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Diagnostic Accuracy of Presepsin (sCD14-ST) for Prediction of Bacterial Infection in Cerebrospinal Fluid Samples from Children with Suspected Bacterial Meningitis or Ventriculitis

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Children with temporary external ventricular drains (EVD) are prone to nosocomial infections. Diagnosis of bacterial meningitis and ventriculitis in these children is challenging due to frequent blood contamination of cerebrospinal fluid (CSF) and the presence of chemical ventriculitis. The aim of this study was to compare diagnostic accuracy of presepsin (sCD14-ST), a novel biomarker of bacterial infection in CSF, to predict bacterial infection in comparison to the accuracy of established biomarkers like those demonstrated in biochemical analysis of CSF. We conducted a prospective study with 18 children with suspected bacterial meningitis or ventriculitis who had 66 episodes of disease. CSF samples were taken from external ventricular drainage. We measured presepsin in CSF, as well as CSF leukocyte count, glucose, and proteins. CSF was also taken to prove bacterial infection with culture methods or with 16S rRNA gene broad-range PCR (SepsiTest; Molzym, Germany). Infection was clinically confirmed in 57 (86%) episodes of suspected meningitis or ventriculitis. Chemical ventriculitis was diagnosed in 9 (14%) episodes of suspected meningitis or ventriculitis. Diagnostic accuracies presented as area under the curve (AUC) for sCD14-ST, leukocytes, and proteins measured in CSF were 0.877 (95% confidence interval [CI], 0.793 to 0.961), 0.798 (95% CI, 0.677 to 0.920), and 0.857 (95% CI, 0.749 to 0.964), respectively. With CSF culture, we detected bacteria in 17 samples, compared to 37 detected with broad-range PCR. It was found that presepsin was present at a significantly higher level in children with clinically proven ventriculitis than in those without meningitis or ventriculitis. Diagnostic accuracies of presepsin were superior to those of leukocytes or proteins in CSF. Presepsin-guided 16S rRNA gene PCR could be used in everyday clinical practice to improve etiological diagnosis of meningitis and ventriculitis and to prescribe more appropriate antibiotics.

Placement of external ventricular drainage (EVD) and draining of the cerebrospinal fluid (CSF) when intracranial pressure (ICP) is elevated are frequently used for treating intracranial hypertension caused by a variety of neurological conditions. However, such an invasive procedure allows free entrance for bacteria and presents an increased risk of infection that can lead to meningitis, ventriculitis, or even death. The incidence of EVD-related ventriculitis ranges from 10% to 27% according to the literature (1–5). The most significant group of microorganisms causing ventriculitis is *Staphylococcus* spp., especially coagulase-negative staphylococci, followed by *Staphylococcus aureus*. Other Gram-positive organisms, such as *Streptococcus* spp., *Enterococcus* spp., *Corynebacterium* spp., and *Propionibacterium* spp., may be involved. Most ventricular infections are a result of contamination during the insertion of the EVD (6, 7). In order to prevent this complication or to detect initiating steps of the disease, early diagnosis and treatment are crucial, yet little has been reported in the literature about its management. The CSF total cell count, differential count, and concentration of proteins and glucose are parameters providing early information pertaining to the diagnosis of bacterial CSF infection. However, cell counts are often unreliable because of blood contamination of the CSF caused by primary or secondary ventricular hemorrhage or by chemical reactions to the drain material. Blood laboratory markers are also frequently elevated because of concomitant bacterial infection (8). C-reactive protein (CRP) and procalcitonin (PCT) were tested for their use to predict infection, but the results were contradictory (8, 9). Bacteriological culture methods such as CSF cultures may take several days until bacterial growth can safely be excluded (9). Furthermore, many patients with EVD are on antibiotic therapy and isolation of bacteria from CSF is often difficult. Thus, there is a need for new markers with higher specificity for early detection of meningitis and ventriculitis. Brain macrophages play a pivotal role during inflammatory reactions of the central nervous system (CNS) parenchyma, ventricles, and meninges, and are involved in the release of soluble CD14 (sCD14) (10). In a study of 91 patients, serum sCD14 levels were measured, and the levels increased during acute bacterial meningitis. Increased CSF and serum sCD14 concentrations were also observed in meningitis caused by viral infection. Repeated measurement of sCD14 in CSF revealed a normalization of sCD14 levels during clinical recovery (10). Determination of presepsin (sCD14-ST) in CSF could overcome problems with time-consuming procedures while measuring sCD14. CD14 is a glycosylphosphatidylinositol (GPI)-anchored glycoprotein

