

Comparison of cerebrospinal fluid lactate with physical, cytological, and other biochemical characteristics as prognostic factors in acute bacterial meningitis

Comparaç o do lactato no l quido cefalorraquidiano com as caracter sticas f sicas, citol gicas e outros marcadores bioqu micos, como fator prognostico nas meningites bacterianas agudas

S rgio Monteiro DE ALMEIDA¹, Nagyla C. BARROS¹, Ricardo PETTERLE¹, Keite NOGUEIRA¹

ABSTRACT

Bacterial meningitis (BM) is associated with a high morbidity and mortality. Cerebrospinal fluid (CSF) lactate may be used as a prognostic marker of this condition. We hypothesized that CSF lactate levels would remain elevated in participants who died of acute BM compared with those who recovered from this disease. **Objective:** To evaluate the potential use of lactate and other CSF biomarkers as prognostic markers of acute BM outcome. **Methods:** This retrospective, longitudinal study evaluated dynamic CSF biomarkers in 223 CSF samples from 49 patients who fulfilled the inclusion criteria of acute BM, with bacteria identified by CSF culturing. The participants were grouped according to outcome: death (n = 9; 18.37%) and survival (n = 40; 81.63%). All participants received appropriate antibiotic treatment. **Results:** In the logistic regression model, lactate concentration in the final CSF sample, xanthochromia, and CSF glucose variation between the first and last CSF samples were predictors of a poor outcome (death). In contrast, decrease in CSF white blood cell count and CSF percentage of neutrophils, increase in the percentage of lymphocytes, and normalization of the CSF lactate concentration in the last CSF sample were predictors of a good prognosis. **Conclusion:** The study confirmed the initial hypothesis. The longitudinal analysis of CSF lactate is an important predictor of prognosis in acute BM.

Keywords: Meningitis, bacterial; cerebrospinal fluid; lactic acid; prognosis.

RESUMO

As meningites bacterianas (MB) est o associadas   alta morbidade e mortalidade. O lactato no l quido cefalorraquidiano (LCR) pode ser usado como biomarcador de progn stico nas MB. A hip tese desse estudo   que os n veis de lactato no LCR se mant m elevados entre pacientes com MB aguda que evoluem para  bito, ao contr rio do que ocorre em pacientes com bom progn stico. **Objetivo:** Avaliar o uso potencial do lactato e outros marcadores no LCR como indicador de progn stico na MB aguda. **M todos:** Foi realizado um estudo retrospectivo longitudinal da din mica dos biomarcadores bioqu micos, celulares e f sicos no LCR. Foram analisadas 223 amostras de 49 pacientes com MB aguda com bact rias identificadas por cultura do LCR. Os participantes foram divididos em dois grupos de acordo com o desfecho:  bito (n = 9; 18,37%) e n o  bito (n = 40; 81,63%). Todos os participantes receberam antibioticoterapia adequada. **Resultados:** No modelo de regress o log stica, as vari veis que diferiram significativamente entre os dois grupos foram concentra o de lactato na amostra final de LCR, xantocromia e varia o da concentra o de glicose entre a primeira e a  ltima amostra de LCR. A altera o desses fatores indicou desfechos negativos ( bito), enquanto a diminui o do n mero de leuc citos e da porcentagem de neutr filos, assim como a normaliza o da concentra o de lactato no LCR foram preditores de bom progn stico. **Conclus o:** O estudo confirmou a hip tese inicial. A an lise longitudinal do lactato no LCR   um importante preditor de progn stico na MB aguda.

Palavras-chave: Meningites bacterianas; l quido cefalorraquidiano;  cido l ctico; progn stico.

¹Universidade Federal do Paran , Hospital de Cl nicas, Curitiba PR, Brasil.

S rgio Monteiro de Almeida  <https://orcid.org/0000-0001-5690-105X>; Ricardo Petterle  <https://orcid.org/0000-0001-7735-1077>; Keite Nogueira  <https://orcid.org/0000-0001-5655-9645>

Correspondence: S rgio Monteiro de Almeida; Hospital de Cl nicas, UFPR, Se o de Virologia, Setor An lises Cl nicas; Rua Padre Camargo, 280; 80060-240 Curitiba PR, Brasil; E-mail: sergio.ma@ufpr.br

Conflict of interest: There is no conflict of interest to declare.

Received 06 February 2019; Received in final form 18 July 2019; Accepted 13 August 2019.



Acute bacterial meningitis (BM) is a life-threatening syndrome, with mortality between 3% and 21%, and may have severe neurological sequelae¹. Early diagnosis and antibacterial therapy are essential for a positive outcome². The differential diagnosis between BM and viral meningitis is crucial for treatment and prognosis^{3,4,5}. The concentration of CSF lactate, in addition to providing information about the differential diagnosis between BM and viral meningitis^{1,6,7}, holds potential as a prognostic marker in acute BM. Experimental animal studies have shown that elevated CSF lactate levels persisting after appropriate antibiotic treatment are associated with increased morbidity and mortality, and may facilitate prognostic prediction⁸. Several studies showed that CSF lactate levels were significantly different in clinical samples obtained from patients with a positive and a negative prognosis^{9,10,11,12,13,14,15}.

We hypothesized that CSF lactate levels remained high among participants with acute BM who died, in contrast to the group who survived. To test this hypothesis, we calculated CSF lactate levels longitudinally, and evaluated whether these values could be used as a prognostic factor in BM with that of other physical, biochemical, and cytological characteristics of the CSF.

METHODS

Participants

A retrospective study was conducted with CSF reports from the Clinical Pathology Laboratory of the Hospital de Clínicas, Universidade Federal do Paraná (HC-UFPR) during the period between 2006 and 2017. The Institutional Research Review Board of the HC-UFPR, Brazil, approved this study.

Patients clinically suspected of having acute BM were selected randomly, based on microbiological identification of the etiological agent. Data regarding the CSF biochemical and cytological characteristics, as well as demographic data, were obtained from the hospital computer records. The inclusion criteria were as follows: 1) CSF samples from patients attending HC-UFPR with clinical suspicion of acute meningitis; 2) CSF cultures positive for bacteria; and 3) quantification of lactate concentration in the CSF. The exclusion criteria of the study were as follows: 1) patients with CSF cultures negative for bacteria; 2) virus identified in the CSF; 3) samples obtained from other services. Lactate was measured in all CSF samples included throughout the study period.

Sample collection

The CSF samples were collected for clinical purposes. To study the dynamics of CSF biomarkers, all CSF samples collected during each patient's hospitalization period were included. Two hundred and twenty-three CSF samples from 49 patients fulfilled the inclusion criteria of the study.

Patients were allocated to two groups according to the outcome: negative outcome (death; $n = 9$; 18.37%) and positive outcome (survival; $n = 40$; 81.63%). All participants received appropriate antibiotic treatment according to the sensitivity of the CSF bacteria isolated.

