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ORIGINAL ARTICLE

Neoptrin and Complement 3 as Immunomodulatory Clues in Serum & CSF for Septic & Aseptic Meningitis

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Key words:
Cerebrospinal fluid; Complement 3; Patients; Brain; Septic versus Aseptic Meningitis

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ABSTRACT

Background: *Distinguishing septic and aseptic meningitis in the emergency department could help limit unnecessary antibiotic use and hospital admissions.* ***Objectives:*** *To assess the potential diagnostic role of serum and cerebrospinal fluid (CSF) neopterin and complement 3 (C3) levels as a rapid diagnostic method for differential diagnosis of septic and aseptic meningitis.* ***Methodology:*** *Forty patients with proven acute meningitis, were categorized into 2 groups, Group I: 20 patients with septic (bacterial) meningitis as confirmed by CSF analysis and positive Gram stain and/or conventional bacterial culture; Group II: 20 patients with proved aseptic meningitis (viral and fungal) as confirmed by CSF analysis with negative culture for bacteria. Serum/CSF neopterin was assayed by Enzyme Linked Immunosorbent Assay (ELISA) while C3 level in serum was assayed by single radial immune diffusion.* ***Results.*** *The simultaneous elevation in serum and CSF neopterin shows high significance in the diagnosis of septic meningitis cases (P < 0.001).* ***Conclusion****. The combined elevated serum and CSF neopterin has a remarkable application in the diagnosis of bacterial meningitis, and can thus help differentiate bacterial from aseptic meningitis*

INTRODUCTION

Despite advances in the diagnosis and treatment of infectious diseases, meningitis and encephalitis are still considered as key causes of mortality and morbidity. Early diagnosis and starting immediate empirical therapy are the key factors to reduce the morbidity and mortality related to bacterial meningitis [1].

CSF analysis is often the diagnostic test of choice for suspected meningitis [2]. The classical CSF picture of turbidity, high tension, white blood cells (WBCs) >1000 with polymorph nuclear leukocyte predominance, elevated protein >40mg/100 ml and depressed glucose is highly predictive of bacterial meningitis [3].

Distinguishing bacterial and aseptic meningitis in the emergency department could help limit unnecessary antibiotic use and hospital admissions[4]. Signs and symptoms are often non-specific, requiring new and rapid diagnostic methods for differential diagnosis of bacterial and aseptic meningitis.

Clinically, subjects with the classic triad of diagnostic symptoms, such as headache, fever and projectile vomiting, and the cluster of nuchal rigidity, positive Kernig's sign; and Brudzinski's sign, or the cluster of fever, coma and purpuric rash, mandate undertaking an immediate lumbar puncture [5].

Neopterin is a product of the guanosine triphosphate pathway that is both cell-restricted and inducible by immune-inflammatory stimuli. Increased neopterin concentrations demonstrate an activated cell mediated immune system [6]. CSF neopterin levels are significantly higher in patients with bacterial than those with aseptic meningitis [7].

Complement C3 is an acute phase reactant produced by the liver, secreted by activated macrophages at inflammation sites and by adipocytes [8] and has a central role in the immune system [9]. C3 contributes to innate immunity, and activation of C3 is required for both classical and alternative complement activation pathways. People with C3 deficiency are susceptible to bacterial infection [10]. C3 is significantly elevated in the CSF of acute bacterial meningitis [11]. We aimed to assess the diagnostic role of each of serum and CSF neopterin and C3 levels, as to evaluate the application of both methods in the differential diagnosis of septic and aseptic meningitis.

METHODOLOGY

Patients:

This cross-sectional study was approved by the ethical committee of the Tropical Medicine- and the

Medical Microbiology Departments-Ain Shams University, and Embaba Fever Hospital, Cairo, Egypt. Informed consents were obtained from all participants before enrollment.

