Usefulness of Various Biomarkers for the Differentiation of Bacterial from Viral Meningitis

Ghada A. Salem[1*] and Hanaa Abd Elmoety[1]

[1]*Department of Tropical Medicine and Clinical Pathology, Faculty of Medicine, Zagazig University, Egypt.*

Authors' contributions

This work was carried out in collaboration between both authors. Author GAS conceived and designed the study were involved in the acquisition of data, analysis, interpretation of data, statistical analysis, drafting the paper, study supervision and critical revision of this paper for important intellectual content. Author HAE was involved in the laboratory designed of the tests involved in this paper. Both authors read and approved the final manuscript.

Article Information

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ABSTRACT

Background and Aim: Meningitis is an emergency condition, particularly bacterial meningitis for young and elderly patients. Differentiation between septic and aseptic meningitis may be difficult, the search for biochemical markers and laboratory tests to help in this task is crucial in order to optimize the treatment and avoid unnecessary use of antibiotics especially in aseptic meningitis. The aim of this study was to evaluate the diagnostic and prognostic utility of sTREM-1, CRP, IL-8 in septic meningitis and their usefulness in early differentiation between septic and aseptic meningitis in Egyptian patients.
Patients and Methods: This work included 70 patients (25 had septic meningitis group I, 30 had aseptic meningitis group II and 15 control individual group III). sTREM-1, IL-8 and CRP measurements were done on admission and after 48-72 h of treatment, in addition to Gram stain, culture of blood and CSF, latex agglutination test of CSF.
Results: Bacterial (septic) meningitis was found in 25 (35.7%) of the studied groups. Patients with septic meningitis had a significant increase in serum sTREM-1 and IL-8 and CRP at the time of

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*Corresponding author: Email: ghadasalem69@gmail.com;

admission (32.99±19.79, 2.46±1.8 and 126±90.5 respectively) while patients with aseptic meningitis had (6.8±5.67, 0.66±0.118 and 35±25.38 respectively), the control group had (6.6±4.6, 0.055±0.07 and 15±4 respectively) (P<0.05). sTREM-1 showed significant higher sensitivity (93.7%) and specificity (94.3%) in the early prediction of sepsis with an area under the receiver operator characteristic (ROC) curve (95% CI) of 88.2 (84-93) at a cut off value of 12.4 ng/ml. Moreover, sTREM-1 level was significantly low (P<0.001) at admission in 6 patients out of 25 patients who had septic meningitis who showed poor outcome.

Conclusion: sTREM-1 and IL-8 are valuable in early distinguishing of septic from aseptic meningitis but with higher diagnostic discriminatory power for sTREM-1 in determining septic meningitis prognosis and this marker would facilitate the clinical decision of interrupting antimicrobial therapy and avoiding unnecessary hospitalization.

ABBREVIATIONS

sTREM-1; serum triggering receptor expressed on myeloid cells-1 – CRP; C-reactive protein – IL-8; Interleukin-8 – CSF; cerebrospinal fluid – ROC curve; receiver operator characteristic curve.

1. INTRODUCTION

Meningitis is an emergency condition, particularly bacterial meningitis for young and elderly patients. Furthermore, recovery is often associated with peristance of serious sequelae, hearing loss and other cranial nerve damage [1].

Bacterial and bacterial products stimulate the production of pro-inflammatory and anti-inflammatory cytokines and prostaglandins in the CNS, all of which have an effect on the occurrence of inflammatory exudates [2].

Acute meningitis particularly in children is mainly aseptic (viral), so early differentiation between septic or aseptic meningitis must be occurred as the mortality of neurological sequelae from septic meningitis are high [3].

Differentiation between aseptic meningitis and acute bacterial meningitis in some instances may be difficult, the search for biochemical markers and laboratory tests to help in this task is crucial in order to optimize the treatment of these conditions [4].

CRP is an important component of innate defence system against infection [5]. It recognizes the pholine on the surface of many bacteria, activates the classical component of innate defence system against infections [6]. Interleukin-8 (IL-8) is a chemoattractant cytokine produced by a variety of tissue and blood cells, IL-8 attracts and activates neutrophils in inflammatory regions, it has high specificity in meningeal affection [7].

The soluble triggering receptor expressed on myloid cells-1 (sTREM-1) marker is a trans-membrane glycoprotein cell-surface receptor of the immunoglobulin superfamily which acts in corporation with toll-like receptors under the control of nuclear factor-κB [8]. The expression of TREM-1 is upregulated on phagocytic cells in the presence of bacteria and fungi triggering the secretion of the proinflammatory cytokines that amplify the host response to these microbial agents [9].