The sites of CSF puncture were comparable between groups. In the first CSF sample, 19 samples (38.78%) were collected by lumbar puncture (15 in the positive-outcome group [37.50%] and four in the negative-outcome group [44.44%]); nine samples were collected by ventricular puncture (18.37%) (six in the positive-outcome group [15.00%] and three in the negative-outcome group [33.33%]); and 21 samples were collected by puncture from the shunt (42.86%) (19 in the positive-outcome group [47.50%] and two in the negative-outcome group [22.22%]) ($p = 0.282$).

The time interval between the last CSF puncture and hospital discharge in the whole group was median (interquartile range [IQR]:) 5 days (2; 13).

Overall, a median of four CSF punctures (IQR: 1; 6) were performed during hospitalization. In the positive-outcome group, the median number of punctures performed was four (1.5; 6); in the negative-outcome group, it was two (1; 7) ($p = 0.518$). The median interval between the first and last CSF sample in all patients who underwent more than one CSF puncture ($n = 36$) was 26 days (13; 44.5). In the positive-outcome group, this median interval was 26 days (13.25; 40.25); in the negative-outcome group it was 34.5 days (8.75; 47.5).

CSF biochemistry and cytology

After CSF puncture, the CSF samples were kept at room temperature and analyzed up to 30 minutes after reception in the clinical pathology laboratory.

Lactate was quantified in the CSF by amperometry (RAPID Point 500, Siemens, New York, NY, USA). Total protein in the CSF was quantified using benzethonium chloride as the precipitation reagent. Glucose in the CSF and the plasma were determined using the hexokinase/G-6-PDH method (Architect, Abbott Diagnostics, Abbot Park, IL). Total white blood cell count (WBC) per mm^3 in the CSF was quantified in fresh, non-centrifuged CSF, in a Fuchs-Rosenthal chamber. Cerebrospinal fluid pleocytosis was defined as a WBC count of > 5 cells/ mm^3 . For differential WBC counts, CSF samples were concentrated using a Shandon cytopsin (Thermo-Shandon; Pittsburgh, PA) and the slides were stained using May-Grünwald-Giemsa staining. Xanthochromia in the CSF¹⁶ was assessed by visual inspection and quantified using the color index by comparing the supernatant after centrifugation with a solution of potassium bichromate¹⁷.

Microbiological diagnostic tests for bacteriology

All CSF samples were subjected to direct microscopic examination (Gram-stain smear) and culturing. The CSF specimens were inoculated onto agar plates (5% sheep's

blood agar and supplemented chocolate agar plates) and incubated at 37°C for 24-48 hours. For positive cultures, bacteria were identified using a VITEK[®] 2 compact system (BioMérieux Vitek, Inc., Hazelwood, MO).

Statistical analyses

The variables studied in the CSF were the color index, WBC, neutrophils (%), lymphocytes (%), glucose (mg/dL), CSF/blood glucose ratio, total protein (mg/dL), and lactate concentration (mmol/L). All variables were studied in the first and last CSF samples. In addition, the mean value and variation (value of first CSF sample subtracted from that of last CSF sample) of all CSF samples from the same participant were assessed. For the variation in the CSF variables, the median of the variable in serial CSF samples was used as a cut-off point.

Results are presented as median (IQR) or number (n) and percentage (%), as appropriate. Categorical variables were compared between groups using Fisher's exact test and continuous variables were compared using the Mann-Whitney or Kruskal-Wallis test for non-parametric data, as appropriate. Dependent continuous variables were compared using the Wilcoxon test.

To estimate survival from lifetime data, Kaplan-Meier curves were constructed and compared using the log-rank test. For the cut-off point of variables, the median of overall samples or the reference values of the laboratory parameters were used.

Logistic regression analysis was used to estimate or predict the value of potential predictors of prognosis, such as sex, race, age, and CSF characteristics (color index, leukocyte, neutrophil percentage lymphocyte percentage, glucose, protein, and lactate levels, and CSF/blood glucose ratio). The odds ratio and 95% confidence intervals (CI) were used to

quantify the strength of the associations. Results were considered significant at the 5% alpha level.

RESULTS

The groups studied were comparable for age, sex, and race. The median (IQR) age was 26.06 years (1.23; 41.33) in the positive-outcome group and 46.36 years (33.18; 53.01) in the negative-outcome group ($p = 0.096$). In the positive-outcome group, 19 patients (47.50%) were male, while in the negative-outcome group, four patients (44.44%) were male ($p > 0.99$). In the positive-outcome group, 39 patients were Caucasian (97.50%), while in the negative-outcome group, eight (88.89%) were Caucasian ($p = 0.337$). The median overall duration of hospitalization was 28 days (13; 49); 30 days (18.25; 49.50) in the positive-outcome group and 10 days (7; 49) in the negative-outcome group ($p = 0.152$).

The bacteria isolated by CSF culture in the first CSF sample for each of the groups are shown in Table 1. Overall, 31 samples (63.26%) were positive for Gram-positive bacteria and 18 samples (36.73%) for Gram-negative bacteria. There was no difference in CSF lactate levels between the groups with Gram-positive or Gram-negative bacteria in the CSF ($p = 0.736$).

Overall, the mortality rate in this series was 18.37%. In the positive-outcome group, 26 patients (65%) had Gram-positive and 14 patients (35%) had Gram-negative bacteria. In the negative-outcome group, five patients (55.55%) had Gram-positive and four patients (44.44%) had Gram-negative bacteria. Thus, mortality was not dependent on the type of bacteria involved ($p = 0.708$).

The physical, cytological, and biochemical characteristics of the CSF in the first and last CSF samples based on the

Table 1. Bacteria identified in cultures of the first cerebrospinal fluid (CSF) sample.

Bacteria	Total	Survival	Death	p-value
<i>Staphylococcus coagulase negative</i>	12 (24.49)	10 (25.0)	2 (22.22)	0.999
<i>Streptococcus pneumoniae</i>	9 (18.37)	8 (20.00)	1 (11.11)	0.999
<i>Staphylococcus aureus</i>	8 (16.33)	6 (15.00)	2 (22.22)	0.638
<i>Klebsiella pneumoniae</i>	5 (10.20)	3 (7.50)	2 (22.22)	0.224
<i>Enterobacter spp.</i>	5 (10.20)	4 (10.00)	1 (11.11)	0.999
<i>Acinetobacter baumannii</i>	2 (4.08)	2 (5.00)	-	-
<i>Escherichia coli</i>	2 (4.08)	2 (5.00)	-	-
<i>Haemophilus influenzae</i>	1 (2.08)	1 (2.08)	-	-
<i>Proteus mirabilis</i>	1 (2.08)	-	1 (11.11)	-
<i>Pseudomonas stutzeri</i>	1 (2.08)	1 (2.50)	-	-
<i>Staphylococcus haemolyticus</i>	1 (2.08)	1 (2.50)	-	-
<i>Streptococcus viridans</i> group	1 (2.08)	1 (2.50)	-	-
Bacilli Gram-negative, oxidase-negative	1 (2.08)	1 (2.50)	-	-

Data presented in n (%).

