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Diagnostic value of the CSF levels of D-Lactate and pro-inflammatory cytokines (TNF-alpha, IL-6, IL-8 and IL-17) in the patients with suspected nosocomial meningitis

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ABSTRACT

Introduction: This study aims to determine the diagnostic value of IL-6, IL-8, IL-17, TNF-α and D-lactate levels in the cerebrospinal fluid (CSF) in nosocomial meningitis.

Methods: CSF levels of cytokines and D-lactate were compared across 29 episodes who were diagnosed with nosocomial meningitis, 38 episodes with pleocytosis but without meningitis and 54 control subjects.

Results: CSF levels of IL-6, IL-8, and D-lactate were higher in the group with nosocomial meningitis compared to the control group and to the group with pleocytosis without meningitis ($p<0.05$). For the levels of IL-6, when the threshold was considered to be > 440 pg/mL, the sensitivity and specificity were 55.17% and 94.74%, respectively. For IL-8 levels, when the threshold was considered to be >1249 pg/mL, the sensitivity and specificity were 44.83% and 84.21%, respectively. In the patients with nosocomial meningitis, when the threshold of D-lactate levels was considered to be >1.05µmol/mL, the sensitivity and specificity were found to be 75.86% and 63.16%, respectively. In the pleocytosis without meningitis CSF samples and in the CSF samples diagnosed with nosocomial meningitis, the highest AUC was calculated for triple combination model of IL-6, IL-8, and D-lactate levels (AUC= 0.801, $p<0.001$), and double combination model IL-6 and IL-8 (AUC= 0.790) ($p<0.001$).

Conclusion: In our study, we have concluded that IL-6, IL-8 and D-lactate levels could be diagnostic markers for nosocomial meningitis.

Keywords: cytokine, D-lactate, IL-6, IL-8, IL-17, nosocomial meningitis, TNF-α

INTRODUCTION

Although not commonly encountered, acute nosocomial meningitis represents a clinical presentation with high rates of morbidity and mortality (20-50%) among the nosocomial infections.[1] Therefore, rapid diagnosis and therapy are essential. The differentiation between the pleocytosis without meningitis and nosocomial meningitis is difficult.[2,3] Since the classic clinical manifestations of meningitis, such as fever, meningismus and altered state of consciousness may also be observed in pleocytosis without meningitis or in the case of underlying disease, they do not present a significant value in the diagnosis of nosocomial meningitis.[4] Changes in CSF cell counts and abnormal protein, lactate and glucose levels may not always result from an infection but also from surgical intervention.[3,5] Moreover, antibiotics that have been pre-operatively used for different purposes may also influence the results of CSF bacterial cultures.[4]

The presence of the D-lactate in sterile body fluids indicates the presence of invading bacteria.[6] There are numerous in vitro studies that investigated the use of lactate to differentiate between bacterial and aseptic meningitis but the data for D-lactate measurements for the rapid diagnosis of the nosocomial meningitis are limited.[7,8]

Cytokines are a suitable marker for the inflammation of meninges and they have a considerable role in the pathophysiology of meningitis.[9,10] In human and animal studies, the investigation of IL-6, IL-8, IL-17 and TNF-alpha in the CSF revealed that levels of these pro-inflammatory cytokines are significant for the differentiation across bacterial meningitis, aseptic meningitis and non-infectious pleocytosis.[9-14] There are few studies that investigated D-lactate and IL-17 in the CSF in the patients with bacterial meningitis.[6,7,14]

In this study, we aimed to determine the diagnostic value of pro-inflammatory cytokines and D-lactate values in CSF for the diagnosis of nosocomial meningitis by comparing the results

from the investigation to CSF cell counts, CSF biochemistry (glucose/concomitant blood glucose and protein levels in the CSF), CSF Gram and Giemsa staining and bacterial cultures.

METHODS

Levels of IL-6, IL-8, IL-17 and TNF-α and D-lactate were measured in the CSF samples obtained for diagnostic analyses in the Department of Neurosurgery, Uludag University Faculty of Medicine hospital and cryogenically stored at -80ºC until the commencement of this study. CSF samples were stored in 4 portions to avoid being affected by freezing and thawing. The study enrolled 121 CSF samples from 104 patients of Neurosurgery Department. Meningitis episodes of the same patient at different times were considered as individual cases. Patients who had not previously taken steroid and antibiotics were included in study. Patient data including age, gender, clinical findings, type of the operation applied, GCS (Glasgow Coma Score), the presence of intracranial catheters (EVD, ICP, V-P shunt), direct microscopic examination and Gram and Giemsa staining of the CSF, CSF biochemistry values was screened. Mortality rates were calculated within the first 28 days after CSF sampling. This study was approved by Uludag University Faculty of Medicine Ethical Committee with the decision no. 2013-19/7 dated November 19th, 2013. Informed consent was not obtained because of the retrospective design.

Nosocomial central nervous system infection was defined based on the definitions suggested by the Center for Disease Control and Prevention (CDC). CDC definition of healthcare associated nosocomial meningitis includes at least one of the following criteria; i. Organism cultured from CSF; ii. Patient has at least two of the following; fever >38℃ or headache, meningeal sign(s), or cranial nerve sign(s); iii. Increased white cells, elevated protein, and decreased glucose in CSF; iv. Organism seen on Gram stain of CSF; v. Organism cultured from blood.[15] Non-culture based diagnostic laboratory tests, antibody titers spesific organism

or molecular tests for bacteria (for example multiplex PCR) was not used in patients in our hospital. As most patients had underwent an intracranial surgery, corrected leukocyte count in the CSF was calculated assuming that in a person with normal findings, the blood passing into the CSF contains 1 leukocyte per 700 erythrocytes per mm^3.[16]

On the day of study, CSF samples were permitted to reach room temperature and thereafter, cytokine (IL-6, IL8, IL 17a and TNF-α) and D-lactate levels were measured using ELISA and colorimetric assay, respectively.

