Research Article

Diagnostic Value Of Tumor Necrosis Factor - Alpha In Cerebrospinal Fluid Differentiates Bacterial From Viral Meningitis In Children

Masayu Lubna Anniazi[1], Fadhillah Tia Nur[1], Sri Lilijanti Widjaja[1]

Author's Affiliation:
1- Medicine Faculty of Sebelas Maret University Pediatric department of Dr. Moewardi Hospital.

Correspondence:
Masayu Lubna Anniazi, Email: lubnamasayu@gmail.com

Received on: 13-Jan-2020 Accepted for Publication: 31-Mar-2020

ABSTRACT

Background: The incidence of pediatric meningitis in Indonesia still arises, with mortality rate between 18-40% and neurological sequelae in developing countries reached between 30-50%. Cytology analysis of cerebrospinal fluid has difficulties in differentiating bacterial meningitis from viral meningitis. Culture, as diagnostic gold standard, only resulted positive in 40-50% of cases. TNF-α cytokine assessment is expected to be able to differentiate bacterial meningitis from viral one in several studies abroad. The value, however, was heterogenic and affected by race, genetic, age and gender.

Objective: To analyze the differences in sensitivity, specificity, positive predictive value, negative predictive value from cut-off value of bacterial and viral meningitis in Indonesian children.

Methodology: Cross-sectional diagnostic study on cerebrospinal fluid samples was conducted in children aged 2 months-18 years from May 2018-June 2019 in Moewardi Hospital, Surakarta.

Results: Out of 46 samples of children with clinical acute meningitis, 23.9% was considered bacterial meningitis, 41.3% viral meningitis, and 30.4% non-meningitis cases. The cut-off value of TNF-α level in bacterial meningitis was 2.61 pg/ml with 90.9% sensitivity, 80% specificity, 58.8% positive predictive value, 96.6% negative predictive value and 0.918 AUC. The cut-off value of TNF-α level in viral meningitis was 1.305 pg/ml with 63.2% sensitivity, 40.7% specificity, 42.9% positive predictive value, 61.1% negative predictive value and 0.434 AUC.

Discussion: The comparison of AUC revealed that TNF-α level in cerebrospinal fluid significantly differed between bacterial and viral meningitis in Indonesian children population. Significant increase of TNF-α level in bacterial meningitis compared to viral meningitis can be used as a diagnostic test of both diseases.

Conclusion: TNF-α cytokine assessment in cerebrospinal fluid can differentiate bacterial meningitis from viral meningitis in Indonesian children.

Keywords: TNF-α, cerebrospinal fluid, bacterial meningitis, viral meningitis

INTRODUCTION

Acute meningitis in children is usually aseptic and did not require specific treatment. Only 4-6% of acute meningitis in children is caused by bacterial infection. The incidence of meningitis in Indonesia children is still high, which placed 9th out of 10 most common disease from the data of 8 educational hospitals in Indonesia. Cases of suspected bacterial meningitis in Indonesia is higher than those of developed countries, comprises of 158 out of 100,000 children per year. In Indonesia, the mortality rate of meningitis in children was 18-40% with disability rate between 30-50%.[1,2,3]

Cerebrospinal fluid analysis is the simplest adjunctive examination. However, several current studies proved that PMNs could also dominate during the early phase of viral meningitis. Therefore, 60-90% of polymorphonuclear cell domination assessment in cerebrospinal fluid as bacterial meningitis indicator can no longer be used. Cerebrospinal fluid culture as the gold standard of diagnosis establishment in meningitis only revealed positive in ± 50% cases in several studies. The sensitivity of culture decreased to 30% after antibiotic therapy before spinal tap procedure. The administration of empirical antibiotic should be halted until the result of culture is

available, which usually took 48-72 hours. Thus, it can cause several factors, including increased antibiotic resistance by pathogenic microorganism, side effects of medication, increased nosocomial infection rate and increased treatment cost.[4,5,6,7,8]