Table 2. Cerebrospinal fluid (CSF) xanthochromia, cytological, and biochemical characteristics of the first and last CSF samples, by outcome.

Variable	First puncture			Last puncture			p-value ⁴	p-value ⁵	p-value ⁶	
	Total	Survival	Death	p-value	Total	Survival				Death
n (%)	49 (100)	40 (81.63)	9 (18.37)		36	30 (83.33)	6 (16.67)			
Color index (%) ¹	0.3 (0; 0.75)	0.3 (0; 1)	0.5 (0; 25.37)	0.483	0.2 (0; 0.5)	0 (0; 0.5)	0.5 (0.35; 0.87)	0.034	0.591	0.735
Color index > 0% (n %)	25 (58.14)	20 (57.14)	5 (62.50)	1.000	25 (83.33)	17 (70.83)	6 (100)	0.068	0.353	0.632
RBC (cells/mm ³)	320 (144; 1565)	295 (104.75; 1035)	510 (225; 10900)	0.653	112 (10.12; 697.5)	112 (7.97; 597.5)	98 (20.25; 602.5)	0.832	0.144	0.145
WBC (cells/mm ³)	67 (5.3; 450)	117.5 (5; 1148.75)	13 (2.8; 68.7)	0.131	24.25 (9.25; 112)	23.75 (9.15; 69.75)	610.5 (19; 1342)	0.959	0.137	0.018
WBC > 5 cells/mm ³ (n %)	37 (75.51)	30 (75.00)	7 (77.78)	1.000	32 (88.89)	27 (90.00)	5 (83.33)	0.535	0.163	0.132
Neutrophils (%) ²	83 (61.5; 92.5)	83 (74; 94)	24 (77.5; 72.5)	0.168	15 (2.5; 58)	10 (1.5; 30)	91.5 (69.25; 94.25)	0.014	0.009	< 0.001
Lymphocytes (%) ²	14 (4.25; 30.25)	12 (4; 23)	71 (46; 87)	0.025	69.5 (42.5; 89.75)	70 (50.5; 90.5)	5 (3; 43)	0.041	0.005	< 0.001
Neutrophil/lymphocyte ratio	5.70 (2.27; 20.75)	7.33 (3.15; 27.71)	0.37 ⁷ (0.01; 9.78)	0.020	0.237 (0.075; 1.415)	0.209 (0.045; 0.506)	18.60 ⁷ (0.09; 98)	0.157	0.058	0.0005
Glucose (mg/dL)	32 (5; 61)	28.5 (5; 53.75)	85 (25; 104)	0.032	42 (27.75; 51)	45 (30; 53)	28 (21.75; 34.25)	0.301	0.697	0.075
Glucose < 45 mg/dL (n %)	31 (63.26)	27 (67.50)	4 (44.44)	0.259	20 (55.56)	15 (50.00)	5 (83.33)	0.196	0.508	0.150
Glucose CSF/blood ³	0.51 (0.05; 0.6)	0.48 (0.05; 0.58)	0.61 (0.44; 0.65)	0.394	0.09 (0.02; 0.55)	0.11 (0.04; 0.53)	0.02 (0.01; 0.33)	0.754	1.125	0.500
Glucose CSF/blood < 0.4 (n %)	10 (40.00)	10 (47.61)	1 (25.00)	0.615	9 (64.29)	7 (63.63)	2 (66.67)	0.999	0.191	0.472
Total protein (mg/dL)	89.5 (34.9; 356.6)	89.4 (35.27; 346.02)	165.2 (29.7; 768.6)	0.767	109.85 (51.8; 228.8)	93.25 (49.4; 174.87)	232 (162; 295)	0.048	0.866	0.647
Total protein increased (n %) ⁸	45 (92)	38 (95)	7 (78)	0.149	34 (94)	28 (93)	6 (100)	0.999	0.999	0.486
Lactate (mmol/L)	4.7 (2.2; 10.7)	4.9 (2.17; 10.95)	3.4 (2.5; 5.4)	0.938	2.75 (1.97; 4.37)	2.45 (1.9; 3.57)	6.75 (4.8; 7.5)	0.002	0.014	< 0.001
Lactate > 3.5 mmol/L (n %)	27 (55.10)	23 (57.50)	4 (44.44)	0.713	13 (36.11)	8 (26.67)	5 (83.33)	0.016	0.123	0.015
Normal (n %)	5 (10.20)	4 (10)	1 (11.11)	1.00	1 (2.78)	1 (3.33)	0	0.999	0.236	0.383

Significant differences ($p < 0.05$) are in bold typeface. Data are presented in median (interquartile range) or n (%). Accordingly: ¹43 samples with color index in the first CSF sample (survival: 35; death: 8); and 42 in the last CSF sample (survival: 34; death: 8); ²35 samples with differential cell count in the first CSF sample (survival: 29; death: 6); and 38 in the last CSF sample (survival: 33; death: 5); ³25 samples with blood glucose on the date of the first CSF sample (survival: 21; death: 4); and 14 in the last CSF sample (survival: 1; death: 3); ⁴Wilcoxon's test between the first and last CSF sample, total group; ⁵Wilcoxon's test between the first and last CSF sample of the survival group; ⁶Wilcoxon's test between the first and last CSF sample of the death group; ⁷maximum, minimum; ⁸total protein increased in accordance with reference values by location of CSF puncture: ventricular 5–15 mg/dL, sub-occipital 10–25 mg/dL, and lumbar 15–45 mg/dL¹⁵; analyzed with Fisher's exact test.

RBC: red blood cell; WBC: white blood cell; CSF: cerebrospinal fluid.