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expressed on the surface of monocytes/macrophages (mCD14) and serves as a receptor for complexes of lipopolysaccharides (LPS) and LPS-binding protein (LPBP) (11). This activates a Toll-like receptor 4 (TLR4)-specific proinflammatory signaling cascade upon contact with infectious agents (12). Simultaneously, CD14 is shed from the cell membrane into the circulation, forming sCD14. Plasma aspartate protease activity, including cathepsin D, also generates presepsin (13, 14). Presepsin is a 13-kDa protein, a truncated N-terminal fragment of CD14 (15). Results of experiments revealed that the production mechanisms of presepsin are related to the phagocytosis process and cleavage of membrane CD14 with lysosomal enzymes of granulocytes in response to bacterial infection (14). Presepsin does not bind to LPS. Its biological function is unknown (15). Increased sCD14-ST values were found in newborns and adults with sepsis (16). Presepsin was also used in postmortem analysis of blood from patients with sepsis (17).

Many studies have confirmed that sCD14-ST is a more specific and sensitive marker for the diagnosis of sepsis than other inflammatory markers, such as CRP, PCT, and interleukin 6 (IL-6) (18, 19). Shozushima et al. (18) demonstrated a correlation between presepsin values and the severity of sepsis. Cutoff values were significantly higher in patients with local infection (721 pg/ml), sepsis (817.9 pg/ml), and severe sepsis (1,992.9 pg/ml) than in patients who did not have an infection (294.2 pg/ml) as a complication. In another clinical study, sCD14-ST was compared with PCT, IL-6, and blood cultures. In this multicenter prospective study, the authors concluded that presepsin is superior to conventional inflammatory blood markers and blood culture for the diagnosis of sepsis (19). Recently, Novelli et al. (20) demonstrated that sCD14-ST can be an early indicator of bacterial infection in patients after surgery.

The objective of this study was to test whether sCD14-ST could be used to diagnose suspected bacterial meningitis and ventriculitis in pediatric patients with EVD in intensive care units (ICUs). The study was designed to evaluate the cutoff concentration of sCD14-ST in CSF samples for positive ventriculitis under routine conditions and determine the diagnostic and prognostic validity of sCD14-ST compared to the established markers: proteins, glucose, PCT, CRP, and counts of neutrophils and leukocytes. Another goal was to see if the use of 16S rRNA gene PCR could be useful to identify the bacteria that caused meningitis and ventriculitis.

MATERIALS AND METHODS

We conducted a prospective, observational pilot study including children presented to a pediatric surgical ward or intensive care unit with possible meningitis or ventriculitis related to EVD. Ethical approval (National Medical Ethics Committee of the Republic of Slovenia) was obtained for this study. The patients for the study were chosen on clinical grounds. Parents of the children included in the study approved additional procedures to take CSF samples to measure sCD14-ST and perform molecular tests to identify bacteria. They were informed that the data will be published and agreed with that.