Tumor necrosis factor alpha (TNF-α) is a proinflammatory cytokine produced by endothelial cells, microglial cells, astrocytes, macrophages, and monocytes in initial phase due to local inflammation in the central nervous system. The increase of TNF-α level in meningitis patients cannot be found in blood serum because this cytokine is produced as a local response to infected brain tissue known as the compartment phenomenon. Two meta-analyses conducted in 2014 showed that TNF-α assessment in cerebrospinal fluid could differentiate bacterial meningitis and viral meningitis in children. Heterogenicity was found in TNF-α assessment results in several studies conducted in several countries, which can be caused by race/ethnic, age, gender, and assessment technique (ELISA/RIA/IF/RT-PCR), thus the diagnostic threshold of TNF-α cannot be determined.[9,10,11]

Currently, TNF-α cytokine assessment in cerebrospinal fluid can only be conducted using the ELISA (Enzyme Linked Immuno Sorbent Assay) technique in Indonesia. The results of this technique can be acquired faster than the gold standard of culture with better accuracy. The purpose of this study was to obtain diagnostic threshold of TNF-α level to differentiate bacterial meningitis and viral meningitis in Indonesian children population and to analyze the differences in sensitivity, specificity, positive predictive value, and negative predictive value.

METHODS

Study Protocol

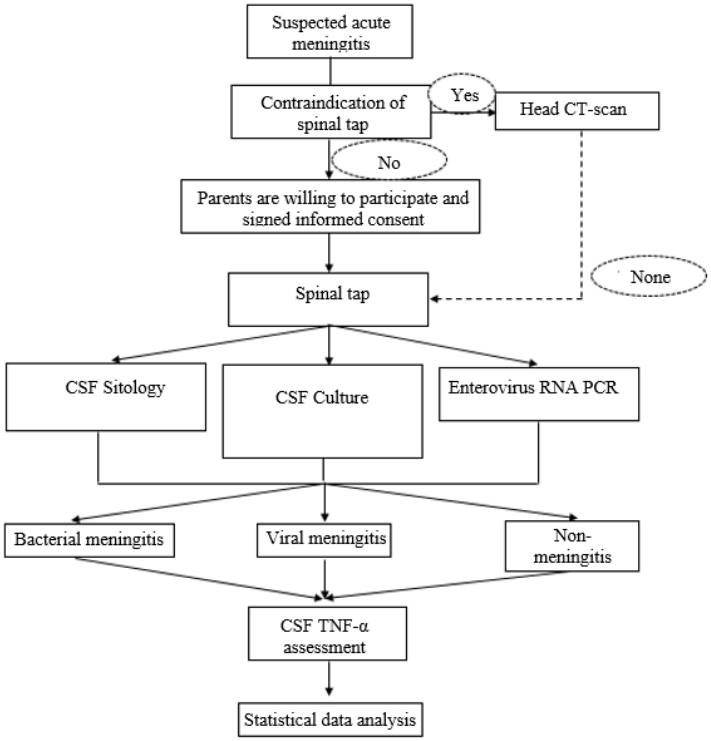

Figure 1. Flowchart of the study process

Sample Population

This study used a single blinded diagnostic test (the appointed laboratory operator did not know whether the CSF samples were the results from positive or negative culture or enterovirus RNA PCR). This study obtained the samples from all children aged 2 months-18 years old with clinical acute meningitis with symptoms onset <

96 hours and admitted to children ward, pediatric High Care Unit, or Pediatric Intensive Care Unit (PICU) of Moewardi Hospital, Solo between May 2018-June 2019.

The samples were selected by consecutive sampling after fulfilling inclusion and exclusion criteria. The exclusion criteria include: history of steroid use within the last 2 weeks, history of antibiotic use that passed through blood-brain barrier for > 24 hours, any contraindication of spinal tap procedure, such as space occupying lesion (SOL), skin infection around puncture location, severe cardiorespiratory disorder, congenital structural anomaly of the spine, blood coagulation disorder, severe immunosuppression condition, and signs of cerebral herniation. The sample size of diagnostic study with AUC outcome:

$$n = \left(\frac{Z\alpha\sqrt{2V_1} + Z\beta\sqrt{V_1+V_2}}{\Theta_1 - \Theta_2} \right)^2$$

n = 45.6 ~ 46 (total samples of suspected acute meningitis)

Operational Definition

Bacterial meningitis

The diagnosis has been classified based on WHO 2003 criteria for bacterial meningitis into:

1. Suspected: sudden fever related with one sign of nuchal rigidity, altered mental status, meningeal sign positive.
2. Probable: suspected case which CSF analysis showed one of cloudy, leukocytosis (>100cell/mm3), Nonne/Pandy test positive, protein level >100mg/dl, glucose ratio CSF/serum < 0.5, Polymorphonuclear domination of leukocyte.
3. Confirmed: CSF-culture found any growth of pathogen bacterial.