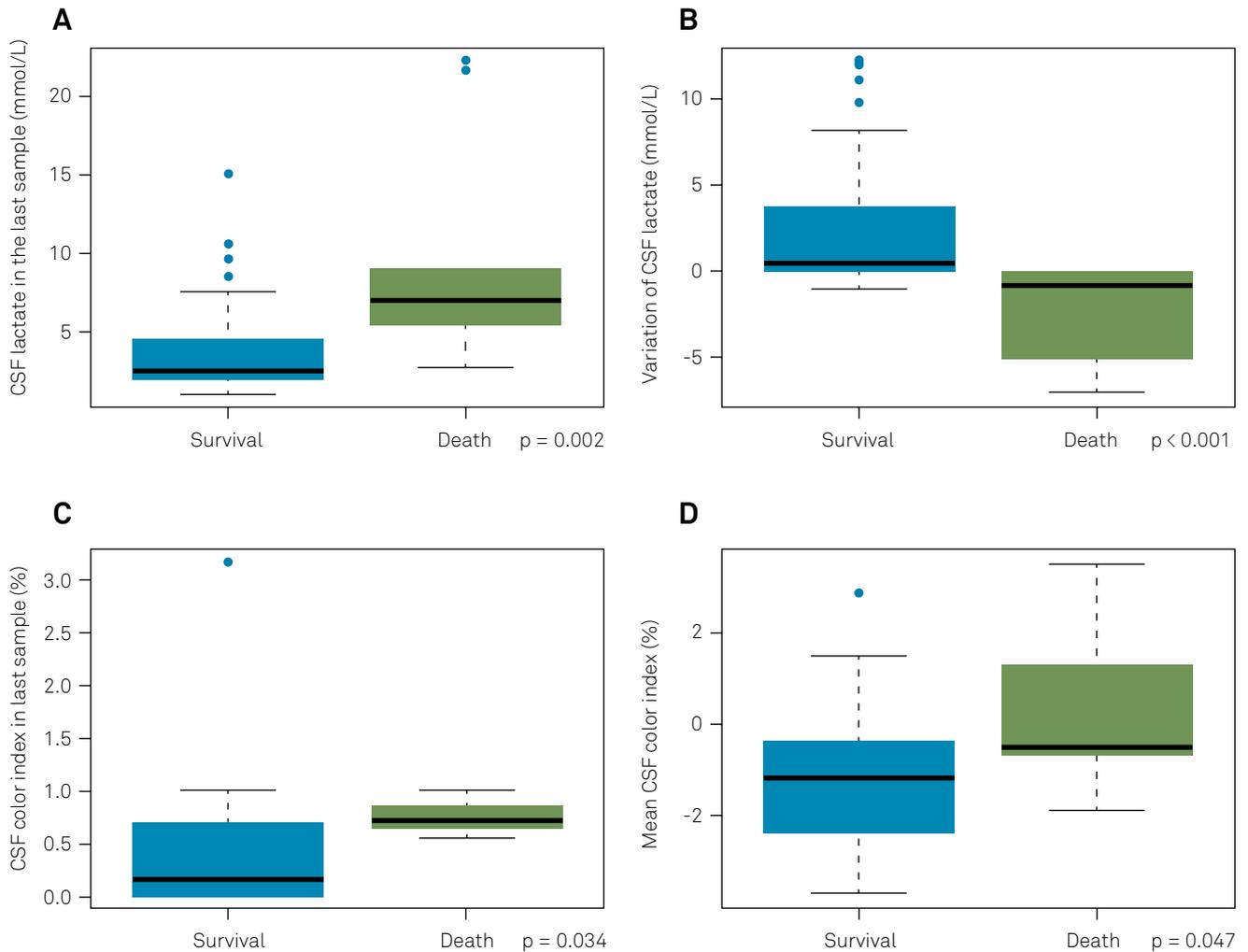


Figure 1. Comparison of cerebrospinal fluid (CSF) variables between the groups with positive (survival) and negative (death) outcomes. A. CSF lactate (mmol/L) in the last sample. B. Variation of CSF lactate concentration between the first and last sample. C. CSF color index (%) in the last sample. D. Mean CSF color index (%) in all longitudinal samples.

outcome are shown in Table 2 and Figure 1. The dynamics of CSF biochemical and cell characteristics, described by the variation and mean of the characteristics of the sequential CSF samples, are shown in Table 3 and Figure 1. The percentage of lymphocytes and glucose levels were higher in the first CSF sample in the negative-outcome group than in the positive-outcome group ($p = 0.025$ and 0.032 , respectively). In the last CSF sample, lactate (Figure 1), color index, neutrophil percentage, and total protein levels were higher in the negative-outcome than in the positive-outcome group ($p = 0.002$; $p = 0.034$; $p = 0.014$; and $p = 0.048$, respectively). The lymphocyte percentage was lower in the negative-outcome group ($p = 0.041$).

The variation between the first and last CSF sample showed increased WBCs and lactate levels in the last CSF sample, and decreased lymphocyte percentage and glucose levels ($p = 0.017$; $p < 0.001$; $p = 0.010$; $p = 0.006$, respectively; Table 3). The mean value of all CSF samples collected per patient was higher in the negative-outcome than in the positive-outcome group for color index ($p = 0.047$). The lactate

concentration was numerically higher in the negative-outcome group, but this difference was not statistically significant (Table 3).

In the negative-outcome group, CSF cultures and Gram-stain smears were positive in the last CSF sample in two of the nine cases (22.22%). Both cases showed the same bacteria that were identified in the first CSF samples. In the positive-outcome group, the CSF culture was negative in the last sample for all participants with more than one CSF sample ($n = 40$). All findings in the last CSF sample of the negative-outcome group suggested a lack of response to the antibiotic therapy.

There was a significant difference in survival time between groups, i.e., more deaths occurred among patients who were older than 29 years, which was the overall median age of patients included in the study ($p = 0.027$). Death was more frequent where the mean lactate concentration in serial CSF samples was higher than 3.5 mmol/L; CSF lactate in the last CSF sample was higher than 3.5 mmol/L; and CSF

Table 3. Dynamics of cerebrospinal fluid (CSF) xanthochromia, and cytological and biochemical characteristics evaluated according to the mean of all longitudinal CSF samples for the same participant, as well as variation between the first and last sample for each participant by outcome.