CSF levels of the pro-inflammatory cytokines IL-6, IL-8 and TNF-α were determined using (BosterImmunoleader® ELISA kits) specific for each cytokine whereas IL-17 levels were determined using (Human IL-17A/F Heterodimer ELISA kit and DuoSet® Ancillary Reagent Kit by R&D Systems). The assays were performed according to the instructions provided by the manufacturer. At the end of the study, the resulting optical densities were read using Sunrise™ micro ELISA plate reader (Tecan Group Ltd. Männedorf, Switzerland) at a wavelength of 450 nm**.** Based on the values obtained from optical reading, CSF cytokine levels observed in the patient and control groups were calculated as pg/mL based on assay kit standards.

D-lactate levels were quantified using commercially available D-Lactate Colorimetric assay kit (D-Lactate Colorimeric assay kit, Biovision, California, USA). The assay was conducted according to the instructions provided by the manufacturer. Spectrophotometric measurements were done at 450 nm. The resulting absorbance values were calculated according to the standard curve plot to determine the concentration of D-lactate present in CSF samples. D-lactate levels of the patient was expressed as µmol/mL after normalizing the data to the sample volume used.

Statistical analyses were performed with IBM SPSS ver.23.0 (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.). Shapiro-

Wilk test was used to examine whether the data showed normal distribution. For the data that did not show normal distribution, Mann-Whitney U test was used for the comparison of two groups and Kruskal Wallis test for the comparison across more than two groups. Categorical data were examined using Pearson's Chi-Squared test, Fisher's Exact Test and Fisher-Freeman-Halton test. Receiver operating characteristics (ROC) curves were plotted for the CSF cytokine and D- lactate levels and cutoff values were determined. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for optimal cutoff value. The performance of the CSF cytokine and D- lactate levels in predicting nosocomial meningitis was determined using ROC curves, with the area under the curve (AUC) being of primary interest. MedCalc® Statistical Software version 19.5.3 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2020) was used for ROC analysis and comparison of ROC curves. Significance level was considered as α=0.05.

RESULTS

Of the 104 patients that have provided CSF samples for our study, 48 (46.2%) were female and 56 (53.8%) were male (Table 1). The total number of 121 samples from 104 patients were divided into three groups (Table 1). The study included 29 (23.8%) episodes diagnosed with nosocomial meningitis, 38 (31.1%) episodes with pleocytosis without meningitis and 54 (45.1%) control subjects without pleocytosis and meningitis. Types of the surgeries undergone by the subjects are summarized in Table 2.

There were 29 patients with pleocytosis due to reasons other than meningitis (trauma, intervention, tumor, hemorrhage), in whom the meningitis was not considered, and 38 CSF samples were obtained from these patients. Of these patients, 10 were operated for a CNS mass, 8 for intracranial hemorrhage and 2 for ventriculoperitoneal shunt whereas 1 had vertebral surgery. In this group, 8 patients with intracranial hemorrhage were not operated (Table 2).

Lastly, 54 patients without pleocytosis and meningitis and 54 CSF samples were enrolled to the study as control group. In the control group, while 17 patients were operated for a CNS mass, 11 were operated for intracranial hemorrhage and 11 underwent ventriculoperitoneal shunt operation, 15 patients were not operated (Table 2). Comparison of the risk factors between the patients and control group was shown in table 3.

Of the subjects with nosocomial meningitis, 16 (55%) showed bacterial growth in culture. Most commonly seen microorganisms (68.75%) were coagulase-negative staphylococci (CoNS) (11/16). Other growing microorganisms encountered in bacterial cultures are summarized in table 4.

In the CSF examination of the patients, leukocyte count and the levels of protein and glucose were evaluated for the diagnosis of nosocomial meningitis. However, when the group with meningitis and the group with pleocytosis without meningitis were compared, there was no significant difference in CSF leukocyte counts (p=0.112) (Table 5).

The median glucose level across the group with nosocomial meningitis was 56 (5-133) mg/dL and 73(38-136) mg/dL across the control group. Glucose levels were significantly lower in the nosocomial meningitis group and the group with pleocytosis without meningitis compared to the control group (p=0.001). The glucose levels did not differ significantly between the group with nosocomial meningitis and the group with pleocytosis without meningitis (p=0.715) (Table 5). Lowest glucose level was detected as 5 mg/dL in a patient with *Acinetobacter baumannii* growth in the CSF.

Protein concentration in the CSF differed significantly across all groups (p<0.001). In all CSF samples evaluated, median protein concentration was 44.8 (4.97-1468.4). Median protein concentration was 141.6 (13.6-1468.4) mg/dL in the group with nosocomial meningitis and 34.9 (6.8-392) mg/dL in the control group. In the pairwise comparisons, protein levels were significantly higher in the group with nosocomial meningitis compared to control group

($p<0.001$). Protein levels were significantly higher in the group with nosocomial meningitis compared to the pleocytic group without meningitis ($p=0.002$) (Table 5).

Median IL-6 concentration was found to be 18 pg/mL (2-7390) in all CSF samples investigated. In the group with nosocomial meningitis, median value for IL-6 was 469 (5-7390) pg/mL and IL-6 level was significantly higher in the meningitis group compared to the other groups ($p<0.001$) (Table 6).

