningitis. Therefore, measuring CSF lactate could be valuable in identifying the most dangerous bacterial causes of CNS infection, which showed high specificity for diagnosing *S. pneumoniae*. Our study recommends the use of a cutoff of CSF lactate level above 7.2 mmol/L. As *S. pneumoniae* was the most prevalent organism in Egypt in the previous years, we recommend using CSF lactate routinely as an early biochemical warning marker and a useful point-of-care test that would trigger a significant escalation of patient care. Furthermore, specific, and sensitive PCR-based molecular methods for assessment of CSF lactate can overcome the limitations of routine CSF culture. PCR is not affected by prior antibiotics intake and provides rapid results within hours versus days for the culture. Thus, results can be readily available to clinicians helping them to reach a prompt diagnosis and start the treatment in the ideal period, especially in CSF culture-negative cases and when patients are on antibiotics therapy before the lumbar puncture.

Author Contributions

MSG, SAE, EMK and NAF conceptualized the main idea. SAE, NAF and ZAAA designed and analyzed the data. SAE, NAF and ZAAA interpreted the results and drafted the manuscript. SAE, NAF and ZAAA conducted the corrections, and language editing. All the authors read and approved the final manuscript.

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Ain Shams University. (Ethical approval number: FMASU MD 297/2019, September 2019).

Informed consent

An informed written consent was obtained from each patient before enrollment in the study.

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