In this study we used probable and confirmed criteria to establish bacterial meningitis diagnosis.12,13

Viral meningitis

Diagnosis has defined based on CSF cytology that showed slight leukocytosis (10-100 cell/mm3), protein level ≤ 100mg/dl, Nonne/Pandy negative, glucose ratio CSF/serum > 0.5, mononuclear domination of leukocyte and PCR RNA positive for enterovirus serotype.7,13

Non-meningitis

The diagnosis has established based on CSF analysis profile that showed normal leukocyte < 5 cell/mm3, protein level ranged 20-45 mg/dl, CSF glucose level 75% serum blood glucose, Nonne/Pandy negative, lymphocyte domination of leukocyte, negative both of PCR RNA enterovirus and CSF-culture.13

Cytokine Assay

Cerebrospinal fluid was divided into 3; four milliliters were poured on the first tube and sent to clinical pathology laboratory for CSF and enterovirus RNA PCR assessments. Three milliliters were poured on the second tube and sent to microbiology laboratory for gram staining and CSF agar culture. One milliliter was poured to the third tube and sent to the clinical pathology laboratory to be centrifuged at 3000 rpm and 0,5 ml of supernatant was obtained in a microtube to be stored in a refrigerator with -70oC temperature. After all samples were collected, TNF-α level was assessed using the ELISA technique. The freeze-stored cerebrospinal fluid will undergo 3% decomposition.9

The reagent used was Luminex Performance Assay Human TNF-α High Sensitivity Kit (Magnetic High Sensitivity Cytokine Panel) produced by R&D System, Minneapolis, USA with detection range limit of 0.82 – 3350 pg/ml. The sensitivity of this reagent assessment is very high and can detect to 0.13 – 0.54 pg/ml (average 0.29 pg/ml) for minimum detectable dose (MDD). The specificity of this reagent is also good, which can detect

natural and artificial (recombinant) human TNF-α, cross-reaction with rat, guinea pig, horse, and dog TNF-α under 0.5% value. Biotin-Streptavidin ELISA was used to obtain quantitative TNF-α level.

STATISTICAL ANALYSIS

The baseline categorical data was presented in frequency distribution (%). The characteristics of patients with bacterial and viral meningitis were subjected to 2 population comparison test; categorical data were subjected to Chi-square/Fisher's Exact test, while numerical data were subjected to independent T-test if the data were normally distributed and Mann-Whitney test if otherwise.

The data were analyzed using SPSS version 20 for diagnostic statistical analysis, ROC curve and graph. The cut-off value of TNF-α level was obtained from the best AUC (Area Under Curve) from sensitivity and specificity sides. The data from AUC value determined sensitivity, specificity, positive predictive value and negative predictive value from TNF-α assessment in diagnosing bacterial and viral meningitis in Indonesian children. The AUC value of each disease was compared to determine the difference of diagnostic cut-off value between bacterial and viral meningitis.

RESULTS

Characteristics	F	%
Age		
<2 years	18	39.1%
2- 5 years	14	30.4%
>5 years	14	30.4%
Gender		
Male	28	60.9%
Female	18	39.1%
Symptom		
Fever	29	63.0%
Seizure	27	58.7%
Loss of consciousness	19	41.3%
Headache	4	8.7%
Nutritional Status		
Overnutrition	3	6.5%
Good nutrition	21	45.7%
Less nutrition	20	43.5%
Poor nutrition	2	4.3%
Diagnosis		
Bacterial meningitis	11	23.9%
Viral meningitis	19	41.3%
TB meningitis	2	4.3%
Non-meningitis	14	30.4%
Onset of disease		
<24	21	45.7%
24-72	19	41.3%
>72	6	13.0%
CSF Culture		
Negative	41	89.1%
Positive	5	10.9%
Enterovirus PCR		
Negative	27	58.7%
Positive	19	41.3%
Neurological sequelae		
Aphasia	1	2.2%
Cerebral palsy	11	23.9%

Epilepsy	4	8.7%
Paresis/plegia	4	8.7%
Mental retardation	1	2.2%
None	25	54.3%

Table 1. Baseline characteristic data of subjects

Meningitis incidence in children increases in < 2 years old with 39.2%. Meningitis patients were mostly male compared to female, with 60.9%. Anthropometric nutrition status of most meningitis patients was good (45.7%) and only 43.5% had poor nutrition. The most common early symptoms of meningitis in children was fever with 63%, followed by seizure with 58.7%, loss of consciousness 41.3% and headache with only 8.7%. Characteristic of both groups showed insignificant difference in baseline condition.