Forty patients, 10-57 years, with proven meningitis recruited from Embaba Fever Hospital, Cairo. The sample size was calculated by Epi Info program (version 6.0) at 95% confidence limit; power of the test is 80%. Subjects were divided into 2 groups: Group I enrolled 20 patients with proved septic (bacterial) meningitis. Group 2 enrolled 20 patients with proved aseptic meningitis (viral and fungal).

Inclusion criteria:

Subjects with clinical picture of acute meningitis, with CSF analysis of septic meningitis (positive Gram stain and/or conventional bacterial culture), and those with aseptic meningitis (with negative culture of CSF and biochemical analysis) were included in the study.

Exclusion criteria:

We excluded subjects with a clinical picture suggestive of cerebro-vascular disease, tuberculous meningitis, malignancies (including brain tumors), and other neurological insults. Subjects with autoimmune diseases, other causes of fever or coma, drug induced meningeal irritation, or subjects receiving antibiotics were excluded from the study.

Methods:

All subjects gave a full medical history and were subjected to thorough clinical examination. The duration of hospital stay was recorded. A routine laboratory investigation included: a complete blood picture (with differential), erythrocyte sedimentation rate (ESR), C-reactive protein, random blood glucose, serum neopterin by ELISA and complement C3 by single radial immune diffusion.

Sampling:

Cerebrospinal fluid and Serum samples obtained from patients fulfilling the previously mentioned inclusion criteria. Samples were put directly in screw capped tubes and refrigerated at -20ºC until they were used for measuring C3 and neopterin.

CSF analysis:

We used the first spinal tap to carry out a physical examination: color and aspect; a chemical examination: protein and glucose, cell count (total and differential), Gram stain and culture, fungal culture using Saboraud's dextrose agar medium and chromogenic selective agar medium (Brilliance agar), CSF/serum glucose ratio, neopterin level by ELISA and C3 level by Radioimmunoassay.

CSF and serum neopterin analysis:

Solid phase-ELISA based on capturing an unknown antigen by monoclonal antibody[11]. We used a commercial kit DRG® neopterin (EIA-1476) International Inc., USA [12].

CSF and serum C3 analysis by single radial immunodiffusion

A commercial kit (Bioscientifica S.A Diffu-Plate, Argentina) was used. Results were read by measuring rings diameters by a ruler and results were obtained from reference table [13,14].

Statistical analysis:

A Statistical Package for Social Science (SPSS 15.0.1 for windows; SPSS Inc, Chicago, IL, 2001) is used for data analysis. Mann Whitney test (U test) was used to assess the statistical significance of the difference of a non-parametric numerical variable between two study groups, Chi-square test was used to examine the relationship between two qualitative variables, and Fisher's exact test was used to examine the relationship between two qualitative variables when the expected count is less than 5 in more than 20% of cells. Pearson's correlation was used to assess the strength of association between two quantitative variables. The correlation coefficient defines the strength and direction of the linear relationship between two variables. The Receiver Operating Characteristic (ROC Curve) was used to evaluate the sensitivity and specificity of CSF and serum neopetrin and C3 in prediction or diagnosis of septic meningitis patients. Because positive and negative predictive values are affected by the prevalence of the disease under study, positive and negative likelihood ratios were calculated. The difference was considered significant at alpha (P) > 0.05. P < 0.01 identified highly significant difference.

RESULTS

The demographic and laboratory data of the bacterial and aseptic groups is shown in Table 1. Both groups were matched for age, sex and residence location. The duration of hospital stay differed significantly between the studied groups, the septic group exhibited longer hospital residency duration (P < 0.001, Figure 1). Also, the meningitis symptoms duration revealed significant difference being longer in aseptic meningitis (P<0.001). The clinical presentations between the aseptic and septic meningitis patients were not significant (P>0.05) (Table 1).