Median IL-8 concentration was found to be 231pg/mL (0.01-1610) in all CSF samples investigated. In the group with nosocomial meningitis, median value was 907 (0.01-1610) pg/mL. IL-8 level was significantly higher in the meningitis group compared to the control group ($p<0.001$). IL-8 level was noticeably higher in the group with pleocytosis without meningitis, compared to the control group ($p<0.001$). A significant difference in IL-8 level was found between the group with nosocomial meningitis and the group with pleocytosis without meningitis ($p=0.045$) (Table 6).

Because patients with intracranial hemorrhage have high levels of pro-inflammatory cytokines in the CSF due to inflammation, only patients who experienced hemorrhage were evaluated. When the groups with and without meningitis (control group and the group with pleocytosis without meningitis) were compared, the significance was found only in terms of IL-6 among the cytokines ($p=0.02$). No significant difference was found between these two groups in terms of IL-8 levels in the CSF ($p=0.084$) (Table 7).

Because TNF-α and IL-17 levels were too low to be measured in most CSF samples, these two parameters were studied by comparing three groups (the group with nosocomial meningitis, the control group and the group with pleocytosis without meningitis). There was no statistically significant difference for IL-17 between three groups ($p=0.077$) (Table 6). But there was statistically significant difference for TNF-α among groups ($p=0.024$). TNF-α levels of the

group with nosocomial meningitis was especially higher than those of the control patients without pleocytosis (p=0.010) (Table 6).

We evaluated the potential use of the proinflammatory cytokines IL-6, IL-8, TNF-α and IL-17 as biomarkers for a new diagnostic test that can be used for the diagnosis of nosocomial meningitis and pleocytosis without meningitis. The sensitivity, specificity, positive predictive value, negative predictive value, cut-off and AUC values for IL-6, IL-8 and D-lactate were shown in table 8. Accordingly, when the performance of IL-6 to differentiate between the group with nosocomial meningitis and pleocytosis without meningitis was examined, the AUC for IL-6 was 0.774 (p<0.001). For IL-8, the AUC was 0.643 (p=0.037) (Figure 1).

We evaluated the potential use of the proinflammatory cytokines IL-6, IL-8, TNF-α and IL-17 as biomarkers for a new diagnostic test that can be used for the diagnosis of nosocomial meningitis. The sensitivity, specificity, positive predictive value, negative predictive value, cut-off and AUC values for IL-6, IL-8 and D-lactate between the group with meningitis and the control group were shown in Table 9. Accordingly, when the performance of IL-6 to differentiate between the group with meningitis and the control group was examined, the area under (AUC) for IL-6 test was statistitically significant (AUC=0.894; p<0.001). Also, for IL-8, the AUC was 0.832 (p<0.001).

In addition, D-lactate levels of patient CSF, which was calculated using colorimetric assay, was found to be statistically significant for the diagnosis of meningitis (p<0.001). When all samples were evaluated, median D-lactate level was found to be 0.807 (0.003-22.04) µmol/mL. In the group with nosocomial meningitis, median level of D-lactate was 2.45 (0.332-22.04) µmol/mL. D-lactate level was significantly higher in the group with meningitis compared to control group (p<0.001). D-lactate level was found to be higher in the group with meningitis compared to the group with pleocytosis without the diagnosis of meningitis (p=0.002) (Table 6). For D-lactate, the AUC was 0.807 (p<0.001) (Table-9). According to

comparison of AUC values in Table-8, there was no statistically significant difference was found.

D-lactate, the area under the ROC curve was AUC=0.723 (p<0.001) (cut-off value for D-lactate is >1.05) (Figure 1). For IL-17 and TNF-α tests, ROC analysis did not yield a significant threshold (p=0.339, p=0.392). Thus, in our study, the potential to use IL-17 and TNF-α as values to differentiate between the group with nosocomial meningitis and the group with pleocytosis without the diagnosis of meningitis was inconclusive.

In CSF samples with pleocytosis without the diagnosis of meningitis and in the CSF samples diagnosed with nosocomial meningitis, the double and triple combinations of IL-6, IL-8 and D-lactate levels were compared. When the individual examination and the combined examination of IL-8 and D-lactate were compared, there was no statistical difference between AUC of both approaches. Also, when the individual examination and the combined examination of IL-6 and D-lactate were compared, there was no statistical difference between AUC of both approaches. Comparing the AUC of IL-6 and IL-8, there was statistical**ly** significant difference between them. But there was no statistical difference the combined examination of IL-6 and IL-8 with individual examination. Similarly, the combined examination of IL-6 and D-lactate showed a specificity (89.47%) (cut-off value for IL-6 is >0.5740) . The combined triple examination of IL-6, IL-8 and D-lactate showed a specificity (94.74%) (cut-off value for IL-8 is >0.60364). PPV was found to be 88.9% (cut-off value for IL-6 and IL-8 >0.5317) using the combined examination of IL-6 and IL-8, 81.8% using the combined examination of IL-6 and D-lactate and 88.9% using the combined examination of IL-6, IL-8 and D-lactate compared to the individual examinations of each parameter. NPV was found to be higher using the combined examination of IL-8 and D-lactate (82.1%) compared to the individual examinations of each parameter (Table 8).

In cases of nosocomial meningitis, assuming the threshold of D-lactate level to be >1.05 µmol/mL, the sensitivity and the specificity were found to be 75.86% (95% confidence interval (CI), 56.5-89.7) and 63.16% (95% CI, 46.0-78.2), respectively (Table 8).