Variable	Bacterial meningitis (n = 11)	Viral meningitis (n = 19)	p-value
Age			0.724
< 2 years	5 (45,5%)	7 (36,8%)	
2-5 years	2 (18,2%)	6 (31,6%)	
> 5 years	4 (36,4%)	6 (31,6%)	
Gender			0.466
Male	8 (72.7%)	11 (57.9%)	
Female	3 (27.3%)	8 (42.1%)	
Onset of symptoms			0.454
< 24 hours	6 (54.5%)	6 (31.6%)	
24-72 hours	4 (36.4%)	11 (57.9%)	
>72 hours	1 (9.1%)	2 (10.5%)	
Neurological examination			
Increased physiological reflex	3 (27.3%)	5 (26.3%)	1.000
Positive pathological reflex	6 (54.5%)	7 (36.8%)	0.454
Positive meningeal stimulus	3 (27.3%)	7 (36.8%)	0.702
CSF routine analysis			
CSF/serum glucose < 0.5	3 (27.3%)	8 (42.1%)	0.466
Positive Nonne/Pandy	10 (90.9%)	9 (47.4%)	0.023
CSF Protein ≥ 100	6 (54.5%)	1 (5.3%)	0.04
Cell dominance	PMN 5 (45.5%)	PMN 5 (26.3%)	1.000
	MN 5 (45.5%)	MN 12 (63.2%)	1.000
CSF Culture			0.001
	Positive 5 (45.5%)	Positive 0 (0 %)	
	Negative 6 (54.5%)	Negative 19 (100%)	
Neurological sequela			0.338
Paresis/plegia	3 (27.3%)	1 (5.3%)	
Aphasia	0 (0%)	1 (5.3%)	
Cerebral palsy	5 (45.5%)	4 (21%)	
Epilepsy	1 (9.1%)	2 (10.5%)	
Mental retardation	0 (0%)	1 (5.3%)	

Table 2. Characteristic difference between bacterial and viral meningitis groups

Predilection of age and gender were similar between bacterial and viral meningitis in children. Bacterial meningitis had a significantly faster onset compared to viral meningitis. Neurological examination did not find any significant difference between both diseases. The result of routine CSF analysis which can differentiate bacterial and viral meningitis depends on Nonne/Pandy examination and CSF protein level. However, these examinations were unspecific for neither. CSF culture result was only found in 45.5% bacterial meningitis cases, which was in line with a literature that assessed positive CSF culture in ± 60% cases of bacterial meningitis. Neurological sequelae was most frequently found in bacterial meningitis compared to viral and most insignificant was cerebral palsy.

Diagnostic Test of TNF-α in Detecting Bacterial Meningitis

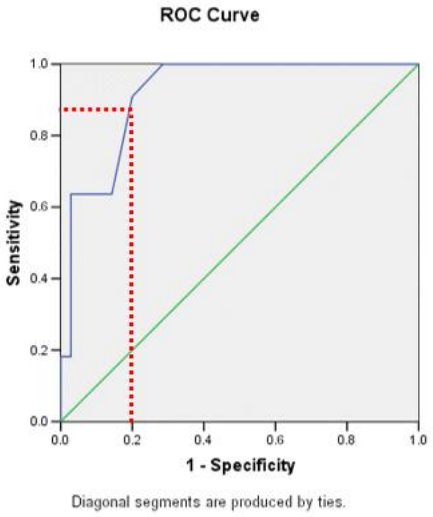

Figure 2. ROC Curve TNF-α in detecting bacterial meningitis

The diagnostic cut-off value of TNF-α was 2.61 pg/ml, AUC = 0.918 (95% CI = 0.839-997) with p value = 0.000.

