Original research article

Polymerase chain reaction based detection of bacterial 16S rRNA gene in the cerebrospinal fluid in the diagnosis of bacterial central nervous system infection in the course of external cerebrospinal fluid drainage. Comparison with standard diagnostics currently used in clinical practice

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ABSTRACT

Background and purpose: External drainage of cerebrospinal fluid (CSF) is a commonly used neurosurgical procedure. Complications of the procedure comprise central nervous system (CNS) bacterial infections, the frequency of which is estimated at around 6–10%. Detection of these infections is ineffective in many cases. The aim of the study was to evaluate the usefulness of a polymerase chain reaction (PCR)-based detection of bacterial 16S rRNA gene (16S rDNA) in the CSF.
Material and methods: The study group consisted of 50 patients. Clinical signs of CNS infection were monitored and routine laboratory and microbiological tests were performed. The results of standard methods were compared with the bacterial 16S rDNA detection.
Results: Using cultures, CNS infection was diagnosed in 8 patients, colonization of the drainage catheter in 6 patients, and sample contamination in 7 patients. In the group of the remaining 29 patients, no positive CSF culture was obtained and 13 of these patients also had all negative results for 16S rDNA detection. For the remaining 16 patients of this group, CNS infection, colonization of the catheter and sample contamination were diagnosed via PCR alone. Routine biochemical CSF tests and blood inflammatory parameters had a supporting value.
Conclusions: Routine hospital tests do not provide rapid and efficient detection of the external drainage related bacterial CNS infection. It is justified to use several diagnostic methods simultaneously. The 16S rDNA determination in CSF can increase the probability of detection of possible pathogens.

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1. Introduction

Bacterial central nervous system (CNS) infections occur in about 6–8% of patients with an implanted external cerebrospinal fluid (CSF) drainage. Predisposing factors include: intraventricular and subarachnoid haemorrhage, cranial fracture with cerebrospinal fluid leak, previous neurosurgical procedures, systemic infections, extended duration of catheterization [1–3]. Fast and accurate identification of the etiological agent and targeted antibiotic therapy are crucial for infection management.

Routine hospital diagnostics are based on time-consuming bacterial cultures whose reliability is further reduced during antibiotic treatment. CSF leucocyte count, biochemical examination, and blood inflammatory parameters have auxiliary value [4]. All of the above methods, therefore, have limited effectiveness [5,6]. Due to the high morbidity and mortality associated with drainage procedures, alternative molecular biological methods were introduced. PCR-based detection of DNA encoding the 16S subunit of the bacterial rRNA (16S rDNA) proved to be useful. Some 16S rDNA fragments and areas located in the vicinity are common to most known bacteria and their detection confirms the presence of microorganisms. The method has been applied to diagnose CNS infection in patients with external CSF drainage [7,8]. Moreover, modern and ready-made laboratory kits are based on quantitative multiplex real-time PCR, e.g., SeptiFast, Roche [9–11]. By choosing the appropriate PCR primer sets, it is possible to detect tens of microorganisms with their species specifications simultaneously. In Poland, the above methods are rarely used for patients with CSF drainage and will require verification in clinical practice. The aim of the study was to evaluate the usefulness of the detection of bacterial 16S rDNA in the CSF in the diagnosis of bacterial CNS infection in patients with CSF external drainage. To achieve this goal the tested method was compared with standard diagnostics currently used in clinical practice.

2. Material and methods

The study group consisted of 50 patients with external CSF drainage hospitalized in the Department of Neurosurgery. External ventricular drainage (EVD) was implanted in a group of 30 patients, external lumbar drainage (ELD) in the remaining 20 patients. During drainage observation, clinical signs of CNS infection (fever, meningeal signs), patient reactivity on the Glasgow coma scale (GCS), and the volume of drained CSF were continuously monitored. Routine laboratory inflammation tests, i.e., white blood cell (WBC) and blood C-reactive protein (CRP) concentration, were performed every 72 h or according to clinical indications. CSF samples were collected daily from the drainage systems and evaluated for the presence of bacteria using Gram stain and microbiological culture, and analyzed for leucocyte count, protein concentration, and glucose concentration. At the same time, the CSF obtained was tested for the presence of bacterial 16S rDNA using PCR. A total of 276 CSF samples were collected.