Variables	Variation between first and last CSF puncture			Mean of all CSF punctures				
	Total	Survival	Death	p-value	Total	Survival	Death	p-value
n (%)	36 (100)	30 (83.33)	6 (16.67)	-	36 (100)	30 (83.33)	6 (16.67)	-
Number of punctures	-	-	-	-	4 (1;6)	4 (1.75;6)	2 (1;5)	-
Color index (%) ¹	0 (-0.15;0.5)	0 (0;0.5)	-0.15 (-0.82;74.62)	0.409	2 (0.1;0.78)	0.3 (0.1;0.71)	0.5 (0.5;0.86)	0.047
Color index > 0 % (n %)	-	-	-	-	32 (86.11)	26 (86.67)	6 (100)	0.999
WBC (cells/mm ³)	15.9 (-15.57;305.95)	27.9 (-7.37;1237.82)	-274.1 (-1026.2;-13.4)	0.017	142.23 (47.5;598.75)	138.34 (4.18;575.06)	195.33 (100;604.2)	0.708
WBC > 5 cells/mm ³ (n %)	-	-	-	-	35 (97.22)	30 (100)	5 (83.33)	0.167
Neutrophils (%) ²	56 (11.25;84.25)	65 (20;87)	1 (-67;10)	0.074	57 (35.4;72)	56.2 (35;74)	61.37 (49.09;63.96)	0.925
Lymphocytes (%) ²	-29.5 (-78.5;4.75)	-59 (-80;-9)	66 (46;86)	0.010	35.43 (18.25;50)	35.43 (17.4;51.14)	34.9 (31.31;43.89)	0.839
Glucose (mg/dL)	-4 (-21.5;18.75)	-8 (-24.5;2.75)	45 (21.25;71.75)	0.006	30.17 (18.5;54.06)	32.87 (15.17;53.13)	28.5 (25.67;57.5)	0.290
Glucose < 45 mg/dL (n %)	-	-	-	-	21 (58.33)	18 (60.00)	3 (50.00)	0.677
Glucose CSF/blood ³	-0.43 (-0.57;-0.22)	-0.43 (-0.57;-0.22)	-	0.500	0.34 (0.06;0.55)	0.36 (0.07;0.54)	0.32 (0.05;0.55)	0.999
Glucose CSF/blood < 0.4 (n %)	-	-	-	-	12 (52.17)	9 (50.00)	3 (60.00)	0.999
Total protein (mg/dL)	3.65 (-93.82;90.97)	4.4 (-65.1;71.9)	-87.65 (-180.75;87.72)	0.291	144.27 (60.9;266.4)	118.91 (48.93;280.76)	171.95 (131.43;195.85)	0.446
Lactate (mmol/L)	1.45 (-0.55;4.3)	2.15 (-0.15;4.97)	-3.15 (-5.62;-0.9)	< 0.001	4.94 (2.85;7.5)	4.05 (2.76;7.39)	5.61 (5.15;10.07)	0.091
Lactate > 3.5 mmol/L (n %)	-	-	-	-	23 (63.89)	18 (60.00)	5 (83.33)	0.634

Significant differences ($p < 0.05$) are shown in bold typeface. Data are presented as median (interquartile range) or n (%). 31 samples with color index in the first and last CSF sample, (survival: 25; death: 6); 26 samples with differential cell count in the first and last CSF sample (survival: 21; death: 5); and 33 patients in more than one CSF sample (survival: 28; death: 5); 4 patients with blood glucose test performed on the date of the first and last puncture (survival: 4; death: 0); 23 patients had blood glucose test performed on the same date as CSF sampling for more than one CSF sample (survival: 18; death: 5).
RBC: red blood cell; WBC: white blood cell; CSF: cerebrospinal fluid.

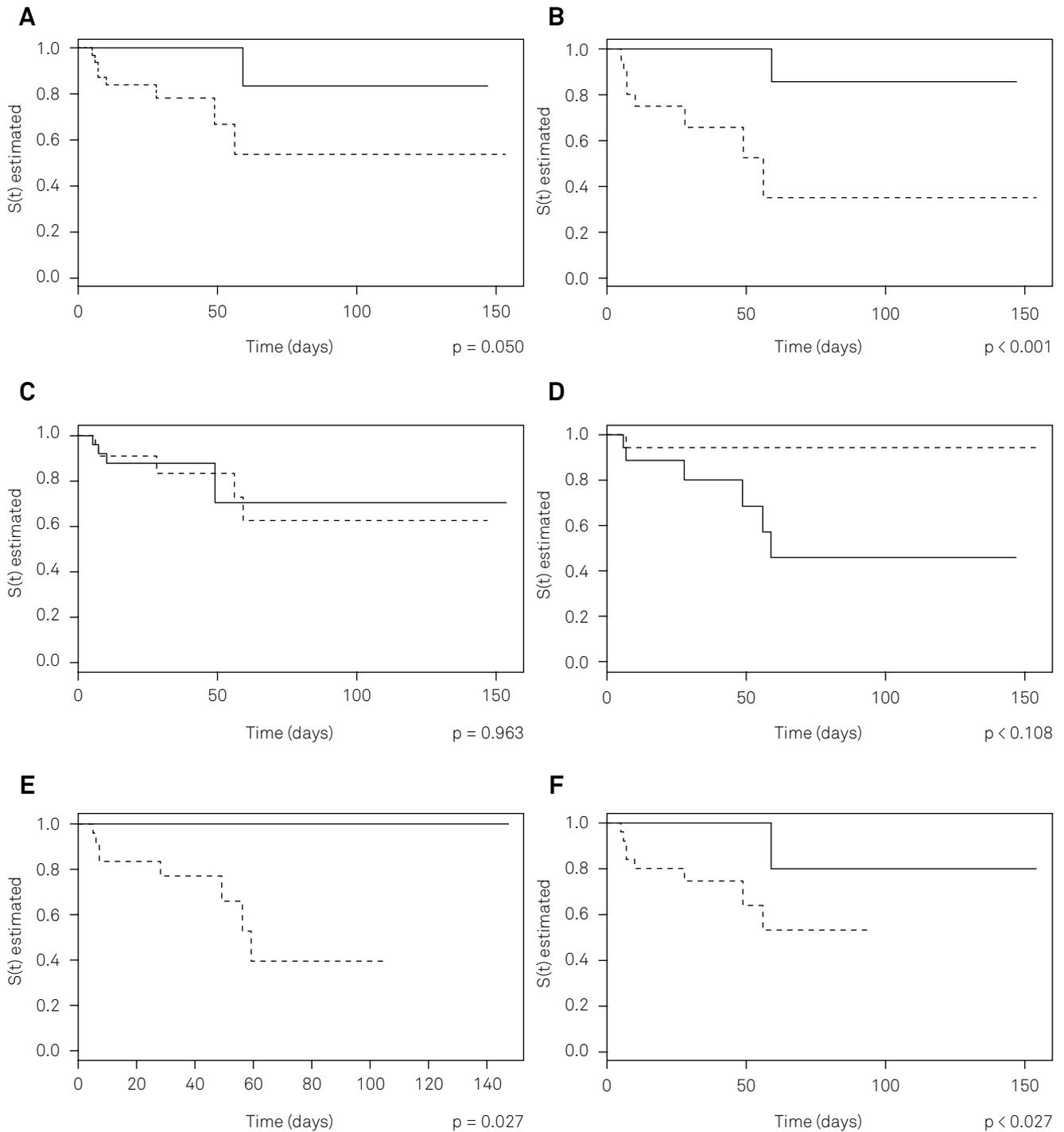


Figure 2. Kaplan-Meier survival curves. A. Survival curve for the mean cerebrospinal fluid (CSF) lactate concentration (mmol/L) in longitudinal CSF samples. B. Survival curve for the CSF lactate concentration (mmol/L) in the last CSF sample. C. Survival curve for the CSF lactate concentration (mmol/L) in the first CSF sample. A, B, C Continuous line CSF lactate < 3.5 mmol/L; dashed line CSF lactate \geq 3.5 mmol/L. D. Survival curve for variation of the CSF lactate concentration between the first and the last sample (mmol/L); continuous line, CSF lactate < 1.4 mmol/L, dashed line CSF lactate \geq 1.4 mmol/L. The cut-off point was calculated by the median of the variation of the CSF lactate concentration in the longitudinal samples in the total group. E. Survival curve for CSF color index (%) in the last CSF sample; continuous line, CSF colorless (color index 0%); dashed line CSF xanthochromia (color index > 0%). F. Survival curve for age (years) of the patients, continuous line age < 29.31 years, dashed line age \geq 29.31 years.

was xanthochromic in the last CSF sample, independent of the value of the CSF color index ($p = 0.050$; $p < 0.001$; $p = 0.027$, respectively; Figure 2). There was no difference in survival time in relation to the other analyzed variables.