Sixty-six samples of CSF were taken from 18 children with multiple episodes of suspected meningitis or ventriculitis. Meningitis or ventriculitis was suspected when one or more signs and symptoms such as fever, nausea, vomiting, rigors, confusion, and temporary loss of consciousness appeared and if ICP was raised. The new episodes were defined with the following rule: at least 1 week had to pass between episodes, and the child's health had to improve during the week. If raised ICP persisted, no bacteria were identified, and the child's health did not improve after antibiotic treatment, it was assumed that chemical ventriculitis (irritation of the meninges not due to infection) was the cause of deterioration of the child's health. CSF for the analysis of several inflammatory biomarkers (sCD14-ST, leukocyte and neutrophil counts, and concentrations of proteins and glucose) and for broad-range 16S rRNA gene PCR analysis was collected from EVD. At the same time, blood with EDTA was drawn to detect CRP (Siemens Healthcare Diagnostics, Germany) and PCT (Brahms, Germany) and to count leukocytes and neutrophils. Biomarker sCD14-ST was measured by a rapid chemiluminescent enzyme immunoassay on the fully automated PATHFAST immunoanalyzer (Mitsubishi Chemical Medience Corporation, Tokyo, Japan). We cultured CSF with standard microbiological methods, and if the culture was positive, identification and susceptibility testing were done. We analyzed CSF with a PCR kit produced by Molzym (Germany), which includes the degradation of human DNA and free bacterial DNA to identify the causing pathogen. DNA sequencing with the manufacturer's primers for Gram-positive and Gram-negative bacteria on an ABI-3500 genetic analyzer (Applied Biosystems, USA) was performed. The sequences of the PCR product were compared with those in GenBank at NCBI using the BLAST algorithm and at the SepsiTest BLAST webpage (Molzym, Germany). Good sequence identity was set at a score of base pairs higher than 97% and a length of >250 bp.

We constructed a "gold standard" on the basis of clinical grounds, culture, and molecular results of bacterial identification from CSF and the results of response to antibiotic treatment and compared all the biomarker values to it.

Statistical analysis was performed using Statistical Package for the Social Sciences 21.0 (SPSS, Chicago, IL). Nonparametric Kruskal-Wallis test and chi-square test were used for comparing quantitative variables between positive infection and the measured biomarker. Chi-square test was also used to determine qualitative statistical significance. *P* values of less than 0.05 were set as statistically significant. We also constructed a receiver operating characteristic (ROC) curve with calculated sensitivity and specificity for the assay.

RESULTS

We analyzed 66 CSF samples from 18 children (median age, 21 months; range, 1 to 167 months) for inflammatory biomarkers. In 57 episodes of meningitis or ventriculitis, infection was the cause of deterioration of a child's health. In 38 (57.6%) CSF samples, infection was confirmed, and 28 (42.4%) samples had no detected pathogens (Table 1). All children had at least one episode of meningitis or ventriculitis of infectious origin. Two patients had 1 episode, 8 patients had 2, and 8 patients had 3 or more episodes of meningitis or ventriculitis. In 9 (14%) CSF samples, no bacteria were identified and chemical ventriculitis (no improvement after antibiotics) was confirmed as a cause of deterioration of a child's health. Twelve out of 18 children had microbiologically confirmed infection in at least one episode. Five children went without antibiotic treatment for at least 48 h before their health worsened and they had another episode of meningitis or ventriculitis.

The mean values for biomarkers of infection divided into episodes of bacterial meningitis and ventriculitis and chemical ventriculitis are presented in Table 2.

We evaluated the diagnostic accuracy of all biomarkers in comparison to the constructed gold standard (clinical symptoms and signs, isolates from CSF, culture and PCR results, and response to treatment). The sCD14-ST value was elevated in all critically ill patients with microbiologically proven infection independent of the bacteria isolated (2,403.08 ± 2,407.56; $P < 0.001$). Even when infection was not microbiologically confirmed, the mean value of presepsin was elevated in comparison to the levels in episodes in which chemical ventriculitis was suspected (Table 3). Leukocytes and proteins in CSF were elevated in patients who had microbiologically confirmed infection ($P < 0.001$ and $P = 0.001$, respec-

TABLE 1 Basic characteristics of 18 children with EVD and suspected meningitis or ventriculitis who participated in this study

Characteristic	Value
No of:	
Females	5
Males	13
Age (mo)	
Female	54
Male	58
No. of episodes/patient	
1	2
2	8
≥3	8
Use of AT[a] during treatment	
No. of children without AT for at least 2 days	5
No. of children with AT	13
No. of episodes (of 66 total) with identification of:	
Gram-negative bacteria	8
Gram-positive bacteria	20
Mixed bacteria	10
No bacteria	28
Chemical ventriculitis	9

[a] AT, antibiotic therapy.