2.1. Implantation of the drainage systems

EVDs were implanted in the operating room, ELDs under sterile conditions at the patient's bedside. For placement of the ventricular catheter, the patient's skull was shaved and prepared with standard sterile techniques. The ventricular catheter was inserted through the burr-hole and tunnelized for a distance of at least 10 cm. Both ventricular and lumbar catheters were connected to a closed external drainage and monitoring system. The CSF samples were obtained aseptically by tapping a valve that is part of the drainage system. If a patient required catheter re-implantation or exchange, then analysis of that patient was finished and only the first external drainage period of the patient was included in the study.

2.2. Definition of infection, colonization, contamination

Similarly as in the study of Schade at al., the bacterial CNS infection associated with the use of external drainage of cerebrospinal fluid was diagnosed when at least one positive CSF culture on one or more consecutive days coexisted in combination with at least one of the clinical signs of bacterial meningitis (fever, nuchal rigidity) [5]. If a patient had two or more consecutive positive CSF cultures with the same pathogen but no clinical signs, the result was defined as bacterial colonization of the drainage catheter. A single positive CSF culture with common skin pathogen and no clinical signs was determined as a sample contamination. Each CSF culture result was compared with 16S rDNA detection and an analysis was performed. In patients with all negative CSF cultures, the same criteria as above were used to determine CNS infection, catheter colonization and sample contamination using only positive PCR. If the outcomes of both methods were negative during the period of external drainage, then the presence of bacteria in CNS was excluded.

2.3. Antibiotic therapy

All patients with EVD received prophylactic antibiotic before placement of the drainage catheter, 1 g of cefazolin was given intravenously and it was continued in a dose of 2×1 g a day for the next 48 h. No antibiotic agents were given prophylactically in the ELD group. In all cases of CNS infection and catheter colonization determined with CSF culture, antibiotics were given in accordance with the received antibiogram, however, in culture-negative patients diagnosed only with positive PCR results, broad-spectrum antibiotics ceftriaxone 2×1 g, amikacin 2×0.5 g and metronidazole 3×0.5 g were given simultaneously. If CSF samples turned out to be contaminated in culture or PCR, no treatment was initiated.

2.4. Routine CSF tests

2.4.1. Direct examination
Leucocyte count was determined using the Fuchs-Rosenthal counting chamber and light microscopy. After centrifugation of the CSF sample, the total protein and glucose concentrations of the supernatants were measured using an automated chemistry analyser.

2.4.2. Standard microbiological testing

The CSF was centrifuged and the sediment was Gram stained and cultured on standard agar plates as well as in enrichment broth. The sediments were also cultured in fluid enrichment media (BACTEC). Standard biochemical tests were used to identify bacteria, and antibiotic susceptibility testing was performed with the Kirby–Bauer disc-diffusion method.

2.5. Detection of bacterial 16S rDNA

The collected CSF was pre-treated with three digesting enzymes: muramidase (Sigma) with a total concentration of 0.002 g/l in a phosphate buffer, pH 4.8, incubation time 3 h at 37 °C, followed by a change in pH to 7.0 using NaOH; lysozyme (Roche) with a total concentration of 0.2 g/l, incubation time 1 h at 37 °C; proteinase K in a suitable buffer system NucleoSpin® Tissue kit (Macherey-Nagel, Germany); and then DNA isolation was carried out according to the procedure set by the manufacturer. The PCR was performed using universal primers for the bacterial 16S RNA gene: forward 5′ AGT TTG ATC CTG GCT CAG and reverse 5′ GAA CTA CCA GGG TAT CTA AT (Oligo Poland), 5 ng DNA, FastStart Taq DNA polymerase Kit, GC rich (Roche) and Anti-inhibitor PCR (DNA Gdańsk), the final volume of 25 µl. The DNA amplification was performed in an MJ Research Thermal Cycler.

The DNA polymerase was activated at 95 °C for 15 min, 60 °C for 45 s, 72 °C for 10 s and amplification was performed using 35 cycles at the following reaction conditions: 95 °C for 45 s, 60 °C for 1 min, 72 °C for 10 s for each cycle. The reaction mixture without the matrix and the mixture treated with DNase were used as a negative control. For a positive control, purified bacterial DNA from Staphylococcus aureus was used. The amplification products were subjected to electrophoresis on a 12.5% polyacrylamide gel (ExcelGel, Amersham Biosciences) and silver staining (DNA Silver Staining Kit, Amersham Biosciences). The expected 789 base pair (bp) bands were compared to the 100 bp ladder (Amersham Biosciences) using the Gene Tools software (Syngene).