The aim of this study was to evaluate the diagnostic and prognostic utility of CRP, IL-8 and sTREM-1 in septic meningitis and their usefulness in early differentiation between septic and aseptic meningitis in Egyptian patients presenting with signs and symptoms suggestive meningitis.

2. PATIENTS AND METHODS

This study was carried out in Al-Humoyat General Hospital Sharkia Governorate, Tropical Medicine Department, Clinical Pathology Department, Faculty of Medicine, Zagazig University, Egypt.

Child patients identified as possible cases of meningitis if they have suspected clinical manifestations of meningitis (Fever, headache, stiff neck, bulging fontanelle or mental status

changes). Patients who had received antibiotics in the past seven days or had co-existing morbidities as other diseases that cause impaired consciousness as intracranial hemorrhage, brain tumor or cerebral malaria were excluded from the study. Informed signed consent was obtained from all participants in the study.

All patients were divided into the following groups:

- Group I: Bacterial meningitis (no = 25), 15 males and 10 females with mean age ±SD 5.3±4.8 years, septic meningitis was defined according to WHO case definition criteria [10]. Children presenting with clinical symptoms of meningitis, fever, headache stiff neck, bulging fontanelle, mental status changes, CSF with an elevated protein (>100 mg/dl), decreased glucose (<40 mg/dl) or leukocytosis (WBC > 100 mm^3) with at least 80% neutrophils. Identification of bacteria directly by gram stain smears or cultures from blood or CSF.
- Group II: Aseptic meningitis (no = 30), 20 males, 10 females with mean age±SD 4.4±4.5 years, aseptic meningitis was defined as presence of acute onset of meningitis symptoms, WBCs of >5 mm^3 of which >50% were mononuclear/ lymphocyte cells with the absence of any bacterial meningitis laboratory criteria.
- Group III: Control group (n = 15), 10 males, 5 females with mean age ±SD 5.1±4.9 years, the control group was defined by absence of inflammatory cells in CSF, WBCs <5 mm^3 and sterile bacteriologic findings in a febrile child with positive meningeal signs.

All participants were subjected to estimation of age, sex, vital signs as well as clinical symptoms and signs additionally, blood and CSF samples were withdrawn. Blood samples were used for routine laboratory investigations including CRP, leucocyte count, blood culture in addition to IL-8 and sTREM-1 measurement. CSF samples were examined for protein, glucose, total and differential CBC. After centrifugation the deposits were subjected to Gram stain examination and microbiological culture. After 48 hours – 72 hours of treatment, CRP, IL-8 and sTREM-1 levels were re-examined for patients with meningitis only. Prognosis of cases was allowed over a period of 7 days.

All patients were subjected to the following: 1- Microbiological cultures: Blood and CSF samples were collected under complete aseptic conditions according to the standardized technique [11].

Blood and CSF samples were subjected to cultures Egyptian diagnostic media EDM Egypt, blood, CSF cultures bottles were incubated at 37ºC and examined each 24 h. for turbidity, subculture were made an sheep and blood agar, incubated both aerobically and anaerobically at 37ºC for 48-72 hours and any growth were identified by colony morphology [12].

Specific serum analysis for:

1- Soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) was measured by commercially available human ELISA kit according to the manufacturer's instructions (Quantikine human TREM-1 immunoassay, R8D systems USA) using monoclonal antibody specific for human TREM-1 coated on a 96-well plate, 50 μl recombinant human TREM-1 standards.
2- Interleukin-8 (IL-8) assessment was occurred from CSF samples by ELISA using specific monoclonal antibodies (Duo set kit-RSD systems) according to the manufacturer instructions.
3- C-reactive protein (CRP) measurement using a nephelometric assay (Dade-behring France) with a detection limit of 0.2 mg/L and intra-assay coefficients of variation at low and high concentration of 3.3% and 2% respectively. The normal value is 6 mg/L.

2.1 Statistical Analysis

It was performed by using SPSS for windows, version 9. Data were expressed as range and mean±standard deviation (SD) or number and percentages. Receiver operator characteristic (ROC) plots were performed using MedCale software to determine the area under the curve with 95% confidence intervals for the three markers to predict septic meningitis. Difference between groups in continuous variables were tested for significance with the one way ANOVA and Mann-Whitney tests while univariate analysis was done by Fisher's exact test, P-value <0.05 was considered significant.

3. RESULTS

Fig. 1 shows the common clinical manifestations at the diagnosis in the meningitis groups were

fever, headache, nausea or vomiting and convulsions while in the control group there is no fever.