In multivariable logistic regression analysis, the following variables differed significantly between the negative-outcome and positive-outcome groups: the lactate concentration in the last CSF sample (odds ratio [OR]: 1.513; 95% CI: 1.178-2.527;

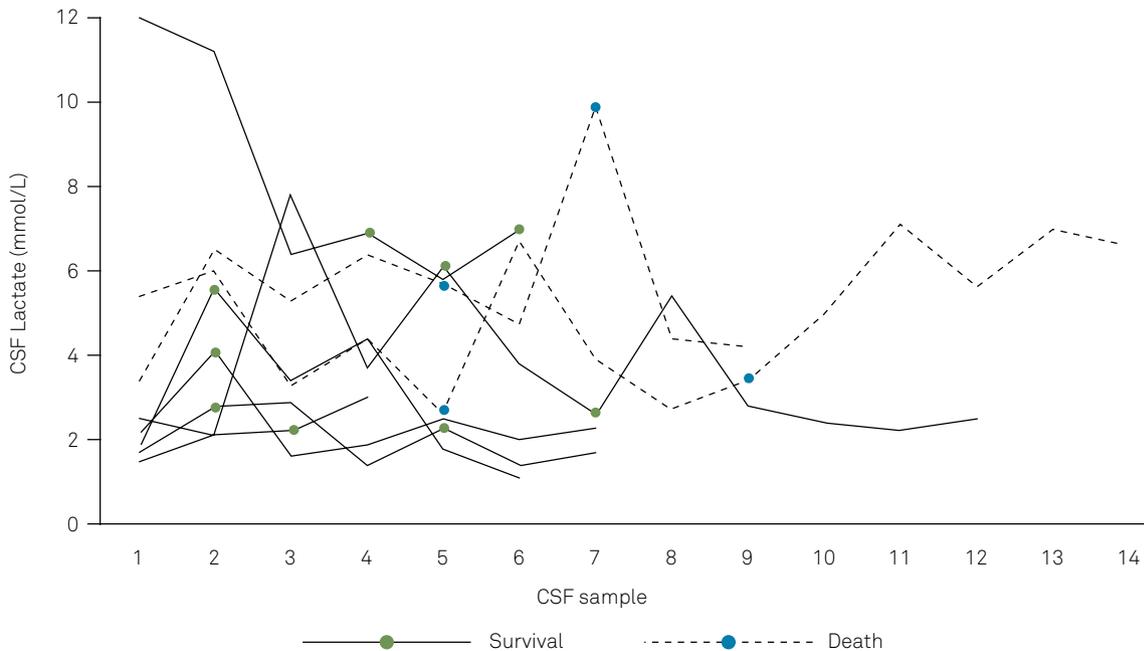


Figure 3. Dynamics of the cerebrospinal fluid (CSF) lactate concentration (mmol/L) in patients with multibacterial meningitis, according to outcome. The continuous line indicates the positive-outcome (survival) group; the dashed line indicates the negative-outcome (death) group. The full circle indicates the CSF sample with positive cultures for bacteria different from that isolate from the first CSF sample. Bacteria identified in multibacterial meningitis cases: *Staphylococcus coagulase-negative* (n = 6), *Klebsiella pneumoniae* (n = 3), *Acinetobacter baumannii* (n = 3), *Proteus mirabilis* (n = 2), *Staphylococcus aureus* (n = 1), *Klebsiella oxytoca* (n = 1), *Serratia marcescens* (n = 1), *Streptococcus viridans* (n = 1), and *Enterobacter cloacae* (n = 1). The highest CSF lactate levels (> 3.5 mmol/L) were associated with a positive culture for bacteria in 31.58% of the CSF samples in which the causal bacteria were identified.

p = 0.017); the mean CSF color index of all serial CSF samples (OR: 1.230; 95% CI: 1.052-1.564; p = 0.023), and CSF glucose variation between the first and last CSF sample (OR: 1.132; 95% CI: 1.040-1.343; p = 0.045).

Multibacterial meningitis

Multibacterial meningitis, i.e. different bacteria isolated from sequential CSF samples, was identified in eight patients (16.33%). Of these, two patients (22.22%) were in the negative-outcome group and six patients (15.63%) were in the positive-outcome group (p = 0.628). In the group with multibacterial meningitis, two patients (25%) died, whereas in the group with a single bacterial strain, six patients (75%) died (p = 0.601).

The relationship between the CSF lactate concentration in patients with multibacterial meningitis and the identified bacteria according to the outcome is shown in Figure 3. A peak of CSF lactate levels (> 3.5 mmol/L) was associated with a positive bacterial culture in six of the 19 (31.58%) CSF samples.

DISCUSSION

In the present series, the mortality rate in patients with acute BM was 18% and this was not dependent on the type of causal bacteria. In developed countries, the mortality rate of acute BM is 10–30%, while in low-income countries it can reach 50%².

In this study, multivariable logistic regression analysis showed that the persistence of a high lactate concentration in the last CSF sample; xanthochromia, indicated by a high mean color index in longitudinal CSF samples; and lower variation in glucose levels between the first and last CSF sample differed significantly between the negative-outcome group and the positive-outcome group.

The value of CSF lactate levels for predicting the outcome of BM is still debatable. In this study, in a univariate analysis, the CSF lactate concentration in the first CSF sample did not discriminate the negative-outcome from the positive-outcome group and was not useful for predicting outcome, in agreement with previous reports^{14,18,19} but in contrast to results from several other studies^{9,10,11,12,13}. In the present study, the CSF lactate levels of the last sample were higher in the negative-outcome than in the positive-outcome group, corroborating the findings of a previous study¹⁴. In addition, we demonstrated that variation in lactate levels, calculated by the difference between the first and last CSF, differed between the two groups.

On the other hand, the present study showed that a rapid decrease in the CSF lactate concentration during treatment was indicative of therapeutic efficacy and good prognosis, confirming the results of previous studies^{14,20,21}. The CSF samples with lactate concentrations higher than 3.5 mmol/L were less frequent in the positive-outcome group and more common in the negative-outcome group. Thus, lactate may be used as a marker of positive as well as negative outcome.