2.6. Statistical methods

We analyzed the diagnostic value of detection of bacterial 16S rDNA and other parameters recorded during the diagnosis of CNS infection during the period of external drainage of the CSF. In the case of binary variables (bacterial 16S rDNA, fever, meningeal signs), the criterion was a number of observed positive results. With regard to continuous variables, we assumed a maximum value of all assays for leucocyte count and protein concentration in CSF, for glucose a minimum value, and for blood CRP concentration and WBC an average value. The analyzed parameter results were evaluated using ROC curves. For each parameter, the area under the curve was estimated with 95% confidence and the null hypothesis was tested, which assumes the value of the field is equal to 0.5. For all analyses, the level of statistical significance was 0.05.

3. Results

The study group consisted of 50 patients, the male/female ratio was 1.8:1, the median patient age was 52.9 years; range 19–81 years. The indications for implantation of external drainage were: intracranial haemorrhage in 18 patients (36%), CSF leakage in 12 patients (24%), traumatic hydrocephalus in 6 patients (12%), post inflammatory complications in 5 patients (10%), brain tumours in 5 patients (10%), cerebellar stroke in 4 patients (8%). The median duration of external drainage was 6 days (range 2–15 days).

3.1. Clinical groups based on positive CSF cultures

3.1.1. Infection
Bacterial CNS infection was diagnosed with culture in 8 patients (16%), the male/female ratio was 1:1; mean age 42.8; range 23–62 years. 54/72 (75%) of the CSF cultures were positive in this group. Acinetobacter baumanni was found in three patients, Staphylococcus epidermidis, Enterobacter cloacae, Escherichia coli, Enterococcus faecium, methicillin-resistant Staphylococcus aureus (MRSA) were found in one patient each. Three patients were febrile on all days of positive CSF cultures. For the remaining patients, periods of fever included at least one day of positive cultures. Meningeal signs were present in 6 patients. The direct CSF examination results were remarkably pathological, the blood CRP concentration and WBC were also elevated (Table 1). In the entire, culture-confirmed CNS infection group 87.5% (63/72) of the PCR results were positive. 82% of the positive cultures were also found positive in PCR (Table 2).

3.1.2. Colonization of the drainage catheter
Colonization was found in six drainages (12%), the male/female ratio was 2:1; mean age 51.3 years; range 30–70 years. Coagulase-negative staphylococci were found in three cases, Proteus mirabilis in one case, Klebsiella pneumoniae in combination with Enterobacter cloacae in one case, and coagulase-negative staphylococcus in combination with Enterococcus faecalis in one

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Table 1 – Average results of direct CSF examination, blood CRP concentration and WBC in the clinical groups based on positive cultures. Range of value is in brackets.

Analyzed parameter	Clinical groups		
	CNS infection	Catheter colonization	Sample contamination
Leucocyte count (/µL)	1115 (10–7850)	126 (1–1877)	37 (1–125)
Glucose concentration (g/L)	0.47 (0.01–1.24)	0.64 (0.22–1.2)	0.74 (0.33–1.23)
Protein concentration (g/L)	2.40 (0.38–7.84)	0.9 (0.1–6.1)	0.41 (0.03–1.90)
Blood CRP concentration (g/L)	0.17 (0.02–0.29)	0.08 (0.001–0.15)	0.08 (0–0.25)
WBC ($\times 10^3$/µL)	12.57 (3–29)	12.25 (7–16)	12.5 (6–14)

Table 2 – Results of CSF cultures vs. PCR in the infection group.

Drainage type	CSF culture		16S rDNA	
	Positive	Negative	Positive	Negative
EVD	16	1	17	0
ELD	38	17	46	9
Total	54	18	63	9

Table 3 – Results of CSF cultures vs. PCR in the colonization of drainage catheter group.

Drainage type	CSF culture		16S rDNA	
	Positive	Negative	Positive	Negative
EVD	6	9	14	1
ELD	16	15	27	4
Total	22	24	41	5

case. The results of direct CSF examination and blood CRP concentration were remarkably less pathological than in the infection group (Table 1). 48% (22/46) of the CSF cultures and 90% (41/46) of the PCR results were positive. 100% (22/22) of the positive cultures were also found positive in PCR (Table 3).