Table 1 shows the laboratory findings of studied groups. Patients with septic meningitis had significantly increased CSF protein, WBC count and neutrophil percentage in comparison with both aseptic meningitis and control groups (P<0.05), while CSF glucose level was significantly lower in patients with septic meningitis than in patients with aseptic meningitis (P<0.05) and controls (P<0.05), the baseline CRP, IL-8 and sTREM-1 levels in the studied groups which showed their levels were significantly higher in patients with septic meningitis than in patients with aseptic meningitis and control groups (P=<0.05).

The bacteriological results in the septic group showed: Neisseria meningitis 11 (44%), Haemophilus influenza type B 8 (32%) and Streptococcus pneumonia 6 (24%), the bacteriological results were done by CSF Gram stain, CSF culture, CSF soluble antigens, and blood culture with sensitivity of 68.7%, 89.1%, 30.1% and 24.9%, respectively.

Table 2 showed the sensitivity specificity, positive and negative predictive value of CRP, IL-8 and sTREM-1. At a cutoff value of 12.4 ng/ml sTREM-1 yielded a sensitivity of 93.7% and a specificity of 94.3% in differentiating patients with septic from those with aseptic meningitis, the use of combination of sTREM-1 and IL-8 didn't affect the sensitivity but increased the specificity to 97.1% with no changes when CRP was added.

Table 3 showed comparison of CRP, IL-8 and sTREM-1 serum levels at admission and after 48-72 h in and between patients with aseptic and bacterial meningitis in aseptic group, there were no significant difference between CRP, IL-8 and sTREM-1 levels, while there were high significant difference between these three markers in septic group between the patients at admission and after 48-72 h of treatment (P<0.005).

Table 1. Laboratory findings in the studied groups

	Bacterial meningitis group (NO=25)	Aseptic meningitis group (NO=30)	Control group (NO=15)	P-value
Protein (mg/dl) mean±SD	162.19±37.5	38.1±10.25	32.8±6.78	P1>0.05 P2<0.05[*] P3<0.05[*]
Glucose (mg/dl) mean±SD	25.6±.4	51.5±11.68	53.6±13.3	P1>0.05 P2<0.05[*] P3<0.05[*]
WBC (/mm^3) mean±SD	297.46±107.6	104.28±26.72	0.0±0.0	P1>0.05 P2<0.05[*] P3<0.05[*]
Neutrophil (%) mean±SD	84±2.6	15±2	0.0±0.0	P1>0.05 P2<0.05[*] P3<0.05[*]
CRP (mg/dl) mean±SD	126±90.5	35±25.38	15±9	P1>0.05 P2<0.05[*] P3<0.05[*]
IL-8 (ng/dl) mean±SD	2.46±1.8	0.66±0.118	0.055±0.07	P1>0.05 P2<0.05[*] P3<0.05[*]
sTREM-1 (ng/ml) mean±SD	32.99±19.79	6.8±5.67	6.6±4.6	P1>0.05 P2<0.05[*] P3<0.05[*]

Values are mean±SD (range); protein, glucose, WBC, neutrophil, CRP, IL-8, sTREM-1
P1 Control group vs aseptic meningitis group;
P2 Control group vs septic meningitis group
P1 Aseptic group vs septic meningitis group;
*P<0.05 is significant

Table 2. Sensitivity, specificity and negative predictive values (%) of admission CRP, IL-8 and sTREM values for septic meningitis

Marker	Cut off value	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
CRP (mg/l)	>29	81.2 (54.4-96)	80 (63.1-91.6)	68.4 (62-71)	90.6 (88-95)
IL-8 (ng/ml)	>1.67	87.5 (61.7-98.4)	88.6 (73.3-96.8)	82.4 (77-85)	94.1 (89-95)
sTREM-1 (ng/ml)	>12.4	93.7 (69.8-99.8)	94.3 (80.8-99.3)	88.2 (84-93)	97.1 (91-99)

Optimum diagnostic cut off values derived from the ROC curve. CRP, C-reactive protein; IL-8; sTREM-1, soluble triggerting receptor expressed on myeloid cells-1; CI, confidence interval; AUC of ROC, area under curve of the receiver operating characteristics curves; PPV, positive predictive value; NPV, negative predictive value