Also, as a result of the comparison of cytokine values between male and female in each meningitis and pleocytosis without meningitis groups, there was no statistically significant difference was found.

DISCUSSION

Majority of postneurosurgical central nervous system infections are of bacterial origin and leads to development of nosocomial meningitis.[17] Although not commonly seen, the incidence of nosocomial meningitis among the neurosurgery patients in the post-operative period is approximately 4%.[1,3]

Clinical signs of meningitis in neurosurgery patients such as new-onset fever and/or consciousness alterations are also observed following neurosurgical operations. Therefore, in neurosurgery patients, the diagnosis of nosocomial meningitis may be mistaken or delayed.[18] Definitive diagnosis of nosocomial meningitis could be made based on the isolation of bacteria from CSF samples. However, in patients with a clinical suspicion of nosocomial meningitis, bacterial growth in the CSF may not always be ensured and the inability to differentiate between nosocomial meningitis and aseptic meningitis results in unnecessary long-term antibiotic use.[1-4,19,20] In some patients, due to the previous use of antibiotics, the negative culture results may be insufficient to exclude the possibility of bacterial meningitis.[4]

Therefore, diagnostic tests that ensure rapid diagnosis of nosocomial meningitis are required. A reliable and rapid test for the diagnosis of nosocomial meningitis other than Gram staining, have not been established for clinical use.[6] In our study, Gram staining of the CSF revealed the presence of bacteria in 11 (9.0%) subjects. Increase in leukocyte counts and protein

levels and decrease in glucose levels of the CSF are commonly used parameters to evaluate for nosocomial bacterial meningitis. In a study performed by Ross et al,[4] among the neurosurgery patients, CSF leukocyte count above 1000/mL had the sensitivity of 61% and the specificity of 68% for the diagnosis of nosocomial meningitis. On the other hand, although the sensitivity of polymorphonuclear leukocyte (PMNL) predominance in the CSF was %94, the specificity was found only 28%. In most patients who experienced intracranial hemorrhage, PMNL predominance in CSF may be observed. Therefore, leukocyte counts are not sufficient for the differentiation between nosocomial meningitis and pleocytosis without meningitis.[1,10] In our study, the sensitivity and the specificity of the CSF leukocyte count for the diagnosis of nosocomial meningitis were 89% and 74%, respectively. Based on the CSF leukocyte count determined using direct microscopic examination, no significant difference was found across the groups. When the group with nosocomial meningitis and the group with pleocytosis without meningitis were compared, the direct microscopic examination did not reveal any significant difference in terms of leukocyte counts.

In the infections of CNS, bacteria and bacterial products cause the production of intrathecal pro-inflammatory and anti-inflammatory cytokines.[11,21] TNF-α, IL-1, IL-6 and IL-8 are cytokines that occur early in infection.[21,22] IL-17 is a potent pro-inflammatory cytokine produced by CD4 memory Th17 cells.[14,23] In a study that demonstrated the elevated levels of IL-6 in acute meningitis, the threshold value of IL-6 for the diagnosis of meningitis was 1,065.96 pg/mL, interleukin-6 had a sensitivity of 76.2% and specificity of 100%.[24] However, in some studies conducted in children, the elevation observed in the level of IL-6 was not found to be statistically significant for the differentiation between aseptic meningitis and bacterial meningitis.[9,25] In another study, with a CSF IL-6 level of>90pg/dL, the specificity and the sensitivity for bacterial meningitis were 100% and 95%, respectively.[16] Similarly, IL-6 level was significantly higher in the nosocomial meningitis group compared to the control group in

our study. IL-6 level was substantially higher in the group with nosocomial meningitis compared to the group with pleocytosis without meningitis.

There are many data to demonstrate increased levels of the pro-inflammatory cytokines TNF-α, IL-1 and IL-6 in traumatic and ischemic intracranial cases during normal recovery period. On the other hand, it was thought that IL-8 levels were high in nosocomial meningitis caused by other causal factors, because IL-8 leads to PMNL chemotaxis.[26,27] However, in two studies performed in patients with bacterial meningitis, no correlation was observed between IL-8 levels and granulocyte counts in the CSF.[14] In the study performed by Seki et al[22] to investigate the level of IL-8 in bacterial meningitis, it was found that IL-8 levels (224±2.57pg/mL) were significantly different between the group with bacterial meningitis and the group with aseptic meningitis and that the diagnostic value of leukocyte counts was as significant as that of protein and glucose levels. In the same study, IL-6 was found to be high in the cases of aseptic meningitis and bacterial meningitis as well and it was considered as a sign of meningeal inflammation. In the study performed by Lopez-Cortes et al,[28] in bacterial meningitis, IL-8 level was high and the threshold value was 2.5ng/dL. In several studies, the threshold values vary by the method and the commercial kit used. IL-8 may be significantly useful as a secondary parameter to reinforce the fast diagnosis of bacterial meningitis in addition to standard markers discussed.[13,29] In our study, for IL-8 testing, when the threshold was considered to be > 90 pg/mL, the sensitivity and specificity were 89.66% and 63.6%, respectively. In addition, negative predictive value was found to be 92.1%.

In the study performed by Lopez Cortes et al[30] following a neurosurgical operation, the sensitivity and the threshold value of the TNF-α was 74% and 150 pg/mL, respectively, in the differentiation between pleocytosis related to aseptic meningitis in the CSF and the pleocytosis due to a bacterial CSF infection.