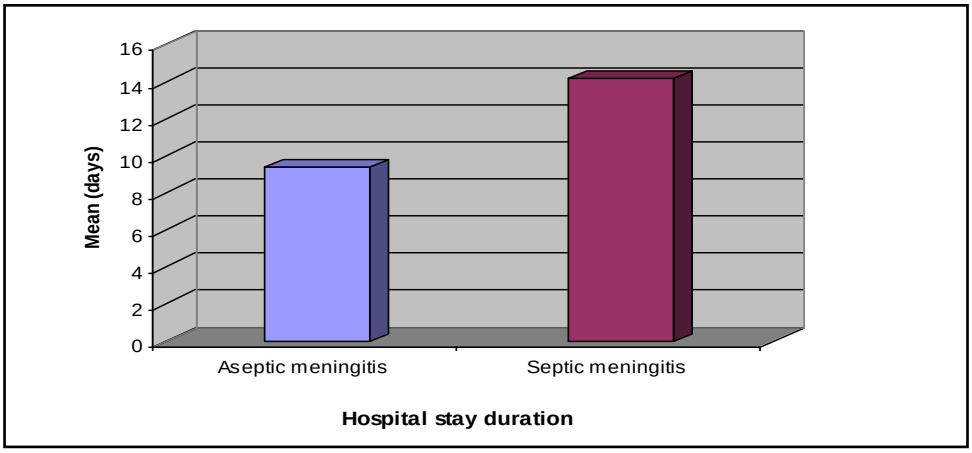

Fig. 1: Hospital stay duration of the patient groups

Table 1: Demographic characteristics and duration of symptoms in patients

Variable			Aseptic meningitis (N=20)	Septic meningitis (N=20)	Test statistics	
					P	Significance
Age (years)	Mean±SD		27.25±17.76	34.80±21.78	0.237‡	NS
Gender	Female	N (%)	4 (20.0 %)	9 (45.0%)	0.091*	NS
	Male		16 (80.0%)	11 (55.0%)		
Residence	Upper Egypt		1 (5.0%)	0 (.0%)	0.797*	NS
	Middle Egypt		2 (10.0%)	2 (10.0%)		
	Lower Egypt		1 (5.0%)	0 (.0%)		
	Greater Cairo		16 (80.0%)	18 (90.0%)		
Duration (days)	Mean±SD		3.80±1.77	1.45±.51	0.001‡	HS

*Chi-Square test, ‡student t test.

A comparison between CSF physical, microbiological, chemical, and cytological characteristics in patients is shown. A significant difference was detected between aseptic and septic meningitis cases as regards CSF analysis (P<0.001. CSF color and CSF gram stain showed no significant difference between the studied groups (P>0.05).

The detected bacteria were *Streptococcus pneumonia* in 16 patients (80%), *Neisseria meningitides* in 3 patients (15%) and *Haemophilus influenzae* in 1 patient (5%) among those with septic meningitis. The detected fungus was *Aspergillus flavus* in 1 patient (5%) with aseptic meningitis. No fungus was detected in 19 (95%) patients (Figure 2).

Table 2: Neopterin and C3 levels of patients

Variables	Aseptic meningitis (N=20)	Septic meningitis (N=20)	Test statistics	
	Mean±SD		P	Significance
CSF Neopterin (nmol/l)	31.60±43.48	59.67±57.85	0.113*	NS
Serum Neopterin (nmol/l)	18.30±19.98	18.64±16.87	0.925*	NS
CSF/Serum Neopterin ratio	2.98±4.84	6.35±10.01	0.167*	NS
CSF C3 (mg/dl)	0.00±.00	0.00±.00	1.0*	NS
Serum C3 (mg/dl)	144.91±73.30	127.21±46.61	0.588‡	NS
Parallel elevation in serum and CSF neopterin	2 (10%)	10 (50%)	0.006**	HS
Increased serum neopterin and decreased serum C3	2 (10%)	3 (15%)	1.00***	NS

* Mann Whitney test (M); ** Chi square; ‡ Student's t test; ***Fisher exact test.