3.1.3. Sample contamination
Single positive CSF cultures with no clinical signs were obtained from 7/50 patients (14%), the male/female ratio was 2.5:1; mean age 50.4 years; range 38–75 years. These patients were actually defined as non-infectious and the CSF routine examination outcomes were usually close to the normal range with only blood CRP concentration still remarkably elevated (Table 1). 5/7 (71%) of the positive CSF culture results were confirmed in 16S rDNA detection.

3.2. Clinical groups with all negative CSF cultures

In the group of 29/50 patients (58%), no positive CSF cultures were obtained. EVD was implanted in 20 patients, ELD in 9 patients. The male/female ratio was 1.9:1; mean age 56.6 years; range 19–81 years. Clinical signs, the results of routine

laboratory tests and PCR outcomes played a major role in treatment management.

3.2.1. Patients with all negative 16S rDNA PCR results
The subgroup consisted of 13 patients with no CNS infection signs; 45 CSF samples were collected. The mean results of general CSF examination showed double-digit leucocyte count, normal glucose concentration and slightly elevated protein concentration; the blood CRP concentration and WBC were abnormal (Table 4).

3.2.2. PCR-confirmed colonization of the drainage catheter
Eight patients had two or more consecutive positive PCR results. Six patients in this group were asymptomatic; two patients had meningeal signs, however, subarachnoid hae-morrhage was diagnosed in these patients. In total, 36 samples were collected and 27 proved to be positive (75%). The mean outcomes of the CSF routine examinations were similar to those of the PCR negative cases; blood CRP concentration was also elevated (Table 4).

3.2.3. PCR-confirmed sample contamination
Three patients had 16S rDNA found in a single CSF sample each; no CNS infection signs were present. The glucose and protein concentration in the CSF was normal; the cell counts of leukocytes were not significantly elevated. Due to peripheral infections, the blood CRP concentration was elevated, however, normal WBC was observed (Table 4).

3.2.4. Symptomatic patients with positive 16S rDNA. PCR-confirmed CNS infection
In 5/29 (17%) of patients with all negative CSF cultures, a fever coexisted with meningeal signs for at least one day of drainage observation. On these days, direct CSF examination showed significant pathological results; the blood CRP concentration and WBC were elevated too (Table 4). Three patients had fever for much of the period of the external drain. In one patient, heavy pneumonia was diagnosed, however, in the rest of them (two EVDs and two ELDs) no remarkable peripheral infection was observed. In total, 27 samples were collected and 12 PCR results were positive (44%). CNS infection was diagnosed in four patients.

Table 4 – Average results of direct CSF examination, blood CRP concentration and WBC in the clinical groups with all negative CSF cultures. Range of value is in brackets.

Analyzed parameter	Clinical groups			
	PCR negative	PCR positive		
		Catheter colonization	Sample contamination	CNS infection
Leucocyte count (/μL)	55 (2–427)	19 (1–80)	20 (4–51)	1115 (1–6180)
Glucose concentration (g/L)	0.79 (0.26–2.6)	0.92 (0.5–2.20)	0.72 (0.32–1.44)	0.59 (0.02–1.33)
Protein concentration (g/L)	0.8 (0.1–7.6)	0.68 (0.10–3.17)	0.52 (0.22–0.96)	1.31 (0.08–3.44)
Blood CRP concentration (g/L)	0.06 (0.003–0.231)	0.1 (0.06–0.24)	0.05 (0.01–0.08)	0.12 (0.06–0.29)
WBC ($\times 10^3$/μL)	12.1 (4–16)	10 (5–31)	8.2 (6–13)	13.8 (7–22)

Table 5 – The results of evaluation of the diagnostic value of the analyzed parameters in bacterial meningitis in the course of external drainage of the cerebrospinal fluid.