Levels of TNF-α were markedly higher in the children with meningitis compared to those without meningitis.[9,25] However, 84.6% of aseptic meningitis patients were positive for TNF-α.[9] It was observed that the levels of TNF-α were decreased following antibiotic therapy.[25] In some studies, TNF-α was not found to be correlated with bacterial meningitis.[9,31-33] In our study, among the patients with nosocomial meningitis, TNF-α levels did not yield significant results when both the group with aseptic meningitis and the group with nosocomial meningitis were compared with the control group.

Contrary to previous studies, the study performed by Asano et al[14] reported high levels of IL-17 in the cases of bacterial and aseptic meningitis. Despite the other cytokines, studies conducted with IL-17 in bacterial meningitis are few. In our study, despite high levels of IL-8 and IL-6 in the patients, IL-17 levels were very low and had no significant threshold value. Accordingly, the performance of IL-17 and TNF-α tests to differentiate between the group with nosocomial meningitis and the control group was not sufficient in our study.

D-lactate is a parameter that can be helpful for the fast diagnosis of bacterial meningitis.[7] High concentrations of D-lactate in the CSF leads to altered mental status and encephalopathy.[34] In addition, CSF levels of lactate do not vary by the presence of erythrocytes.[1] In a study performed by Chen et al,[7] for the diagnosis of bacterial meningitis, D-lactate was found to have sensitivity and specificity equal to 94.7% and 79.7%, respectively. Similarly, in our study, concentrations of D-lactate were higher in the patients with nosocomial meningitis compared to other groups.

When IL-6 and D-lactate were concomitantly evaluated, the specificity (89.47%) and PPV (81.8%) were higher, whereas the concomitant evaluation of IL-8 and D-lactate showed higher NPV (82.1%). In the pleocytosis without meningitis CSF samples and in the CSF samples diagnosed with nosocomial meningitis, when the double and triple combinations of IL-6, IL-8 and D-lactate levels were compared, there was statistically significant difference

between them ($p<0.001$). We thought that more significant results could be obtained in terms of the diagnosis if the parameters were collectively evaluated. Zhang et al[35] also evaluated the combination of cerebrospinal fluid procalcitonin, lactate, interleukin-8 and interleukin-10 concentrations for the diagnosis of postneurosurgical bacterial meningitis; they concluded the combination of several markers may improve the diagnostic accuracy in detecting postneurosurgical bacterial meningitis.

Consequently, we investigated the contributions of the IL-6, IL-8, IL-17 and TNF-α cytokines and D-lactate for the diagnosis of nosocomial meningitis. Although IL-6 and IL-8 levels were high in pleocytosis resulting from neurosurgical interventions, we observed that this increase was greater in bacterial CNS infections. In neurosurgery patients, the specificity and the sensitivity of D-lactate, IL-8 and IL-6 were found to be high in the differentiation between aseptic and infection-related pleocytosis. We believe that these parameters are valuable for the fast differential diagnosis between nosocomial meningitis and aseptic meningitis. However, studies that will be conducted on larger patient groups are required to obtain definitive results.

There is no conflict among the authors. The study was approved by Uludağ University, Faculty of Medicine, Medical Researches, Ethical Committee with the decision no. 2013-19/7 dated November 19th, 2013 and conducted with the support and decision of the Scientific Research Project declared in the session no. 2014-3 dated 03.24.2014.

Our study has several limitations that need to be considered. First, sampling number was small. Second, non-cultured-based diagnostic laboratory test, antibody titers specific organism or molecular tests for bacteria (for example multiplex PCR) was not used in patients, because of molecular tests are not used routinely in our hospital.

REFERENCES

1. Tavares WM, Machado AG, Matushita H, Plese JPP. CSF markers for diagnosis of bacterial meningitis in neurosurgical postoperative patients. Arq Neuropsiquiatr 2006; 64:592-5.
2. Zarrouk V, Vassor I, Bert F, et al. Evaluation of the management of postoperative aseptic meningitis. Clin Infect Dis 2007; 44:1555-9.
3. Leib SL, Boscacci R, Gratzl O, Zimmerli W. Predicitive value of cerebrospinal fluid (CSF) lactate level versus CSF/blood glucose ratio for the diagnosis of bacterial meningitis following neurosurgery. Clin Infect Dis 1999; 29:69-74.
4. Ross D, Rosegay H, Pons V. Differentiation of aseptic and bacterial meningitis in postoperative neurosurgical patients. J Neurosurg 1988; 69:669-74.
5. Cemal B, Tekiner A, Yetkin A ve ark. [Evaluation of hospital-acquired meningitits after neurosurgery interventions]. Hastane Enfeksiyon Derg 2005; 9: 218-24. Turkish
6. Smith SM, Eng RH, Campos JM, Chmel H. D-lactic acid measurements in the diagnosis of bacterial infections. J Clin Microbiol 1989; 27:385-8.
7. Chen Z, Wang Y, Zeng A, et al. The clinical diagnostic significance of cerebrospinal fluid D-lactate for bacterial meningitis. Clin Chim Acta 2012; 413:1512-5.
8. Marcos MA, Vila J, Gratacos J, Brancos MA, Jimenez de Anta MT. Determination of D-lactate concentration for rapid diagnosis of bacterial infections of body fluids. Eur J Clin Microbiol Infect Dis 1991; 10:966-9.
9. Mukai AO, Krebs VLJ, Bertoli CJ, Okay TS. TNF-alpha and IL-6 in the diagnosis of bacterial and aseptic meningitis in children. Pediatr Neurol 2006; 34:25-9.
10. Asi-Bautista MC, Heidemann SM, Meert KL, Canady AI, Sarnaik AP. Tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-6 concentrations in cerebrospinal fluid predict ventriculoperitoneal shunt infection. Crit Care Med 1997; 25:1713-6.
11. Vázquez JA, del Carmen Adducci M, Coll C, Monzón DG, Iserson KV. Acute meningitis prognosis using cerebrospinal fluid interleukin-6 levels. J Emerg Med 2012; 43:322-7.