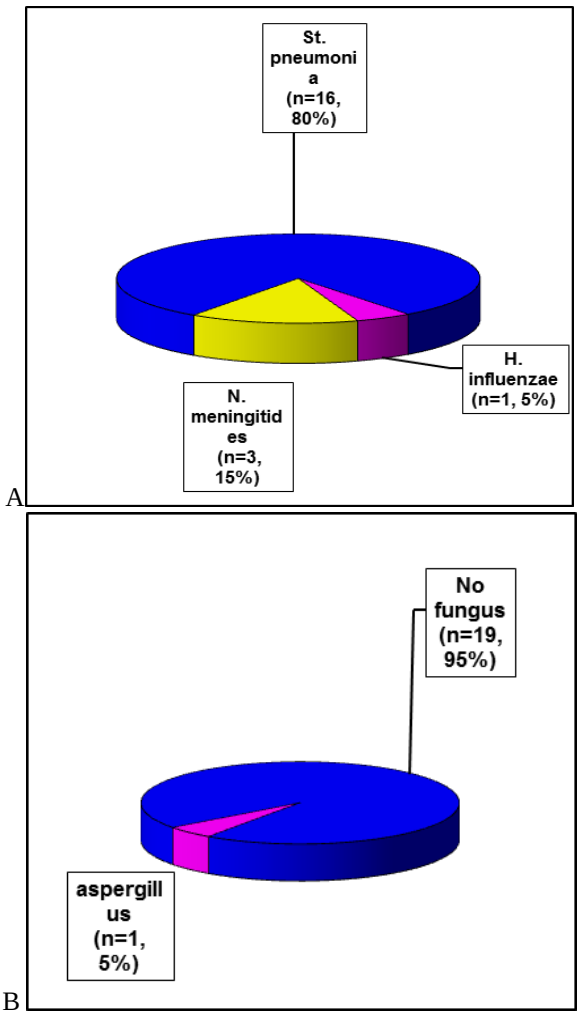

Fig. 2: Frequency of distribution of different identified pathogen in positive CSF- bacterial culture of (a) group 1; and (b) fungi in CSF- fungal culture of group 2 patients.

CSF neopterin levels, CSF/serum neopterin ratio and serum C3 levels (31.60±43.48, 2.98±4.84, 144.91±73.30) were higher in septic than aseptic meningitis (59.67±57.85, 6.35±10.01, 127.21±46.61), however, this was not significant (P > 0.05). The combined elevated serum and CSF neopterin showed a highly statistically significant value in the diagnosis of septic meningitis subjects ((P < 0.001), although the combined increment in serum neopterin and decrease in C3 was non significant (P > 0.05).

CSF neopterin was strongly correlated with CSF/serum neopterin ratio among aseptic patients (P<0.001). In septic patients, significant correlations were found between patients' characteristics and CSF neopterin; patients' characteristics and serum neopterin; patients' characteristics and C3. Significant correlations were found between serum neopterin and age; serum neopterin and CSF glucose (Figure 3a). Septic patients revealed a significant correlation between CSF neopterin and CSF/serum neopterin ratio (P<0.001)**;** between CSF neopterin and the course of illness (P<0.001); and between serum neopterin and TLC (P<0.05) (Table 3).