In the present study, xanthochromia, quantified by color index, differed significantly between the negative-outcome and positive-outcome groups; specifically, xanthochromia was associated with a negative prognosis. Color index is a visual comparative method for quantifying CSF xanthochromia; it is easy and is not expensive to perform, although it has a low sensitivity (47%)¹⁷. Xanthochromia has not been investigated in the past as a prognostic marker in acute BM; however, this finding must be interpreted with caution, as the present study included CSF samples collected from different puncture sites. Further studies are necessary to confirm our findings. The causes of CSF xanthochromia in acute BM are increased CSF WBC (> 200 cells/mm³), bacterial concentration $> 10^5$ CFU/mL, and high protein concentration (> 150 mg/dL). Additionally, other causes of xanthochromia can be considered as the presence of red blood cells (> 400 red blood cells/mm³), high intake of carotenoids, hyperbilirubinemia, and meningeal melanomatosis¹⁶.

In the present study, the CSF WBC count was numerically lower in the negative-outcome than in the positive-outcome group, although the difference was not statistically significant. Previous studies showed that a low CSF WBC count is related to a poor prognosis²². A low CSF WBC count was also described in sepsis in adults with pneumococcal meningitis²³. In our study, the last CSF sample showed a persistent elevation of WBC in both groups. In the positive-outcome group, the differential WBC showed predominance of lymphocytes, while in the negative-outcome group, neutrophils predominated. All findings in the last CSF sample in the negative-outcome group suggested a lack of response to antibiotic therapy, independent of the presence of bacteria in the last sample. The CSF findings in patients who have received previous antibiotics may differ^{24,25}; the positivity of conventional bacteriological diagnostic methods (CSF culture and Gram stain) in partially-treated BM is reduced from 50% to 20%; the relationship between polymorphonuclear cells and lymphocytes in CSF may be reversed²⁶⁻²⁸.

In this study, multibacterial meningitis was not related to an increased rate of mortality, although the number of participants was small. Multibacterial infection is reported as a negative prognostic factor by some authors^{6,29,30}. In the present study, the dynamics of the CSF lactate concentration in patients with multibacterial meningitis showed a peak of CSF lactate in those samples with positive bacterial cultures.

In the present study, older age was also considered as a factor of a negative prognosis, confirming the results of

previous studies. Nevertheless, the age of participants considered in previous studies was higher than in our study^{29,30}.

In this study, in a univariate analysis, decreased lactate levels, CSF WBC count, CSF percentage of neutrophils, increased lymphocyte percentage, and inversion of the CSF neutrophil/lymphocyte ratio were identified as factors predicting a good prognosis in a longitudinal CSF analysis, corroborating the findings of an earlier study²⁰. In agreement with this previous study²⁰, we did not find the CSF total protein levels to have a prognostic value.

Several studies addressing prognostic factors in BM have been performed; all were retrospective and relatively small. The present study differed from other studies in that it examined the dynamics of several CSF biomarkers to evaluate mortality-predicting factors from serial CSF samples. Other strengths of this study were the high number of overall CSF samples. Furthermore, in contrast to previous studies, our analysis included communitarian as well as nosocomial bacterial meningitis cases.

The main limitations of this study are its retrospective design; the inclusion of children and adults; the limited number of patients in the negative-outcome group; and the inclusion of only patients with definite acute BM, although the latter point could also be considered a positive point. The aim of this study was to investigate the impact of CSF biomarkers on prognoses, and thus no other measures of disease severity were analyzed. Meningitis prognosis is associated with several factors of diverse nature. Another important limitation was that this study included CSF samples collected from different sites of puncture; therefore, the results of total protein and xanthochromia should be interpreted with caution, as there is a gradient of CSF total protein from the ventricular to the spinal CSF. To address this, we performed a qualitative analysis, using the reference values for the location of the CSF puncture⁵. Concerning lactate, the site of CSF puncture did not interfere with the lactate results, as there was no difference in the reference values of lactate between ventricular and lumbar CSF³¹.

In conclusion, this longitudinal analysis adds important information about lactate and glucose levels as risk factors in the prognosis of acute BM. The current study suggests that persistently elevated CSF lactate and lower variation in glucose levels in patients with acute BM receiving adequate antibiotic therapy may indicate a negative prognosis, with increased mortality. The present study enforces the importance of longitudinal studies of CSF biomarkers and the fact that dynamic studies may provide more useful information for predicting disease outcome.

References

1. Sakushima K, Hayashino Y, Kawaguchi T, Jackson JL, Fukuhara S. Diagnostic accuracy of cerebrospinal fluid lactate for differentiating bacterial meningitis from aseptic meningitis: a meta-analysis. *J Infect.* 2011 Apr;62(4):255-62. <https://doi.org/10.1016/j.jinf.2011.02.010>
2. McGill F, Heyderman RS, Panagiotou S, Tunkel AR, Solomon T. Acute bacterial meningitis in adults. *Lancet.* 2016 Dec;388(10063):3036-47. [https://doi.org/10.1016/S0140-6736\(16\)30654-7](https://doi.org/10.1016/S0140-6736(16)30654-7)