12. 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; 62:255-62.
13. Pinto VL Jr, Rebelo MC, Gomes RN, et al. IL-6 and IL-8 in cerebrospinal fluid from patients with aseptic meningitis and bacterial meningitis: their potential role as a marker for differential diagnosis. Braz J Infect Dis 2011; 15:156-8.
14. Asano T, Ichikia K, Koizumi S, et al. IL-17 is elevated in cerebrospinal fluids in bacterial meningitis in children. Cytokine 2010; 51:101-6.
15. National Healthcare Safety Network (NHSN). Surgical site infection (SSI) event. Procedure associated module SSI 2015. Available at: https://www.cdc.gov/. Accessed May 2, 2015.
16. Hopkins SJ, McMahon J, Singh N, et al. Cerebrospinal fluid and plasma cytokines after subarachnoid haemorrhage: CSF interleukin-6 may be an early marker of infection. J Neuroinflammation 2012; 9:255.
17. Morris A, Low DE. Nosocomial bacterial meningitis, including central nervous system shunt infection. Infect Dis Clin North Am 1999; 3:735-50.
18. The management of neurosurgical patients with postoperative bacterial or aseptic meningitis or external ventricular drain associated ventriculitis. Infection in Neurosurgery Working Party of the British Society for Antimicrobial Chemotherapy. Br J Neurosurg 2000; 14:7-12.
19. Weisfelt M, van de Beek D, Spanjaard L, de Gans J. Nosocomial bacterial meningitis in adults: a prospective series of 50 cases. J Hosp Infect 2007; 66:71-8.
20. Kleine TO, Zwerenz P, Zöfel P, Shiratori K. New and old diagnostic markers of meningitis in cerebrospinal fluid (CSF). Brain Res Bull 2003; 61:287-97.
21. van Furth AM, Roord JJ, van Furth R. Roles of proinflammatory and anti-inflammatory cytokines in pathophysiology of bacterial meningitis and effect of adjunctive therapy. Infect Immun 1996; 64:4883-90.

22. Seki T, Joh K, Oh-ishi T. Augmented production of interleukin-8 in cerebrospinal fluid in bacterial meningitis. Immunology 1993; 80:333-5.
23. Mei FJ, Ishizu T, Murai H, et al. Th1 shift in CIDP versus Th2 shift in vasculitic neuropathy in CSF. J Neurol Sci 2005; 228:75-85.
24. Dano ID, Sadou H, Issaka B, Oukem-Boyer OOM. Measurement of interleukin-6 in cerebrospinal fluid for the diagnosis of bacterial meningitis. Pak J Biol Sci 2016; 19:185-90.
25. Taskin E, Turgut M, Kilic M, Akbulut H, Aygun AD. Serum procalcitonin and cerebrospinal fluid cytokines level in children with meningitis. Mediators Inflamm 2004; 13:269-73.
26. Sprenger H, Rösler A, Tonn P, et al. Chemokines in the cerebrospinal fluid of patients with meningitis. Clin Immunol Immunopathol 1996; 80:155-61.
27. Yokohama T, Oda M, Ogura S, Horiuchi T, Seino Y. Relationship of interleukin-8 and colony-stimulating factors to neutrophil migration in aseptic meningitis. Acta Paediatr 1996; 85:303-7.
28. López-Cortés LF, Cruz-Ruiz M, Gómez-Mateos J, et al. Interleukin-8 in cerebrospinal fluid from patients with meningitis of different etiologies: its possible role as neutrophil chemotactic factor. J Infect Dis 1995; 172:581-4.
29. Chen X, Zhang G, Li Y, et al. Circulating B7-H3(CD276) elevations in cerebrospinal fluid and plasma of children with bacterial meningitis. J Mol Neurosci 2009; 37:86-94.
30. López-Cortés LF, Marquez-Arbizu R, Jimenez-Jimenez LM, et al. Cerebrospinal fluid tumor necrosis factor-alpha, interleukin-1beta, interleukin-6, and interleukin-8 as diagnostic markers of cerebrospinal fluid infection in neurosurgical patients. Crit Care Med 2000; 28:215-9.
31. Huy NT, Thao NT, Diep DT, et al. Cerebrospinal fluid lactate concentration to distinguish bacterial from aseptic meningitis: a systemic review and meta-analysis. Crit Care 2010; 14:R240.
32. Paul R, Koedel U, Winkler F, et al. Lack of IL-6 augments inflammatory response but decreases vascular permeability in bacterial meningitis. Brain 2003; 126(Pt 8):1873-82.

33. Nadal D, Leppert D, Frei K, et al. Tumour necrosis factor-alpha in infectious meningitis. Arch Dis Child 1989; 64:1274-9.
34. Uribarri J, Oh MS, Carroll HJ. D-lactic acidosis. A review of clinical presentation, biochemical features, and pathophysiologic mechanisms. Medicine (Baltimore) 1998; 77:73-82.
35. Zhang G, Yang C, Kang X, et al. The combination of cerebrospinal fluid procalcitonin, lactate, interleukin-8 and interleukin-10 concentrations for the diagnosis of postneurosurgical bacterial meningitis: a prospective study. Ann Clin Biochem 2019; 56:133-40.