TABLE 2 Differences in values of biomarkers according to classification into infectious versus chemical ventriculitis

Biomarker (unit)	Value in cases of meningitis or ventriculitis (no. of episodes = 57)	Value in cases of chemical ventriculitis (no. of episodes = 9)	*P* value
sCD14-ST (pg/ml)	1,776.5 ± 2,179.2	451.7 ± 399.6	0.014
CSF leukocytes (no./μl)	415.3 ± 668.2	162.1 ± 192.0	0.456
CSF neutrophils (%)	48.8 ± 27.7	37.2 ± 26.3	0.374
Glucose (mg/dl)	2.3 ± 1.3	3.4 ± 1.3	0.031
Proteins (mg/dl)	2.0 ± 2.0	1.0 ± 1.6	0.059
CRP (mg/liter)	85.15 ± 124.37	78.13 ± 79.35	0.841
PCT (μg/liter)	0.54 ± 0.85	0.52 ± 0.32	0.108
Leukocytes ($Í0^9$/liter)	11.6 ± 4.8	12.5 ± 4.6	0.668
Neutrophils (%)	62.5 ± 19.4	56.5 ± 15.4	0.425

tively). Meanwhile, glucose concentrations were significantly lower in patients with ventriculitis than in patients without infection ($P = 0.008$). Leukocytes from blood and neutrophils from blood or CSF samples were not statistically elevated ($P = 0.912$, $P = 0.219$, and $P = 0.068$).

According to our analysis, the best cutoff for presepsin for positive infection in CSF would be 625 pg/ml (Table 4). The best diagnostic accuracy for meningitis and ventriculitis was achieved with presepsin and proteins in CSF, with areas under the curve (AUCs) of 0.877 (95% confidence interval [CI], 0.793 to 0.961) and 0.857 (95% CI, 0.749 to 0.964), respectively. The count of leukocytes in CSF also performed well, with an AUC of 0.789 (95% CI, 0.677 to 0.920), a sensitivity of 77.4%, and a specificity of 77.3%. The worst predictive value for positive infection was calculated for PCT and a leukocyte count from blood and for glucose level in CSF. The AUCs for these markers were equal to or lower than 0.50 (Table 4).

We analyzed CSF to confirm infection with standard cultures and with broad-range PCR that amplifies the 16S rRNA gene. Broad-range PCR already detected the pathogens in the first episode of meningitis or ventriculitis. Meanwhile, cultures of CSF were negative in most cases after the first sampling and turned positive after the second or even third episode. We confirmed bacterial etiology in 37 out of 38 samples of CSF with broad-range 16S rRNA PCR, in comparison to 17 samples with culture methods. In 6 samples, we identified a mixed infection with classic culture of CSF, and 10 samples were found to have mixed infection with broad-range 16S rRNA PCR. In 9 samples and episodes, we were able to diagnose additional bacteria with broad-range 16S rRNA PCR. The pathogen could not be identified with broad-range 16S rRNA PCR in only one sample of CSF that was culture positive (Table 5).

DISCUSSION

We have evaluated the use of sCD14-ST in clinical settings at an intensive care unit that takes care of children after surgery. Our study is the first study to check the values of sCD14-ST in CSF in children. It is also the first study to use broad-range 16S rRNA PCR to identify a pathogen, the cause of infection, in children with meningitis or ventriculitis. We had a special population of children that had EVD because of raised ICP. EVD is an entry point for bacteria, and children can have several episodes of meningitis or ventriculitis during their hospitalization. Bacteria that are usually considered contaminants can be responsible for ventriculitis (5). Previously, sCD14 was measured in CSF and proved to be good marker of bacterial meningitis in adults, but with time-consuming procedures, its diagnostic value in ICUs can be problematic (10).

We have concluded that the use of sCD14-ST is beneficial in predicting infection for children with bacterial ventriculitis. The diagnosis of bacterial infection is more certain if sCD14-ST is used. AUC, sensitivity, and specificity are higher than in the case of counting cells in CSF, which is usually used when ventriculitis is suspected (the AUC for sCD14-ST was 0.877, compared to 0.798 for leukocytes in CSF; sensitivity and specificity were 84.2% and 82.1% for sCD14-ST and 77.4% and 77.3% for leukocytes in CSF, respectively). The positive and negative predictive values were also much higher for sCD14-ST. Diagnostic utility of sCD14-ST in comparison to leukocyte count in CSF was better and statistically significant ($P < 0.001$).