Table 3. Comparison of CRP, IL-8 and sTREM-1 serum levels at admission and after 48-72 h in and among patients with aseptic and bacterial meningitis
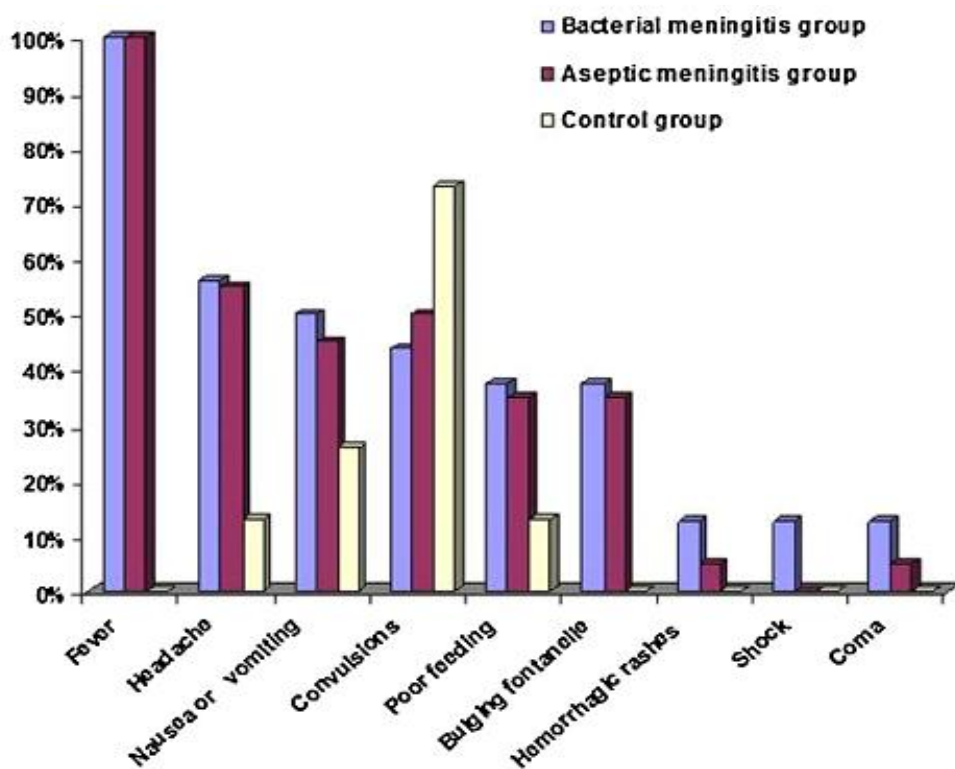
	Septic meningitis (no=26)		Aseptic meningitis (no=32)	
	On admission	After 48-72 h	On admission	After 48-72 h
CRP (mg/l)	126±90.5 P1<0.001*	51±42.6	35±25.38 P2 = 0.058	35.4±19 P3 = 0.016*
IL-8 (ng/ml)	2.46±1.85 P1 = 0.01*	0.24±0.14	0.66±0.118 P2 = 0.163	0.21±0.13 P3 <0.001*
sTREM-1 (ng/ml)	32.99±19.79 P1 = 0.002*	17.69±13.5	6.8±5.67 P2 = 0.09	4.65±3.8 P3<0.001*

P1: Septic meningitis on admission vs bacterial meningitis after 48-72 h.
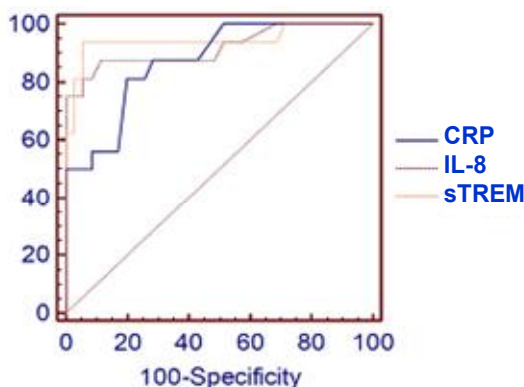*P2: Aseptic meningitis on admission vs aseptic meningitis after 48-72 h.*
P3: Septic meningitis after 48-72 hs vs aseptic meningitis after 48-72 h.
*P<0.05 is significant using t-test and Mann-Whitney rank sum test

Fig. 1. Clinical manifestation of the different studied groups

Fig. 2 shows the receiver operating characteristic curve (ROC) curve compairing admission CRP, IL-8, sTREM-1 for prediction of septic meningitis (95% CI) was 0.87 (0.75-0.95) for CRP, 0.99 (0.81-0.98) for IL-8 and 0.94 (0.84-0.99) for sTREM-1.