Indicator	The area under the ROC curve (95% confidence interval)	p	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Kappa	Agreement
16S rDNA	0.91 (0.83; 1.00)	<0.001	100	78.6	47.1	100	0.54	Medium
Fever	0.85 (0.68; 1.00)	0.002	87.5	88.1	58.3	97.4	0.63	High
Meningeal signs	0.76 (0.55; 0.98)	0.021	75.0	78.6	40.0	94.3	0.40	Low
CSF leucocyte count	0.95 (0.87; 1.00)	<0.001	100	90.5	66.7	100	0.75	High
CSF glucose	0.84 (0.63; 1.00)	0.003	87.5	69.0	35.0	96.7	0.35	Low
CSF protein	0.91 (0.83; 1.00)	<0.001	100	78.6	47.1	100	0.54	Medium
Blood CRP	0.85 (0.73; 0.97)	0.002	100	64.3	34.8	100	0.37	Low
WBC	0.50 (0.26; 0.75)	0.979	–	–	–	–	–	–

3.3. Statistical analysis

In the statistical analysis, we used CSF culture results combined with CNS infection clinical signs as a reference. The highest diagnostic value was found for CSF leucocyte count >512. The area under the ROC curve was 0.95 and was significantly greater than 0.5 ($p < 0.001$) (Table 5). The sensitivity and specificity were respectively 100% and 90.5%. For this parameter, good agreement was found (kappa = 0.75). A slightly smaller diagnostic value was found for the 16S rDNA detection and also for the CSF protein concentration. For both indicators, we received the same values of evaluation criteria. The area under the ROC curve was 0.91 ($p < 0.001$), sensitivity and specificity 100% and 78.6%, kappa agreement 0.54. The minimum (zero) diagnostic value was found for WBC. The estimated area under the ROC curve was 0.5 ($p = 0.98$) and, therefore, no cut-off value for this indicator was established.

4. Discussion

Despite technological advances in the types of materials used and in the production of draining equipment, e.g., anti-bacterial filters, drains coated with antibiotics and agents that increase the smoothness of the surface, drainage procedures still remain invasive and accompanied by a large percentage of complications of which the most important are bacterial CNS infections [12,13]. Fast and precise diagnosis remains the key issue but even the definition of infection is extremely difficult and ambiguous [14]. In their historical study, Mayhall et al. defined infection as the positive culture of the cerebrospinal fluid obtained from the ventricular catheter [15]. Some authors used the same criteria, other recognized external drainage related bacterial meningitis after receiving at least two positive CSF cultures [16–19]. Infection was also diagnosed when positive CSF cultures coexisted with high CSF leucocyte count, low glucose or high protein concentration [20]. Only a few authors drew attention to the clinical symptoms and signs, e.g., fever or consciousness disorders. In 2006, Schade et al. published the results of a research group of 220 patients. They relied on CSF plating and clinical signs and simultaneously questioned the usefulness of the CSF direct examination as a prognostic and diagnostic factor in CNS infection in the course of external CSF drainage [5]. The number and diversity of diagnostic methods and definitions of infection are, therefore, proof of the diagnostic difficulties. Molecular biology started to

be an additional option, especially the detection of the bacterial genome. In our study, we analyzed the results of clinical observations and the most frequently performed laboratory and microbiological tests and compared them with the detection of 16S rDNA. Clinical signs combined with CSF culture results had a decisive role, however, in culture-negative patients we used 16S rDNA to confirm or exclude infection. During the analysis of the results, a significantly higher percentage of positive PCR results (57%) in comparison with positive CSF cultures (31%) was observed. In the study of Banks et al., it was respectively 73% and 21%, and the proportion of PCR (+)/culture (−) was 49% of the total number of samples [7]. In our material, 84 16S rDNA positive samples were not positive in plating (30% of all samples tested). This raises the suspicion of a large number of false positive results as, e.g., Deutch et al. had a comparable total number of positive PCR and culture results at about 10% each [8]. However, the clinical data and the circumstances in which the individual fluid samples were collected are important. Banks et al. investigated samples of CSF derived only from patients with suspected infection of the CNS. This explains the very high number of positive PCR outcomes. In our study, a large number of positive 16S rDNA results was also obtained in patients with culture-confirmed CNS infection and colonization of the drainage catheter. On the other hand, there was also a group of asymptomatic patients with all negative PCR and culture results. In asymptomatic CSF culture (−)/PCR (+) patients, colonization of the drainage catheter and sample contamination were recognized using 16S rDNA detection alone. The most important PCR application in the study was to diagnose the CNS infection in symptomatic culture-negative cases. Four patients were diagnosed and treated with broad-spectrum antibiotics; one patient died as a consequence of underlying neurological damage, two patients obtained a permanent infection cure, one patient died as a result of CNS infection complication. In above cases, qualitative broad range PCR made it possible to detect 16S rDNA and to diagnose the CNS infection however no species determination was performed. Multidrug antibiotic treatment was used subsequently and although it sometimes proved to be effective, however, this can lead to the accumulation of drug resistant microbes. For this reason, in recent years, there are new methods based on quantitative real-time PCR [21,22]. Carefully selected groups of PCR primers allow the simultaneous detection of a few tens of microorganisms (bacteria and fungi) that most commonly cause hospital infections, including infections related to the