The calculated cutoff for the sCD14-ST was higher than suggested by the assay manufacturer. The value of 500 pg/ml is suggested to be indicative for sepsis measured in plasma in adults. Values below 200 pg/ml are considered negative for infection confirmed in an independent cohort (20–22). Our value of 625 pg/ml in CSF is still in accordance with the suggested value. Given the fact that sCD14-ST is a protein that is released in blood in bigger quantities by immune cells that participate in the innate immune response to bacteria (19), we think that sCD14-ST could also be helpful in cases of suspected meningitis to quickly identify possible patients that have infection and would benefit from faster administration of broad-spectrum antibiotic therapy, since phagocytic immune cells (especially macrophages) are also present in brain.

TABLE 3 Differences in mean values with standard deviation for biomarkers

Biomarker (unit)	Value in cases positive for infection	Value in cases negative for infection	*P* value	Value in cases of Gram-positive infection	Value in cases of Gram-negative infection	Value before AT[a]	Value after AT	*P* value
sCD14-ST (pg/ml)	2,403.08 ± 2,407.56	500.35 ± 545.05	<0.001	1,646.85	1,699.36	2,654.77	1,456.25	0.217
CSF leukocytes (no./μl)	579.84 ± 750.62	91.36 ± 132.14	0.004	413.23	432.39	488.65	842	0.258
CSF neutrophils (%)	54.12 ± 26.44	37.78 ± 26.69	0.051	47.09	42.84	56.39	43.23	0.375
Glucose (mg/dl)	2.03 ± 1.08	3.02 ± 1.47	0.008	2.42	2.38	2.44	0.76	<0.001
Proteins (mg/dl)	2.64 ± 2.14	0.79 ± 1.05	0.001	1.85	2.04	2.59	2.78	0.843
CRP (mg/liter)	106.21 ± 135.50	52.48 ± 81.13	0.095	87.06	86.41	103.04	118	0.8
PCT (μg/liter)	0.65 ± 1.01	0.39 ± 0.26	0.332	0.56	0.46	0.61	0.75	0.77
Leukocytes (×10^9/liter)	11.78 ± 5.17	11.60 ± 4.27	0.893	11.78	11.23	11.26	13.59	0.303
Neutrophils (%)	66.94 ± 18.15	55.32 ± 17.98	0.053	61.68	60.52	69.05	61.67	0.258

[a] AT, antibiotic therapy.

When the values of sCD14-ST in infection episodes were compared to the values of other biomarkers in chemical ventriculitis episodes, the difference was found to be statistically significant ($P = 0.014$) (Table 2). We also determined that after the initiation of antibiotic therapy or a change in the therapy, the values of sCD14-ST in CSF dropped almost 2-fold, but the difference was not statistically significant (Table 3) ($P = 0.217$). The other two factors that are also measured in CSF, the levels of glucose and proteins, were also statistically significant to predict infection ($P = 0.033$ and $P < 0.001$) but less accurate, especially glucose, with an AUC of 0.324, while proteins had an AUC of 0.857 (Tables 3 and 4).

Ventriculitis is a type of low-grade infection that develops gradually and is not necessarily accompanied by a quick rise of proteins and a decrease of glucose as we see in typical bacterial meningitis (23–25). As we expected, statistical analysis showed a positive correlation with meningitis or ventriculitis and the level of sCD14-ST and counts of leukocytes and proteins in CSF in our study. Furthermore, when patients had bacterial infection, there was a statistically significant decrease of glucose in CSF. Based on these findings, we could conclude that sCD14-ST is useful to predict bacterial meningitis and ventriculitis and might be used to monitor the effectiveness of antibiotic therapy.

Although our study is the first one in which the sample used was CSF, other studies have been performed with children with sepsis. Presepsin is seen as a good biomarker of neonatal sepsis, specifically for very early diagnosis, and can be used as a predictor of complications and death (26). In pediatric oncology patients with chemotherapy-induced febrile neutropenia and sepsis, PCT and soluble IL-2 receptor (sIL-2R) levels were considerably higher in a group with bacteremia or sepsis than in a group with fever of unknown origin, whereas levels of sCD14-ST between investigated groups did not differ significantly (27). The neutropenia might be the cause of the different conclusions from those of the other studies. The mean blood sCD14-ST level in 26 preterm newborns with suspected sepsis in a study from Italy was 643.1 pg/ml. The results suggested that ranges from adults could be used also in newborns to predict sepsis, but with cutoff modifications (28).