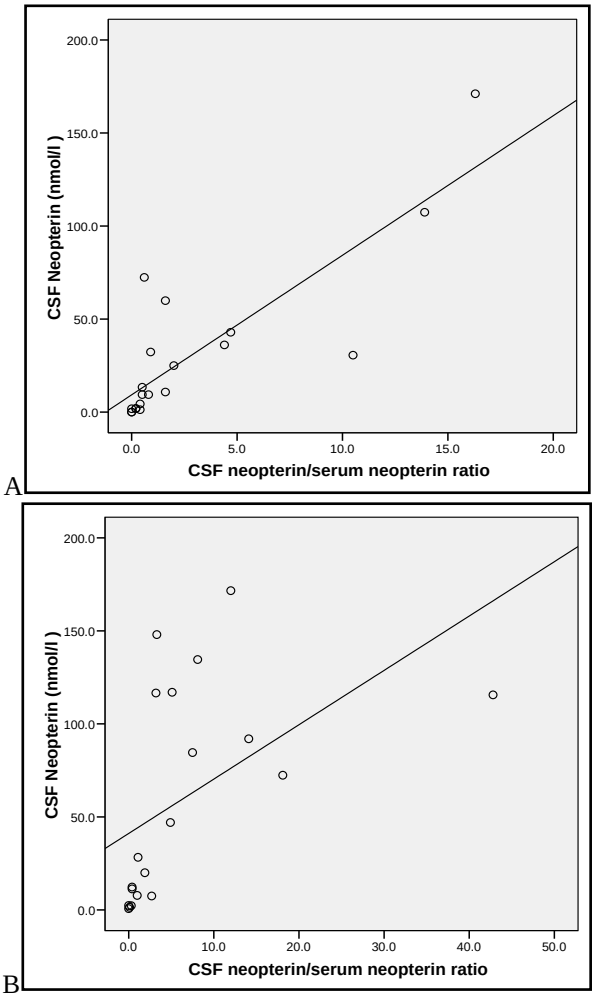

Fig. 3: CSF/serum neopterin among (a) aseptic; and (b) septic patients.

Table 3: Correlations between serum neopterin, CSF neopterin, CSF/serum neopterin ratio and serum C3 among septic cases

Variable	Test statistics	CSF neopterin (nmol/l)	CSF/ Serum neopterin ratio	Serum C3 (mg/dl)
Serum neopterin (nmol/l)	R	0.032	-0.367	-0.034
	P	0.894	0.112	0.888
	Sig	NS	NS	NS
CSF neopterin (nmol/l)	R		0.505	-0.342
	P		0.023	0.140
	Sig		S	NS
CSF/Serum neopterin ratio	R			0.251
	P			0.286
	Sig			NS
Duration (days)	R	0.642	-0.033	-0.286
	P	0.002	0.891	0.221
	Sig	HS	NS	NS
TLC (cells/ml)	R	-0.036	0.450	-0.102
	P	0.879	0.046	0.668
	Sig	NS	S	NS

The ROC curve differentiating between aseptic and septic meningitis by CSF neopterin; serum neopterin and serum C3 with detection of the sensitivity (50%, 45%, 90% respectively) and specificity (80%, 70%, 30% respectively) of each test is presented in (Table 4) and (Figure 4).

Table 4: ROC Curve to differentiate between aseptic and septic meningitis from CSF neopterin, serum neopterin and serum C3

Variable	AUC	95% CI	Sensitivity	Specificity	P	Significance
CSF neopterin septic if ≥ 1 - 44.95	0.646	0.473-.819	50%	80%	0.114	NS
Serum neopterin septic ≥ 13.35	0.509	0.326-691	45%	70%	0.925	NS
Serum C3 septic if ≤ 181.8	0.451	0.269-633	90%	30%	0.598	NS

AUC=area under curve. CI= confidence interval.

