Original Article

Correlation between leukocyte aggregation score of cerebrospinal fluid and bacterial meningitis in children

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ABSTRACT

Background Bacterial meningitis is one of life-threatening diseases and carries a risk of sequelae in affected children. In terms of cost and rapid differentiation between bacterial and non-bacterial meningitis, several tests have been proposed.

Objective This study aimed to determine the use of leukocyte aggregation score (LAS) of cerebrospinal fluid (CSF) in diagnosing bacterial meningitis.

Methods A prospective analytic study was done from October 2001 to July 2002 in the Department of Child Health, Medical School, Sam Ratulangi University/ Manado General Hospital. Children presenting with symptoms of meningitis, aged between 28 days and 13 years were enrolled. LAS was counted in percentage. Regression analysis was used to determine the correlation between LAS and diagnosis of bacterial meningitis.

Results CSF examinations were done on 35 meningitis patients. Three patients were excluded. The remaining 32 patients comprised of 11 with bacterial meningitis and the other 21 with non-bacterial meningitis. The mean of LAS in bacterial meningitis was significantly higher than that of non-bacterial meningitis (p<0.001). The cut off value of LAS to diagnose bacterial meningitis was 12.35%.

Conclusion LAS may be used as a fast and simple alternative diagnostic tool to confirm the diagnosis of bacterial meningitis **[Paediatr Indones 2004;44:61-65].**

Keywords: bacterial meningitis, leukocyte aggregation score, cerebrospinal fluid

Bacterial meningitis is one of life-threatening neurologic diseases, especially in neonatal and childhood, that carries a high risk of neurologic sequelae and still has high mortality.[1-3] Early diagnosis and prompt treatment are important in the management of bacterial meningitis. But early symptoms and signs are not specific, mainly in infant. There had been no single examination that could early distinguish the etiology of meningitis in infant and children.[1-3] Yet, there are many reports about the efforts to diagnose bacterial meningitis as early as possible, such as CSF lactate level,[4] C-reactive protein (CRP),[5-7] lactoferrin, α-1 antitrypsin, immunoglobulin GA,[7] tumor necrosis factor (TNF),[8-10] interleukin (IL) 1α,[10-11] IL-6,[12] granulocyte colony stimulating factor,[13] bacterial antigen detection test,[14] and CSF leukocyte aggregation score (LAS).[15-16] CSF LAS examination is simple, fast, not expensive, and does not need sophisticated equipment or skills.[16]

The objective of this study was to evaluate the use of LAS examination in establishing early diagnosis of bacterial meningitis and to compare it with standard examinations.

Methods

A prospective analytic study was conducted at the Department of Child Health, Manado General Hospital from October 2001 to July 2002. The inclusion criteria were infants and children aged between 28 days and 13 years with symptoms and signs

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of meningitis who were admitted to our department. The exclusion criteria were infants or children who had contraindication of lumbar puncture (LP) (shock, severe general condition, skin infection around the location of LP, space occupying lesion, hydrocephalus, blood dyscrasia, hyperventilation, irregular respiration, and apnea), blood-contaminated CSF whether grossly or microscopically, and other conditions such as meningismus, malignancy, collagen-vascular syndromes, toxin exposure, and focal infection of the central nervous system (i.e., brain abscess, parameningeal infection, and subdural empyema). Informed consent was obtained from the parents.

The diagnosis of bacterial meningitis is based on symptoms and signs of meningitis i.e., fever, seizure, and unconsciousness, plus at least one of the followings: 1) biochemistry and cytology results of CSF examination were opalescent to turbid in color, Nonne (+), Pandy (+), protein >40 mg/dL, glucose <40 mg/dL, leukocyte count 1,000-10,000/mm^3 with predominant PMN, and positive Gram staining; 2) positive bacterial growth on culture.