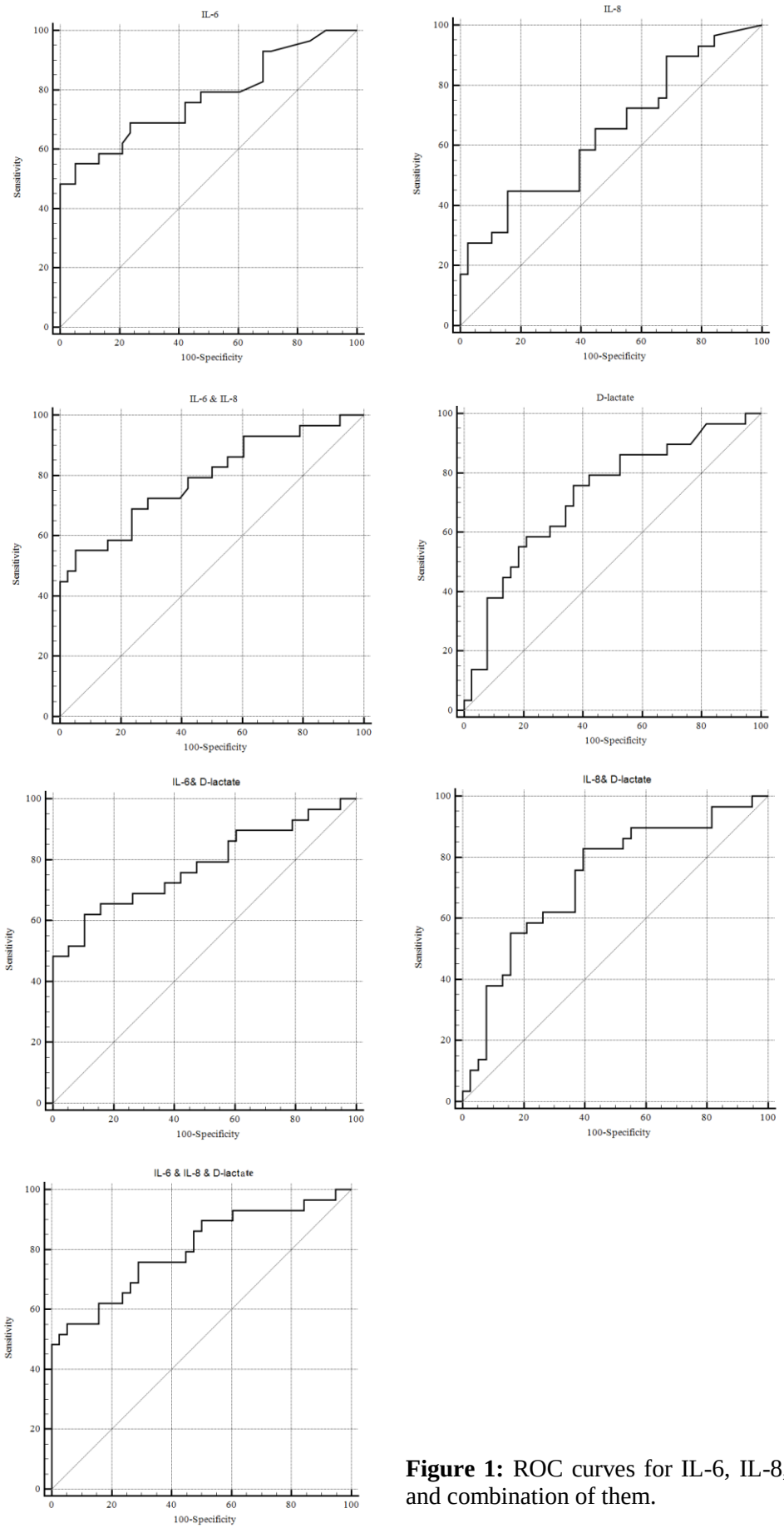

Figure 1: ROC curves for IL-6, IL-8, D-lactate and combination of them.

Table 1. Patient and control group demographics

Variables	Meningitis (n=21)	Pleocytosis without meningitis (n=29)	Patients without pleocytosis (control) (n=54)	Overall p	p (Meningitis vs. Pleocytosis without meningitis)
Gender (Female)	8 (%38)	13 (%45)	27 (%50)	0.641	0.634
Age (years)	46.14±14.93	48.10±15.22	54.76±14.79	0.039*	0.652

*Although overall p statistically significant, there was no significant difference in pairwise comparisons.
Descriptive statistics were given as mean ±standard deviation and frequency with percentage.

Table 2. Types of the surgeries (Patients underlying diseases)

Types of the surgeries	Meningitis (n=21)	Pleocytosis without meningitis (n=29)	Patients without pleocytosis (control) (n=54)
Tumor surgery	10	10	17
Intracranial bleeding surgery	8	8	11
Ventriculoperitoneal shunt operation	1	2	11
*Foreign body surgery	1	-	-
Vertebral surgery	1	1	-
Without operation	-	8	15

*****intraorbital foreign body surgery

Table 3. Comparison of risk factors in patient and control groups

Variables	Meningitis (1)(n=21)	Pleocytosis without meningitis (2) (n=29)	Patients without pleocytosis (control) (3) (n=54)	Overall p
Craniotomy	14 (66.7)[b]	11 (37.9)[ab]	10 (18.5)[a]	**<0.001**
Dural defect	16 (76.2)	19 (65.5)	32 (59.3)	0.384
ICP catheter	4 (19.0)[b]	0 (0)[a]	4 (7.4)[ab]	**0.043**
EVD catheter	9 (42.8)	6 (20.7)	21 (38.9)	0.169
V-P shunt	3 (14.3)	3 (10)	13 (24.1)	0.264

Descriptive statistics were given as frequency (n) with percentage(%).