In the adult population, several studies proved the usefulness of sCD14-ST to predict sepsis. In a comparative study of diagnostic markers of sepsis based on ROC curves, the AUC of sCD14-ST was 0.845, greater than the AUCs of PCT, CRP, and IL-6. Influenced by the study, the manufacturer of the assay calculated the value of 500 pg/ml to predict sepsis and the value of 1,000 pg/ml to predict septic shock. Levels of sCD14-ST further correlated with the severity of sepsis during follow-up in comparison with other conventional sepsis biomarkers, like CRP and PCT (16, 18). The cutoffs from this study are in accordance with our cutoff for CSF.

Other established biomarkers of bacterial infection, like CRP or PCT measured in blood, did not prove useful in our study of children with meningitis or ventriculitis. Diagnostic accuracies of PCT and CRP to predict meningitis and ventriculitis were not satisfying. Bacteria that caused the infection are usually considered contaminants and caused ventriculitis in the case of EVD, which is not an on-off infection like acute bacterial meningitis; the immune response is not necessarily systemic, and PCT is not necessarily raised, as seen in our study. CRP was raised irrespective of the etiology of inflammation and had a low AUC. It is known that PCT and CRP can be raised after surgery and after multiorgan failure, in the case of autoimmune diseases. Its use is not helpful,

TABLE 4 Values of ROC analysis to detect suspected bacterial infection for tested biomarkers[a]

Biomarker (unit)	AUC	95% CI	Sensitivity (%)	Specificity (%)	Cutoff	PPV (%)	NPV (%)	*P* value
sCD14-ST (pg/ml)	0.877	0.793–0.961	84.2	82.1	625	86.5	79.3	<0.001
CSF leukocytes (no./μl)	0.798	0.677–0.920	77.4	77.3	102.0	82.8	70.8	<0.001
CSF neutrophils (%)	0.663	0.494–0.832	62.5	78.9	55.0	78.9	62.5	0.068
Glucose (mg/dl)	0.324	0.168–0.481	58.6	36.4	1.90	54.8	40.0	0.033
Proteins (mg/dl)	0.857	0.749–0.964	82.8	77.3	0.94	82.8	77.3	<0.001
CRP (mg/liter)	0.682	0.537–0.827	78.8	60.9	6.5	73.0	68.4	0.021
PCT (μg/liter)	0.451	0.258–0.643	38.1	50.0	0.33	60.0	44.4	0.613
Leukocytes (í10^9/liter)	0.506	0.350–0.662	61.3	47.8	10.25	56.5	61.9	0.944
Neutrophils (%)	0.689	0.518–0.860	81.0	61.1	55.35	80.0	50.0	0.044

[a] Units apply to AUCs and cutoffs only. *P* value was determined by Kruskal-Wallis and chi-square tests. PPV, positive predictive value; NPV, negative predictive value.

TABLE 5 Identified bacteria from CSF in episodes of meningitis or ventriculitis

Bacterial identity	
Standard CSF cultivation	16S rRNA PCR
Staphylococcus epidermidis	*Staphylococcus epidermidis*
Enterococcus gallinarum	*Enterococcus gallinarum*
Staphylococcus haemolyticus	*Staphylococcus haemolyticus*
Serratia marcescens	*Serratia marcescens*
Escherichia coli	*Escherichia coli*
Pseudomonas aeruginosa	*Pseudomonas aeruginosa*
Staphylococcus aureus	*Staphylococcus aureus*
Streptococcus mitis	*Streptococcus mitis*
Streptococcus cristatus	*Streptococcus cristatus*
Enterococcus casseliflavus	*Staphylococcus sciuri*
	Pseudomonas extremorientalis
	Enterococcus cecorum
	Granulicatella elegans
	Enterococcus mundtii
	Propionibacterium acnes
	Streptococcus pneumoniae
	Corynebacterium pseudotuberculosis
	Pseudomonas otitidis
	Bacillus siralis
	Corynebacterium amycolatum

since the dynamics of its production are influenced by any kind of systemic inflammation (29–31).