After LP had been performed, the CSF obtained was divided into 2 tubes. One tube containing 1-2 mL CSF was sent to Prodia Clinical Laboratory for biochemistry, cytology, and Gram staining examination within 90 minutes. The second one containing 1 mL CSF was sent as soon as possible to the Department of Microbiology, Medical School, Sam Ratulangi University for bacterial culture. The procedure to make LAS slide was as follows: one to two drops of CSF was put on an object glass, then it was laid on a 45° declined base for 3-4 seconds. The CSF was precipitated by gravitation, leaving a thin layer. Then, it was dried on a horizontal position, fixed by absolute methanol and stained with hematoxyllin-eosin. Two skillful pathologists from the Department of Clinical Pathology, Medical School, Sam Ratulangi University, read it under a light microscopes without knowing either the patient's condition or the results of CSF biochemistry, cytology, and culture. The score was then given in percentage.

Data were analyzed descriptively and analytically. Regression model was used to determine the correlation between two variables, with formula: y = Bx+E (y for dependent variable i.e., diagnosis of bacterial meningitis; x for independent variable, i.e., LAS; B for regression coefficient; and E for other variable).

Fisher's exact test was used to compare two categorical variables.

Results

From October 2001 to July 2002, CSF examinations were conducted on 35 patients with symptoms and signs of meningitis who were admitted to the Department of Child Health, Medical School, Sam Ratulangi University, Manado General Hospital. Three patients were excluded due to technical faults i.e., one without Pandy and protein level examination results, one with biochemistry, cytology, and Gram staining examination performed in more than 90 minutes after LP, and the other one with insufficient CSF sample for bacterial culture. The remaining 32 patients comprised of 11 patients with bacterial meningitis and 21 with non-bacterial meningitis.

The characteristics of the patients showed that most were in the age of <2 year-old. The ratio of male to female was 1:1.

Table 1 shows that positive bacterial culture was found mostly in the age of <2 years (6 from 11 patients).

TABLE 1. DISTRIBUTION OF PATIENTS WITH POSITIVE CULTURE RESULTS ACCORDING TO BACTERIAL ETIOLOGY AND AGE GROUP

Bacterial etiology	Age Group			
	< 2 yrs	2-5 yrs	> 5 yrs	Total
Enterobacter aerogenes	1	2	0	3
Staphylococcus epidermidis	3	0	0	3
Alkaligenes faecalis	0	1	1	2
Citrobacter difersus	1	0	0	1
Acinetobacter sp.	0	1	0	1
Serratia marsecen	1	0	0	1
Total	**6**	**4**	**1**	**11**

Table 2 shows the mean and standard deviation of age, protein, glucose, leukocyte, and LAS of CSF according to the diagnosis. It was obvious that only the mean of LAS that was significantly higher in bacterial meningitis than that in non-bacterial meningitis (p<0.001).

The correlation between diagnosis and the results of CSF examinations including biochemistry, cytology, and culture is shown in **Table 3**; only LAS that was significantly correlated to bacterial meningitis. Accord-

TABLE 2. MEAN AND STANDARD DEVIATION (SD) OF VARIABLES ACCORDING TO DIAGNOSIS.

| Variables | Diagnosis | | p |
	Bacterial Meningitis (mean±SD)	Non-bacterial Meningitis (mean±SD)	
Age (years)	2.77±2.32	4.06±3.62	0.296
Protein (mg/dL)	247.02±614.42	52.81±51.42	0.320
Glucose (mg/dL)	40.36±29.26	59.48±32.28	0.111
Leukocyte (/mm³)	377.00±441.16	100.86±257.16	0.077
LAS (%)	20.27±8.26	1.95±3.37	0.000

TABLE 3. THE CORRELATION BETWEEN CSF EXAMINATIONS AND DIAGNOSIS

| CSF examinations | Diagnosis | | Total (n) | Fisher's exact test |
	Bacterial meningitis (n)	Non-bacterial meningitis (n)		
Color				p=0.148
Turbid	4	2	6	
Clear	7	19	26	
Nonne test				p=0.681
Positive	4	5	9	
Negative	7	16	23	
Pandy test				p=0.645
Positive	8	12	20	
Negative	3	9	12	
Protein (mg/dL)				p=0.703
>40	8	13	21	
<40	3	8	11	
Glucose (mg/dL)				p=0.053
<40	7	5	12	
>40	4	16	20	
Leukocyte (/mm³)				p=0.266
>1,000	2	1	3	
<1,000	9	20	29	
Bacterial culture				p=0.000
Positive	11	0	11	
Negative	0	21	21	
LAS (%)				
>12.35	11	0	11	p=0.000
<12.35	0	21	21	
>15.0	9	0	9	p=0.000
<15.0	2	21	23	

ing to our equation, the cut off value of LAS for the diagnosis of bacterial meningitis was 12.35%.