Table 4. The culture results in meningitis group

Microorganism	**n (%)**
Coagulase Negative Staphylococcus	11 (68.75)
Acinetobacter baumannii	2 (12.5)
Enterococcus faecalis	1 (6.25)
Klebsiella pneumoniae	1 (6.25)
Staphylococcus aureus	1 (6.25)
Total	**16 (100)**

Descriptive statistics were given as frequency (n) with percentage(%).

Table 5. Comparison of CSF examination results of patient and control groups

	Meningitis (1) (n=29)	**Pleocytosis without meningitis (2) (n=38)**	**Patients without pleocytosis (3) *(n=54)**	**Overall p**	**Pairwise Comparison**
CSF leukocyte count /mm³	160 (0-48000)	25 (10-4600)	0 (0-110)	**<0.001**	1-2 0.112 1-3 <0.001 2-3 <0.001
CSF erythrocyte count/mm³	6900 (0-230000)	1320 (0-70200)	245 (0-141000)	**0.008**	1-2 0.041 1-3 0.004 2-3 0.135
Glucose mg/dL	56 (5-133)	62.5 (17-107)	73 (38-136)	**0.001**	1-2 0.715 1-3 0.009 2-3 0.001
Protein mg/dL	141.6 (13.6-1468.4)	45.6 (4.97-189)	34.9 (6.8-392)	**<0.001**	1-2 0.002 1-3 <0.001 2-3 0.060

Descriptive statistics were given as median(minimum-maximum).

* This was secondary to the operation or caused by bloody touching.When corrected, WBC counts were not pleocytosis

Table 6. Comparison of serum cytokines and D- lactate levels in patients and control groups

	Meningitis (1) (n=29)	Pleocytosis without meningitis (2) (n=38)	Patients without pleocytosis (3) (n=54)	Overall p	Pairwise Comparison
IL-6 (pg/mL)	469 (5-7390)	49(2-596)	9(2-468)	**<0.001**	1-2 <0.001 1-3 <0.001 2-3 <0.001
IL-8 (pg/mL)	907(0.01-1610)	520(0.01-1468)	0.01(0.01-1390)	**<0.001**	1-2 0.045 1-3 <0.001 2-3 <0.001
D-lactate (µmol/mL)	2.45(0.332-22.04)	0.801(0.03-5.81)	0.541(0.11-5.945)	**<0.001**	1-2 0.002 1-3 <0.001 2-3 0.012
IL-17 (pg/mL)	0.01(0.01-1203)	0.01(0.01-0.01)	0.01(0.01-2378)	0.077	-
TNF-α (pg/mL)	0.01(0.01-16428)	0.01(0.01-293)	0.01(0.01-413)	**0.024**	1-2 0.105 1-3 0.010 2-3 0.381

Descriptive statistics were given as median(minimum-maximum).

Table 7. Comparison of cytokine and D- lactate levels of patients with meningitis and pleocytosis without meningitis

	Meningitis (n=8)	Pleocytosis without meningitis (n=19)	p
IL-6 (pg/mL)	448 (9-760)	94 (9-423)	**0.020**
IL-8 (pg/mL)	1370 (29-1518)	633 (0.01-1468)	0.084
D-lactate (µmol/ml)	3.59 (0.525-5.74)	1.375 (0.11-5.945)	0.058
IL-17 (pg/mL)	0.01 (0.01-1203)	0.01 (0.01-2378)	0.481
TNFα (pg/mL)	0.01 (0.01-1168)	0.01 (0.01-413)	0.515

Descriptive statistics were given as median(minimum-maximum).

Table 8. ROC analysis of CSF cytokine and D- lactate levels between patients with nosocomial meningitis and pleocytosis patients without meningitis

	Sensitivity (%)	Specifity (%)	PPV (%)	NPV (%)	Cut-off value	AUC	p
IL-6 (pg/mL)	55.17	94.74	88.9	73.5	>440	0.774	**<0.001**
IL-8 (pg/mL)	44.83	84.21	68.4	66.7	>1249	0.643	**0.037**
D-lactate (µmol/mL)	75.86	63.16	61.1	77.4	>1.05	0.723	**<0.001**
IL-6 & IL-8	55.17	94.74	88.9	73.5	>0.5317	0.790	**<0.001**
IL-6 & D-lactate	62.07	89.47	81.8	75.6	>0.5740	0.779	**<0.001**
IL-8& D-lactate	82.76	60.53	61.5	82.1	>0.3134	0.730	**<0.001**
IL-6 & IL-8& D-lactate	55.17	94.74	88.9	73.5	>0.60364	0.801	**<0.001**

AUC: Area under curve, PPV: Positive predictive value, NPV: Negative predictive value

Table 9. ROC analysis of CSF cytokine and D- lactate levels between patients with nosocomial meningitis and control

	Sensitivity (%)	Specifity (%)	PPV (%)	NPV (%)	Cut-off value	AUC	p
IL-6 (pg/mL)	79.31	87.27	76.7	88.9	>70	0.894	<0.001
IL-8 (pg/mL)	89.66	63.64	56.5	92.1	>90	0.832	<0.001
D-lactate (µmol/mL)	79.31	72.73	60.5	87.0	>0.861	0.807	<0.001
Model	79.31	22.51	85.2	89.5	>0.186	0.873	<0.001

AUC: Area under curve, PPV: Positive predictive value, NPV: Negative predictive value
Model refers to multivariate ROC analyses of the combinations of IL-6 (pg/mL) and IL-8 (pg/mL)