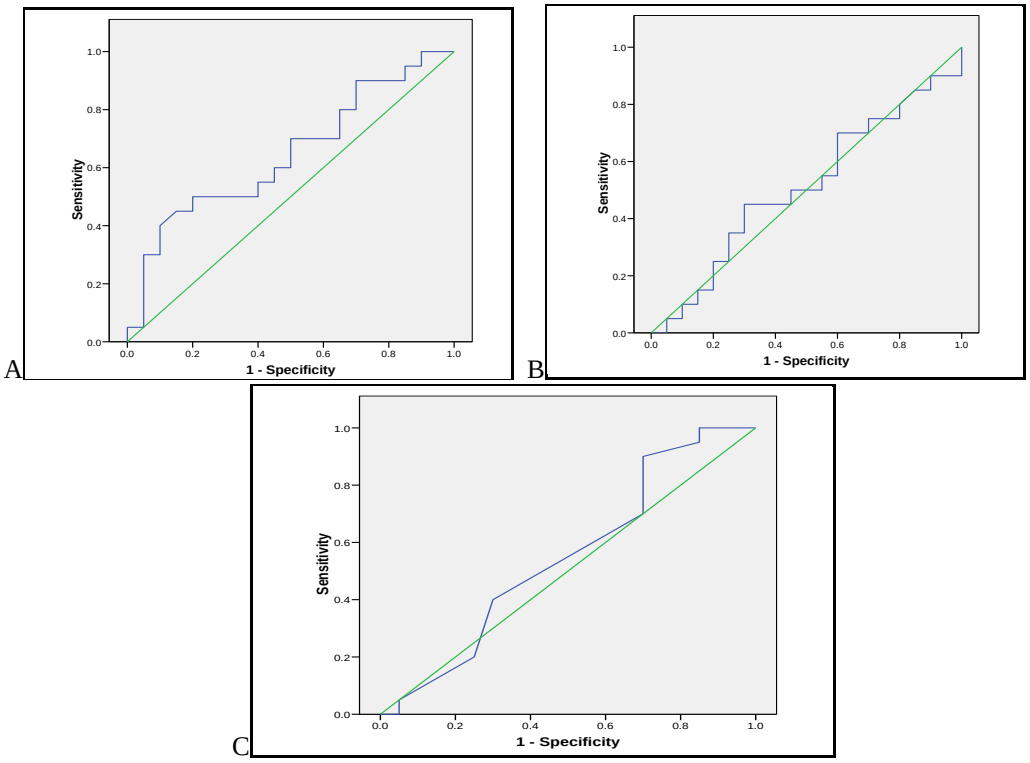

Fig. 4: ROC curve to differentiate between aseptic and septic meningitis from CSF neopterin (a), serum neopterin (b) and serum C3 (c).

DISCUSSION

The discrimination between patients with bacterial meningitis and other causes by clinical feature alone is often very difficult [15]. However, the distinction between septic and aseptic meningitis during the acute phase of the disease is critical, as this helps to avoid the complications and to limit unnecessary antibiotic use and hospital admissions [16].

The CSF gram stain can be negative and misleading because of the small number of organisms present in CSF or if therapy has been started. Culture often require a delay of one or more days and may be negative [17]. The diagnostic significance of neopterin, C3, CSF serum levels, and their combinations in differentiating septic from aseptic meningitis in Egypt.

Concerning the clinical presentations of bacterial and aseptic meningitis, the course of the disease differed significantly between bacterial and aseptic group (P<0.001), the septic group having the shorter course. Similar symptoms and signs were manifested by the septic and aseptic groups (P> 0.05). These results went in agreement with those of Holub et al.[20], who found a highly significant difference in course of symptoms in a similar study population. The acute onset encountered in bacterial meningitis may be explained by the bacterial pathogen being more virulent than the agents of aseptic meningitis, thus causing more rapid illness and deterioration.

In the current study, the duration of hospitalization between both groups was significantly different (P<0.001), similar to results by Abro et al.[19]. Østergaard and Benfield reported similar findings in patients with purulent meningitis[21].

Our mean CSF neopterin concentrations and C/S neopterin ratio were not significantly higher in patients with acute bacterial meningitis than in patients with aseptic meningitis (P>0.05). The mean serum neopterin concentrations were almost the same in septic and aseptic meningitis. This may be explained by serum neopterin release from human monocyte-derived macrophages and dendritic cells upon stimulation with the proinflammatory cytokine interferon-γ, which may be stimulated by both bacterial and viral infections.

This study revealed an undetectable level of C3 in the CSF of bacterial and aseptic meningitis by radioimmunoassay method. The mean concentrations of C3 were lower in septic compared to aseptic groups but this reduction was not significant, (P>0.05). The integrated increase in serum and CSF neopterin and C3 was highly significant and efficient in diagnosis of septic meningitis (P<0.001), with sensitivity 50% and specificity 90%.