Discussion

In this study, we excluded neonates and we did not perform any culture for *Mycobacterium tuberculosis*, fungi, and virus; we just categorized the diagnosis as bacterial and non-bacterial meningitis.

Bacterial meningitis was found more frequently in female than male and most commonly at the age of <2 years. These findings were similar to those reported by Klein *et al.*[1] Vince *et al*[17] reported that bacterial meningitis might occur in all ages, but most frequently in the first year of life.

We did not find bacteria in all Gram staining of CSF. According to literatures, Gram staining may reveal bacteria in about 70% of bacterial meningitis, but it may show no bacteria if the number of bacteria is less than 10^4–10^5 organism/ml although the culture shows bacterial growth.[18]

The etiologies of bacterial meningitis in our study were *Enterobacter aerogenes* and *Staphylococcus epidermidis*

(3 patients each), *Alkaligenes faecalis* (2 patients), *Citrobacter difersus*, *Acinetobacter*, and *Serratia marcesen* (1 patients each). Wenger *et al* [19] reported that the etiologies of bacterial meningitis were *Enterobacter* (9%), *Serratia* (9%), *S. epidermidis* (8%), and *Acinetobacter* (6%).

Michelow *et al*[16] reported that compared to LAS, other CSF examinations (leukocyte count, Gram staining, and culture) and blood culture were less significant in diagnosing bacterial meningitis. In this study, there was no significant correlation between the color of CSF, Nonne/Pandy test, protein, glucose, leukocyte count and the diagnosis of bacterial meningitis. It means that those examinations might not be used as a single diagnostic tool without any additional examinations.

There were only several hundreds of leukocytes per mm^3 CSF in the early stage of bacterial meningitis, even mononuclear cell may be more prominent.[20] On the other hand, the CSF of non-bacterial meningitis, such as viral or aseptic ones, in their early stage, may be dominated by PMN.[20-22] Polk *et al*[23] reported several cases of bacterial meningitis with normal leukocyte count in their CSF.

Protein level in CSF may vary depending on age, number of cell, etiology, and damaged cells. High protein level may be found in either bacterial meningitis or other conditions.[22] Thus, we can not confirm the diagnosis of bacterial meningitis based on protein level only.

CSF glucose level may be low in tuberculous meningitis or if sample is examined in more than 90 minutes after LP due to the consumption of glucose by bacteria for their metabolism. The level of glucose in CSF is influenced by blood glucose level.[24] Thus, it is better to examine both blood and CSF glucose level, and then determine their ratio. It was not done in our study because of our limitation.

There was a very significant correlation between LAS and the diagnosis of bacterial meningitis (p<0.001). Based on these data and by regression analysis model, we found the cutoff value of LAS was 12.35%. Garty *et al* reported that the cutoff value of LAS was 15%.[15]

In our study, we found that with the cutoff value of LAS at 12.35%, the sensitivity and specificity were 100% each. Meanwhile, with the cutoff value at 15%, they were 81.8% and 100% respectively. Michelow *et al*[16] reported that the higher the cutoff value, the lower the sensitivity is. They showed the sensitivity of LAS

was 92.5%-98.5% and its specificity was 64.3%-88.1%. Garty *et al*[15] reported the sensitivity and specificity of LAS to diagnose bacterial meningitis were 88%-94% and 100% respectively.

We concluded that there was a very significant correlation between either LAS or CSF culture and the diagnosis of bacterial meningitis. Therefore LAS may be used as a fast and simple alternative diagnostic tool to confirm the diagnosis of bacterial meningitis.

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