TNF-α	Bacterial Meningitis		Total
	Yes	No	
>2.610	10	7	17
<2.610	1	28	29
Total	11	35	46
Sensitivity	90.9%		
Specificity	80%		
PPV	58.8%		
NPV	96.6%		
PLR	4.545		
NLR	0.114		

Table 3. Dummy tables of diagnostic test bacterial meningitis

The above ROC curve reveals that TNF-α examination in cerebrospinal fluid is highly sensitive in detecting bacterial meningitis with significant moderate specificity with p value < 0.05. AUC score of 0.918 means that it is good diagnostic in bacterial meningitis significantly with sensitivity of 90.9%, specificity of 80%, positive predictive value of 58.8%, and negative predictive value of 96.6%.

Diagnostic Test of TNF-α in Detecting Viral Meningitis

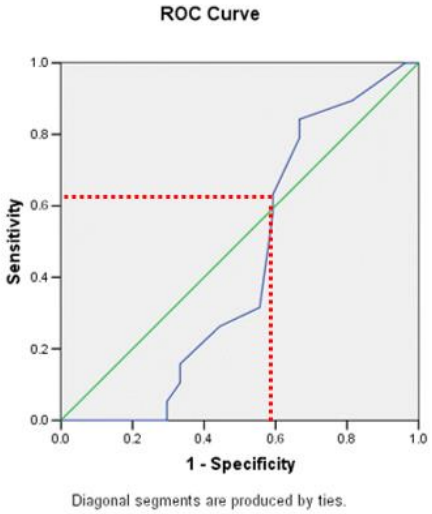

Figure 3. ROC Curve TNF-α in detecting viral meningitis

The diagnostic cut-off value of TNF-α = 1.305 pg/ml with AUC = 0.434 (95% CI = 0.266 – 0.601) and p value = 0.448.

TNF-α	Viral Meningitis		Total
	Yes	No	
>1.305	12	16	28
<1.305	7	11	18
Total	19	27	46
Sensitivity	63.2%		
Specificity	40.7%		
PPV	42.9%		
NPV	61.1%		
PLR	1.066		
NLR	0.904		

Table 4. Dummy tables of diagnostic test viral meningitis

The above ROC curve analysis revealed less sensitive TNF-α examination in cerebrospinal fluid in detecting viral meningitis with low specificity (AUC = 0.434) and statistically insignificant with p value = 0.448. The AUC score of 0.434 means that it was poor in diagnosing viral meningitis with sensitivity of 63.2%, specificity 40.7%, positive predictive value of 42.9%, and negative predictive value of 61.1%.

AUC Comparison of Diagnostic TNF-α Value

AUC was considered poor in diagnosing with < 0.7 score. Otherwise, it was considered good with 0.7-0.9 score, and very good with 0.9-1. The diagnostic ability of TNF-α in bacterial meningitis was very good with AUC score of 0.918, with wide confidence interval range and p value of < 0.05. This showed that there was a significant difference of TNF-α level between bacterial and viral meningitis which only had 0.434 AUC. This examination can be performed as a diagnostic test in bacterial meningitis cases.

DISCUSSION

The incidence distribution of meningitis was mostly found in < 2 years old population. Older age seems to reduce meningitis incidence. The implication from this certainly increases neurological sequel incidence, whereas

early age group should have brain neuron cell myelinization, thus affecting long term cognitive or motoric ability. It was seen by high neurological sequel complication of cerebral palsy in both meningitis groups.[14,15]

According to Barbara in 2000, the result of CSF sitology still poses difficulties in differentiating bacterial and viral meningitis. Leukocyte dominance in viral meningitis revealed more mononuclear cells such as lymphocytes and monocytes, whereas bacterial meningitis showed proportional results between polymorphonuclear and mononuclear cells. Protein level and Nonne/Pandy can differentiate bacterial and viral infection, although non-specific because it can be found in all inflammatory process of central nervous system due to trauma, autoimmune disease or degenerative disease. Comparison of CSF glucose and serum could not differentiate bacterial and viral meningitis. Results of CSF culture, which is a gold standard in diagnosing bacterial meningitis, only revealed positive in 45.5% of cases.[5,16,17]