We found that the best cut off point for serum neopterin for diagnosis of bacterial meningitis was 13.35 nmol/L with area under curve (AUC) 0.51, 95% CI (0.33-69), sensitivity 45% and specificity 70%. For CSF neopterin, the best cutoff point for the diagnosis of bacterial meningitis was 44.95 nmol/L with AUC of 0.65, 95% CI (0.47-0.82), sensitivity 50% and specificity 80%. For serum C3, the best cutoff point for the diagnosis of bacterial meningitis was 181.8 nmol/L with AUC 0.45, 95% CI (0.27-63), sensitivity 90% and specificity 30%.

This data is in agreement with Hagberg et al.[22], who reported that the mean CSF neopterin concentrations were 63.0 nmol/L in patients with acute bacterial meningitis, 32.5 nmol/L in patients with viral meningitis. They concluded that CSF neopterin concentrations cannot be useful in discriminating viral from bacterial infections. However, the analysis of CSF levels of neopterin may be a useful guide in the following clinical course and effect of treatment. Azumagawa et al.[23], reported that neopterin concentrations were significantly high in bacterial meningitis, and also in encephalitic patients, especially those with serious neurological sequelae.

Kawakami et al.[7] reported that the CSF neopterin levels on admission were significantly higher in patients with bacterial meningitis (82.4±37.0 nmol/L) than in those with aseptic meningitis (32.3±22.1 nmol/L). Nakazawa[23] reported that CSF neopterin levels markedly increased during the acute phase of bacterial meningitis, aseptic meningitis, and encephalitis as compared with those in patients without neurological diseases and suggested that CSF neopterin is a useful marker of inflammatory central nervous diseases. Zaknun et al. [25] attributed normal neopterin levels in serum to the lack of systemic infection, and concluded that neopterin determinations in CSF and serum were useful in the differential diagnosis of bacterial and viral meningitis.

Concerning complement C3 levels in meningitis, Tuomanen et al.[26] found a significantly higher level of C3 in the CSF of patients with acute bacterial meningitis, and this finding is in agreement with the findings of Zwahlen et al. [11] who reported increased C3 levels in the CSF in bacterial meningitis.

In the present study, we found that aseptic meningitis cases showed a highly significant correlation between CSF neopterin and CSF/serum neopterin ratio (P<0.001), and a significant correlation between serum neopterin and age; and between serum neopterin and CSF glucose (P<0.05). For bacterial meningitis subjects, we found a significant correlation between CSF neopterin and CSF/serum neopterin ratio (P<0.001), and a highly significant correlation between CSF neopterin and symptoms duration (P<0.001). Another significant correlation was found between serum neopterin and TLC (P<0.05). However, no correlation was found between C3 and neopterin in serum and/or CSF in both patient groups and other indicators as ESR, C reactive

protein (CRP), CSF glucose, CSF protein, and CSF WBCs. In addition, we did not find a correlation between serum and CSF neopterin in both patient groups.

Our results are inconsistent with Kawakami et al.[7], who reported no correlation between CSF neopterin levels and CSF cell count or CSF protein. The study by Kawakami et al. reported no correlation between serum neopterin levels and serum CRP or peripheral leukocyte count. Nakazawa [24] found no correlation between CSF neopterin and other CSF values, such as TLC, mononuclear cell count and protein. Millner et al.[27] highlighted the diagnostic validity of neopterin as an aid in differential diagnosis of inflammatory versus non-inflammatory diseases, and confirmed that CSF neopterin concentrations did not correlate with serum neopterin concentrations, accordingly CSF neopterin was suggested to be produced intrathecally.

CONCLUSION

The quantification of C3 or neopterin levels in serum or CSF alone is not recommended for the differential diagnosis of acute bacterial and aseptic meningitis. A parallel elevation in serum and CSF neopterin is a putative remarkable indicator in the diagnosis of bacterial meningitis.

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