use of external drainage of cerebrospinal fluid. It is possible to determine the specification of species and the drug resistance genes. Several authors have described the doubtful usefulness of such methods; others regard the methods as a viable alternative [9–11]. Excluding the presence of bacteria in the CSF using the two independent methods (culture and PCR) can be an effective diagnostic tool in neurosurgery patients, e.g., in cases of external CSF drainage related CNS infection during antibiotic therapy, when we expect microorganism eradication before ventriculoperitoneal shunt implantation. Culture results alone can be false negative in such circumstances and PCR outcomes enable to choose the appropriate moment for surgery. Similarly, in coexisting systemic or peripheral and CNS infection, the application of the PCR is to determine whether bacterial CSF infection still exists when patients receive antibiotics for other indications so that CSF cultures may be negative due to a partially treated CNS infection. DNA amplification techniques could provide rapid diagnosis, which would guide the clinician in antimicrobial therapy decisions [22–25]. The 16S rRNA gene PCR is useful than for diagnosis of culture-negative bacterial infections in patients pretreated with antibiotics [26].

The statistical analysis confirmed a usefulness of the method of PCR in detecting bacteria in CSF during the course of external drainage, particularly in cases of doubt. This method can, therefore, be a supplement to the traditional bacterial culture, which still remains the gold standard. The advantages of PCR are: shorter analysis time and the independence of the results from treatment with antibiotics. Limitations of the method are: inability to distinguish whether the detected bacterial DNA comes from living or dead microorganisms, possible contamination of samples and difficulties to determine the drug susceptibility of detected bacteria. In Poland, PCR-related methods of CNS infection detection in the course of CSF external drainage are not widely used in clinical practice. Their introduction could have influence on the effectiveness of treatment. However, the obtained data have to be always interpreted with caution and in connection with the clinical data, outcomes of other investigations and with the result of both the sample and the controls.

Other diagnostic parameters, e.g., general examination of the cerebrospinal fluid have a supporting value while not directly detecting microorganisms, however, in contrast to Lozier et al., we found the highest diagnostic value for the CSF leucocyte count, and slightly lower for both protein and glucose concentration. Undoubtedly, these are also necessary for the definitive diagnosis of infection. Blood leucocytosis and CRP concentration have low diagnostic value. To summarize, because of the diagnostic difficulties, it is justified to use several diagnostic methods simultaneously.

In our study we used a surveillance cultures protocol, which was necessary to detect the incidence of bacterial colonization and infection. CSF samples were collected in the consecutive days of observation and it enabled to compare efficiently the tested diagnostic methods. However, in clinical practice the utility of surveillance cultures must be thoroughly weighed against the risk of catheter colonization and CNS infection this may imply. Although in the past some authors suggested that there was no relation between infection and the number of manipulations of the external CSF drainage [16], the routine

CSF sampling is no longer used since it is felt that the risks outweigh any possible benefits [27]. Routine surveillance cultures of CSF were no more likely to detect infection than cultures obtained when clinically indicated [28]. CSF samples should be sent only if there is fever and other sources have been excluded or there are clinical signs of CNS infection [29].

5. Conclusions

(1) Effective detection of CNS infection in the course of external drainage of the cerebrospinal fluid is an important clinical problem. It is difficult to clearly determine the definition of infection as this varies between authors.

(2) It is recommended to use several diagnostic methods simultaneously. CSF samples should be obtained only when clinically indicated.

(3) PCR-based detection of bacterial 16S rDNA in CSF is a possible supplement to routine hospital diagnostics, especially in doubtful cases. Despite the important restrictions of the method, it has a relatively short time for analysis limited to a few hours. The results do not depend on the antibiotic treatment used.

(4) Modern diagnostic kits based on quantitative real-time PCR makes it possible to screen for a number of microorganisms at the same time and also to identify species.

(5) Dissemination of PCR-based methods may increase the detection of bacterial infections associated with the use of CSF drainages and can influence on treatment outcomes.

Conflict of interest

None declared.

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