A meta-analysis study conducted by Lv and Panato in 2014 showed that TNF-α examination in cerebrospinal fluid had very good diagnostic value in differentiating bacterial and viral meningitis in children. Lv Meta-analysis showed that this examination had 83% sensitivity and 92% specificity with accuracy rate of AUC = 0.9317. Similar to a meta-analysis conducted by Panato who obtained diagnostic test of TNF-α with 80.5% sensitivity and 94.9% specificity with accuracy rate of AUC = 0.942. The diagnostic threshold obtained in both meta-analyses were different in each country.[10,11]

The difference of TNF-α level diagnostic value indicated an influence of race and genetic in this proinflammatory cytokine production stimulation by the body immune system in cerebrospinal fluid. TNF-α as proinflammatory cytokine is produced locally in subarachnoid space as a response to meninges inflammation known as compartment phenomenon. Therefore, the increase of its level in cerebrospinal fluid is not affected by the level of TNF-α in blood serum due to blood vessel permeability or leakage of blood-brain barrier in central nervous system infection. TNF-α had a peak level at 48 hours after early invasion of pathogen to target tissue and started to decrease after the first 96 hours. This provides a narrow period of examination after acute infection. A lot of factors affecting immune cell production also affects cytokine level, such as nutritional status, immunity system competence, and administration of antibiotic drugs. Poor nutritional status highly affects the ability of leukocytes in producing inflammatory cytokines in fighting infection. Primary and secondary immunodeficiency condition also affects the production of inflammatory cytokine produced by lymphocytes. The administration of antibiotics after the first 24 hours also affects cytokine level produced by T-lymphocytes against pathogen. Bias had been controlled since the start of subject selection in inclusion and exclusion criteria.[18,19,20]

TNF-α is considered new in Indonesia and can only be performed with cerebrospinal fluid using ELISA method. The results of this study showed TNF-α value of 2.61 pg/ml as the diagnostic threshold of bacterial meningitis in Indonesian children population with 90.9% sensitivity, 80% specificity, 58.8% positive predictive value, 96.6% negative predictive value, and 0.918 AUC. The comparison of diagnostic AUC results revealed that TNF-α level could differentiate bacterial meningitis from viral meningitis significantly, whereas the AUC of viral meningitis was only 0.434 while bacterial meningitis reached 0.918 with p value < 0.05.

These results were also in line with a previous study conducted by Kothur in 2016 who compared the levels of several proinflammatory cytokines in various central nervous system disorders. TNF-α level was proven to increased significantly in all cases of bacterial meningitis, contrary to viral meningitis which tend to stay normal. Other study by Agrawal in 2017 showed that TNF-α level can also determine the success of therapy response and the possibility of neurological sequel of bacterial meningitis.[15,17]

A prospective cohort study is needed to assess the benefit of serial examinations of cytokine level in determining the success of antibiotic therapy response and prognosis outcome of death or complication of neurological sequel that may rise after infection. More studies conducted on TNF-α cytokine level will develop meningitis diagnostic ability in children, thus accelerate diagnosis establishment.[21]

CONCLUSION

Diagnostic value of TNF-α level in bacterial meningitis in Indonesian children was 2.61 pg/ml with 90.9% sensitivity, 80% specificity, 58.8% positive predictive value, 96.6% negative predictive value, and 0.918 AUC. Comparison of AUC revealed that TNF-α assessment can differentiate bacterial meningitis from viral meningitis significantly, whereas the AUC value of viral meningitis was 0.434 and bacterial meningitis was 0.918 with p value < 0.05.

FUNDING ACKNOWLEDGEMENT
The authors received no specific grants from any funding agency in the public, commercial, or not-for-profit sectors.