Fig. 2. Receiver operating characteristic (ROC) curves comparing admission, CRP, IL-8 and sTREM-1 for prediction of septic meningitis

The use of the current parameters as prognostic markers was also evaluated. Six (27.1) of 25 patients with septic meningitis showed poor outcome and died. Serum sTREM-1 level (10$\pm$7.5 ng/ml vs 32.99$\pm$19.79 ng/ml, Z=2.7; P<0.001) but not IL-8 (120.97$\pm$81.65 ng/ml vs 99.97$\pm$88 mg/ml, z = 1.13, P=0.58) or CRP levels (114$\pm$48.84 ng/ml vs 130$\pm$102.49 mg/ml, Z = 0.17, P=0.69).

The AUCs of ROC for CRP, IL-8 and sTREM-1 in predicting septic meningitis poor outcome were 0.82 (95% CI 0.41-0.59), 0.61 (95% CI 0.57-0.69) and 0.88 (95% CI 0.79-0.91) respectively.

4. DISCUSSION

Meningitis is an emergency condition, particularly bacterial (septic) meningitis [1].

Differentiation between aseptic meningitis and septic in some instances may be difficult, the search for biochemical markers and laboratory tests to help in this task is crucial in order to optimize the treatment of these conditions [4].

CRP, recognizes the phosphocholine on the surface of many bacteria and activates the classical component of innate defence system against infections [6]. IL-8 attracts and activates

neutrophils in inflammatory regions, it has high specificity in meningeal affection [7]. sTREM-1 is upregulated on phagocytic cells in the presence of bacteria and fungi triggering the secretion of the proinflammatory cytokines that amplify the host response to these microbial agents [9].

We conducted this study to evaluate the diagnostic and prognostic utility of CRP, IL-8 and sTREM-1 is septic meningitis and their usefulness in early differentiation between septic and aseptic meningitis in Egyptian patients.

Our result showed that CRP, IL-8 and sTREM-1 were significantly higher in patients with septic meningitis (group I) than in patients with aseptic meningitis (group II) or control group (group III) P = <0.005.

A cut off value of 12.4 ng/ml of sTREM-1 and a specificity of 94.3% in differentiation of septic from aseptic meningitis groups and the use of combination of sTREM-1 and IL-8 didn't affect the sensitivity but increased the specificity 97.1% with no changes when CRP was added. So sTREM-1 levels appeared to be the most helpful parameter in differentiating between septic or aseptic meningitis. sTREM-1 was concluded to be upregulated in the CSF of patients with bacterial meningitis [13].

In our study, we determined the value of 1.67 ng/dl for IL-8, as the best cut-off performing a ROC curve. CSF IL-8 concentrations above 1.67 ng/dl would indicate bacterial meningitis confirming other clinical and laboratory findings.

One of our main goals in this work was to evaluate the role of measuring serum sTREM-1 as an easy and non invasive procedure in early differentiating septic from aseptic meningitis. Similar to IL-8 but with markedly discriminative power, serum sTREM-1 showed significantly higher concentration early in septic meningitis compared to patients with aseptic meningitis and controls, and after 48-72 hours of treatment their levels significantly decreased in comparison with the admission levels.

Our results agreed with Su et al. [14] who reported that on ICU day, the sepsis group had higher serum sTREM-1 and CRP compared with non-infectious systemic inflammatory response syndrome group, while sTREM-1 level was significantly low at admission in patients with poor outcome with nearly stationary value after 48-72 h, the low baseline sTREM-1 level was

found to be the prognostic factor of the poor outcome and elevated baseline sTREM-1 level could be a valuable protective marker. The mechanism by which sTREM-1 modulate the immune response is still unclear. However in experimental model, blockade of sTREM-1 signaling reduced but didn't abolish NF-κB activation and cytokine production through competing with the natural ligand of TREM-1 and/or impairing TREM-1 dimerization, thus protecting septic animals from hyper-responsiveness and death [15].

5. CONCLUSION

In conclusion, IL-8 and sTREM-1 are valuable in early distinguishing of septic from aseptic meningitis but with markedly higher diagnostic discriminatory power for sTREM-1. Moreover, this study pointed to the significant value of sTREM-1 in determining septic meningitis prognosis and these laboratory marker would facilitate the clinical decision of interrupting antimicrobial therapy and avoiding unnessary hospitalization. Further study of biochemical markers as CSF lactate, serum procalcitonin, IL-6, can be recommended on larger number of patients.

ETHICAL APPROVAL

Ethical approval of the manuscript is: Informed consent was obtained from all participants, and the study was approved by the Ethical committee of faculty of medicine, Zagazig University Egypt.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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