3. Putz K, Hayani K, Zar FA. Meningitis. *Prim Care*. 2013 Sep;40(3):707-26. <https://doi.org/10.1016/j.pop.2013.06.001>
4. Giulieri S, Chapuis-Taillard C, Jaton K, Cometta A, Chuard C, Hugli O, et al. CSF lactate for accurate diagnosis of community-acquired bacterial meningitis. *Eur J Clin Microbiol Infect Dis*. 2015 Oct;34(10):2049-55. <https://doi.org/10.1007/s10096-015-2450-6>
5. Fishman RA. *Cerebrospinal fluid in diseases of the nervous system*. 2nd ed. Philadelphia: Saunders; 1992.
6. Almeida SM, Faria FL, Fontes KG, Buczenko GM, Berto DB, Raboni SM, et al. Quantitation of cerebrospinal fluid lactic acid in infectious and non-infectious neurological diseases. *Clin Chem Lab Med*. 2009;47(6):755-61. <https://doi.org/10.1515/CCLM.2009.160>
7. Huy NT, Thao NT, Diep DT, Kikuchi M, Zamora J, Hirayama K. Cerebrospinal fluid lactate concentration to distinguish bacterial from aseptic meningitis: a systemic review and meta-analysis. *Crit Care*. 2010;14(6):R240. <https://doi.org/10.1186/cc9395>
8. Giampaolo C, Scheld WM, Savory J, Sande MA, Wills MR, Boyd JC. A multivariate approach to prognostication in experimental bacterial meningitis. *Am J Clin Pathol*. 1981 Oct;76(4):442-9. <https://doi.org/10.1093/ajcp/76.4.442>
9. Wu YM, Hsu PC, Yang CC, Chang HJ, Ye JJ, Huang CT, et al. *Serratia marcescens* meningitis: epidemiology, prognostic factors and treatment outcomes. *J Microbiol Immunol Infect*. 2013 Aug;46(4):259-65. <https://doi.org/10.1016/j.jmii.2012.07.006>
10. Lu CH, Chang WN, Chang HW. Adult bacterial meningitis in Southern Taiwan: epidemiologic trend and prognostic factors. *J Neurol Sci*. 2000 Dec;182(1):36-44. [https://doi.org/10.1016/S0022-510X\(00\)00445-7](https://doi.org/10.1016/S0022-510X(00)00445-7)
11. Lu CH, Chang WN, Chuang YC, Chang HW. The prognostic factors of adult gram-negative bacillary meningitis. *J Hosp Infect*. 1998 Sep;40(1):27-34. [https://doi.org/10.1016/S0195-6701\(98\)90021-4](https://doi.org/10.1016/S0195-6701(98)90021-4)
12. Lu CH, Chang WN, Wu HS. *Klebsiella pneumoniae* meningitis: analysis on clinical features of thirty-two adult patients. *Zhonghua Yi Xue Za Zhi (Taipei)*. 1997 Dec;60(6):296-302.
13. Imuekehme S, Obi J, Alakija W. Cerebro-spinal lactate status in childhood pyogenic meningitis in Nigeria. *J Trop Pediatr*. 1997 Dec;43(6):361-3. <https://doi.org/10.1093/tropej/43.6.361>
14. Genton B, Berger JP. Cerebrospinal fluid lactate in 78 cases of adult meningitis. *Intensive Care Med*. 1990;16(3):196-200. <https://doi.org/10.1007/BF01724802>
15. Baird DR, Whittle HC, Greenwood BM. Mortality from pneumococcal meningitis. *Lancet*. 1976 Dec;2(7999):1344-6. [https://doi.org/10.1016/S0140-6736\(76\)91985-1](https://doi.org/10.1016/S0140-6736(76)91985-1)
16. Petzold A, Sharpe LT, Keir G. Spectrophotometry for cerebrospinal fluid pigment analysis. *Neurocrit Care*. 2006;4(2):153-62. <https://doi.org/10.1385/NCC:4:2:153>
17. Arora S, Swadron SP, Dissanayake V. Evaluating the sensitivity of visual xanthochromia in patients with subarachnoid hemorrhage. *J Emerg Med*. 2010 Jul;39(1):13-6. <https://doi.org/10.1016/j.jemermed.2007.09.052>
18. Dzupova O, Rozsypal H, Prochazka B, Benes J. Acute bacterial meningitis in adults: predictors of outcome. *Scand J Infect Dis*. 2009;41(5):348-54. <https://doi.org/10.1080/00365540902849391>
19. Tang LM, Chen ST. *Klebsiella pneumoniae* meningitis: prognostic factors. *Scand J Infect Dis*. 1994;26(1):95-102. <https://doi.org/10.3109/00365549409008596>
20. Sumanth Kumar AS, Sahu BP, Kumar A. Prognostic value of cerebrospinal fluid lactate in meningitis in postoperative neurosurgical patients. *Neurol India*. 2018 May-Jun;66(3):722-5. <https://doi.org/10.4103/0028-3886.232330>
21. Cunha BA. The clinical and laboratory diagnosis of acute meningitis and acute encephalitis. *Expert Opin Med Diagn*. 2013 Jul;7(4):343-64. <https://doi.org/10.1517/17530059.2013.804508>
22. de Fátima Magalhães Acioly Mendizabal M, Bezerra PC, Guedes DL, Cabral DB, de Barros Miranda-Filho D. Prognostic indicators in bacterial meningitis: a case-control study. *Braz J Infect Dis*. 2013 Sep-Oct;17(5):538-44. <https://doi.org/10.1016/j.bjid.2013.01.016>
23. Weisfelt M, Beek D, Spanjaard L, Reitsma JB, Gans J. Attenuated cerebrospinal fluid leukocyte count and sepsis in adults with pneumococcal meningitis: a prospective cohort study. *BMC Infect Dis*. 2006 Oct;6(1):149. <https://doi.org/10.1186/1471-2334-6-149> PMID:17038166
24. Rothrock SG, Green SM, Wren J, Letai D, Daniel-Underwood L, Pillar E. Pediatric bacterial meningitis: is prior antibiotic therapy associated with an altered clinical presentation? *Ann Emerg Med*. 1992 Feb;21(2):146-52. [https://doi.org/10.1016/S0196-0644\(05\)80149-0](https://doi.org/10.1016/S0196-0644(05)80149-0)
25. Nigrovic LE, Malley R, Macias CG, Kanegaye JT, Moro-Sutherland DM, Schremmer RD, et al.; American Academy of Pediatrics, Pediatric Emergency Medicine Collaborative Research Committee. Effect of antibiotic pretreatment on cerebrospinal fluid profiles of children with bacterial meningitis. *Pediatrics*. 2008 Oct;122(4):726-30. <https://doi.org/10.1542/peds.2007-3275>
26. Feldman WE. Effect of prior antibiotic therapy on concentrations of bacteria in CSF. *Am J Dis Child*. 1978 Jul;132(7):672-4.
27. Blazer S, Berant M, Alon U. Bacterial meningitis. Effect of antibiotic treatment on cerebrospinal fluid. *Am J Clin Pathol*. 1983 Sep;80(3):386-7. <https://doi.org/10.1093/ajcp/80.3.386>
28. Shohet I, Shahar E, Meyerovich J, Barzilay Z. Diagnosis of bacterial meningitis in previously treated children. *South Med J*. 1985 Mar;78(3):299-301. <https://doi.org/10.1097/00007611-198503000-00016>
29. Jonge RC, Furth AM, Wassenaar M, Gemke RJ, Terwee CB. Predicting sequelae and death after bacterial meningitis in childhood: a systematic review of prognostic studies. *BMC Infect Dis*. 2010 Aug;10(1):232. <https://doi.org/10.1186/1471-2334-10-232>
30. Vibha D, Bhatia R, Prasad K, Srivastava MV, Tripathi M, Singh MB. Clinical features and independent prognostic factors for acute bacterial meningitis in adults. *Neurocrit Care*. 2010 Oct;13(2):199-204. <https://doi.org/10.1007/s12028-010-9396-4>
31. Almeida SM, Nogueira MB, Nogueira K. Cerebrospinal fluid lactate levels according to the site of puncture. *Clin Chem Lab Med*. 2019 Aug;0(0):/j/cclm.ahead-of-print/cclm-2019-0726/cclm-2019-0726.xml. <https://doi.org/10.1515/cclm-2019-0726>