Conflicts Of Interest
None declared

REFERENCES

1. Dimyati Y. Central nervous system infection. In: Samik A, Setyati A, Ardianto B, Braghmandita WI, Rusmawatiningtyas D, Herini ES, editor. Implementing advances in pediatrics for better child health. Kongres Nasional Ilmu Kesehatan Anak XVII. Yogyakarta. 2017. p. 622-32.
2. Arydina, Herini E, Triono A. Bacterial meningitis score sebagai indikator diagnosis meningitis bakterialis di RSUP dr. Sardjito Yogyakarta. Sari Pediatri, 2014;15: 274-80.
3. Brouwer MC, Tunkel AR, Van de Beek D. Epidemiology, diagnosis and antimicrobial treatment of acute bacterial meningitis. Clin Microbiol Rev. 2010;23:467-92.
4. Grandgirard D, Gaumann R, Coulibaly B, Dangy J, Sie Ali, Junghanss T. The causative pathogen determines the inflammatory profile in cerebrospinal fluid and outcome in patient with bacterial meningitis. Mediators Inflamm. 2013;31:2476.
5. Barbara N, Kelleher K, Wald E. Cerebrospinal fluid findings in aseptic versus bacterial meningitis. Pediatrics. 2000;105:316-9.
6. Swansons PA, McGavern DB. Viral disease of the central nervous system. Curr Opin Virol.2015;11:44-54.
7. Bonthius DJ, Bale JF. Viral infections of the nervous system. In: Swaiman KF, Ashwal S, Ferriero DM, Schor NF, Finkel RS, editor. Swaiman's pediatric neurology principle and practice. Sixth edition. Philadelphia: Elsevier Saunder.2017. p. 895-906.
8. Nigrovic L, Malley R, Kuppermann N. Meta-analysis of bacterial meningitis score validation studies. Arch Dis Child. 2012;97:799-805.
9. Prasad R, Kapoor R, Srivastava R, Prakash O, Bali Singh T. Cerebrospinal fluid TNF-α, IL-6, and IL-8 in children with bacterial meningitis. Pediatr Neurol J.2014;50:60-65.
10. Lv S, Zhao J, Zhang J, Kwon S, Han M, Bian R, et al. Tumor necrosis factor α level in cerebrospinal fluids for bacterial and aseptic meningitis: a diagnostic meta-analysis. European Journal of Neurology. 2014;21:1115-23.
11. Panato AP, Tomasi L, Simon C, Madeira K, Simoes L, Medeiros L. Metaanalysis identifies tumor necrosis factor- alpha and interleukin-1β as diagnostic biomarkers for bacterial and aseptic meningitis. Current Neurovascular Research. 2014;11:1-9
12. Weinberg AG, Thompson-Stone R. Bacterial infection of the nervous system. In: Swaiman KF, Ashwal S, Ferriero DM, Schor NF, Finkel RS, editor. Swaiman's pediatric neurology principle and practice. Sixth edition. Philadelphia: Elsevier Saunder.2017. p. 883-94.
13. Fischbach. Analysis of cerebrospinal fluid. In: Ravel, editor. Laboratory medicine. Edisi ke-3.London:Wiley.2009.p.4-20.
14. Barichello, T., Generoso, J. S., Milioli, G., Elias, S. G., & Teixeira, A. L. Pathophysiology of Bacterial Infection of the Central Nervous System and its Putative Role in the Pathogenesis of Behavioral Changes. Revista Brasileira de Psiquiatria.2013;35(1):81–87.
15. Kothur K, Wienholt L, Brillot F, Dale R. CSF cytokines / chemokines as biomarkers in neuroinflammatory CNS disorder: a systematic review. Cytokine.2016;77:227-37.
16. Mukai A, Krebs V, Bertoli C, Okay T. TNF-α and IL-6 in the diagnostic of bacterial and aseptic meningitis in children. Pediatr Neurol. 2006;34:25-29.
17. Agrawal P, Agrawal A, Prasad R. Cerebrospinal fluid cytokine in diagnosis of acute bacterial meningitis. Austin Pediatr. 2017;4:1029.
18. Hao Liu Z, Hsun Tu P, Yu Chen N, Ping KY, Bowes A, Chi Lee C. Raised proinflammatory cytokines production within cerebrospinal fluid precedes fever onset in patient with neurosurgery-associated bacterial meningitis. Critical Care Med J. 2015;43:2416-2428.
19. Sulik A, Kroten A, Wojkowska M, Oldak E. Increased level of cytokines in cerebrospinal fluid of children with aseptic meningitis caused by mumps virus and echovirus 30. Scand J Immunol.2014;79:68-72.
20. Srinivasan L, Kilpatrick L, Shah S, Abbasi S, Harris M. Cerebrospinal fluid cytokines in the diagnosis of bacterial meningitis in infants. Pediatr Res J.2016;38:117-23.
21. Lv S, Han M, Yi R. Anti-TNFα therapy for patient with sepsis : a systematic meta-analysis. Int J Clin Pract. 2014;68:520-528.