We also used conventional microbiological methods, such as culture, to confirm bacterial infection. We compared the results of culturing with the results of broad-range 16S rRNA PCR. The broad-range PCR proved to be very useful, as it detected far more bacterial pathogens than culturing alone. We confirmed etiology of ventriculitis in 37 out of 38 samples of CSF, in comparison to 17 cases with culture methods. We also confirmed more mixed infections with broad-range 16S rRNA PCR ($P < 0.001$). In 9 cases, we were able to diagnose additional bacteria that could influence the administration of other antibiotics with a different spectrum that could be more suitable for mixed infection, which can generally be expected if EVD is an entry point to the sterile cerebrospinal compartment. Bacterial skin flora contaminations of samples that were taken through EVD could not be excluded, since EVD was also the entry point for bacteria in all episodes of meningitis and ventriculitis. The use of broad-range PCR improved the diagnostic process in the case of sepsis, especially since more sepsis cases are confirmed with its use than with blood cultures alone (32). The culture methods are still indispensable, because sensitivity testing is needed. However, we are able to clarify more cases of ventriculitis with broad-range PCR, identify the pathogen, and prescribe a more suitable 16S rRNA gene PCR-guided antibiotic therapy.

We additionally found that there were no significant differences in sCD14-ST or any other used biomarker levels between the Gram-positive and Gram-negative bacterial infection groups. Our findings are in accordance with those of Endo et al. (19), who measured sCD14-ST in the blood of suspected sepsis patients and did not find any important differences between different bacteria.

The main drawback of our study is that no such measurements of sCD14-ST have ever been done, and as a result, the test is not validated for CSF. However, measurements of sCD14 have been done in CSF, and the results of experiments indicate that CD14 may represent a member of a multireceptor complex responsible for the establishment of immune responses to *S. aureus* in brain abscesses (33). Intracerebral bacterial infection caused only a minor increase of sCD14 levels in the serum; elevated concentrations of sCD14 were detected in CSF in another experimental study (34). In patients with Lyme disease, sCD14 levels in CSF were no different from those in control subjects. However, concentrations of sCD14 in synovial fluid from joints of patients with Lyme disease were elevated compared with levels in normal serum, which may play a role in the pathogenesis of arthritis (35). These data confirm our decision to measure a subtype of sCD14 in CSF to diagnose bacterial meningitis or ventriculitis.

We could not measure blood sCD14-ST levels in our group of children since the blood was discarded after measurement of biomarkers in a biochemical laboratory. With such measurements performed, we might have some insight into the severity of the disease and prognostic values of sCD14-ST in comparison to other biomarkers such as CRP and PCT. Evaluation of sCD14-ST in CSF was also not done in an independent cohort, since that would be ethically inappropriate for children without suspected meningitis or ventriculitis. The study was conducted on a relatively small number of patients since the uniqueness of EVD-related meningitis and ventriculitis prevented us from enrolling more patients. In the case of 16S rRNA broad-range PCR and culturing of CSF, the procedures are prone to contamination, which cannot be ruled out with certainty.

Conclusions. The use of sCD14-ST can be added to the diagnostic process in the case of meningitis and ventriculitis in children in conjunction with a biochemical analysis of CSF. The added value of sCD14-ST is its simplicity to measure because it can be done on automated assay by the patient's bedside. If we wish to improve the microbiological diagnostic process for patients with suspected meningitis or ventriculitis after EVD, sCD14-ST could be useful to identify the patients that would benefit the most from broad-range 16S or 18S rRNA gene PCR.

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D.S. and A.N.K. equally contributed to the article and wrote the manuscript. D.S. and A.N.K. carried out the molecular genetic analysis and statistically analyzed all the data. M.G.-G. enrolled the patients and collected CSF samples. K.S. and T.F. measured laboratory biomarkers for all patients. M.S. proposed the study, wrote the protocol, was involved in drafting the manuscript and revising it critically, and gave final approval of the manuscript to be published.

We declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interests.

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