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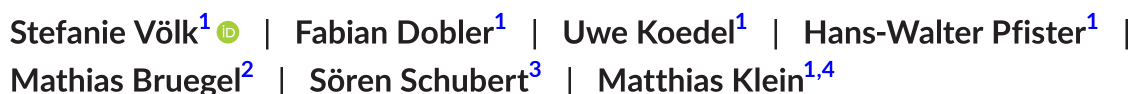
ORIGINAL ARTICLE

Cerebrospinal fluid analysis in emergency patients with suspected infection of the central nervous system



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Abstract

Background and purpose: Meningitis and encephalitis are potentially life-threatening diseases that require fast and accurate diagnostics and therapy. The value of polymerase chain reaction (PCR) multiplex testing in clinical practice is still a matter of debate. This study aims to evaluate its benefits and limitations in emergency patients.

Methods: We assessed the value of a meningoencephalitis PCR array in the clinical routine of an emergency department.

Results: Of 1578 emergency patients who received a lumbar puncture, 43% received it for a clinically suspected central nervous system (CNS) infection. After initial workup for cerebrospinal fluid (CSF) cell count, protein and glucose, a CNS infection was still considered likely in 307 patients. In these patients, further microbiologic workup was performed. A total of 230 samples were examined by PCR and a pathogen was detected in 66 of these samples. In the case of a positive microbiologic result, a comparison between PCR array and standard method was available for 59 samples, which demonstrated an overcall agreement of 80% ($n = 47/59$). Of interest, exclusively array-positive results were observed for patients with meningitis found to be positive for *Streptococcus pneumoniae*; four out of five patients had been treated with antibiotics before the lumbar puncture. In samples with normal CSF cell count only two positive array results were obtained, both for human herpesvirus 6, and these were not clinically relevant.

Conclusion: Our data suggest that the array substantially contributes to a detection of pathogens in patients with suspected CNS infection and seems of particular interest in patients with acute bacterial meningitis under empiric antibiotic treatment. In CSF samples with normal cell count, it might be dispensable.

KEYWORDS
encephalitis, meningitis, meningoencephalitis, polymerase chain reaction

INTRODUCTION

Meningitis and encephalitis are potentially life-threatening diseases, therefore, fast and accurate diagnosis is crucial for the treatment of patients with these conditions [1]. The recommended diagnostic approach in patients with suspected bacterial meningitis is initial evaluation of cerebrospinal fluid (CSF) according to standard measures (cell count, protein, and glucose), followed by gram stain and

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culture if CSF cell count, CSF protein, and/or CSF serum-glucose-index or CSF lactate indicate bacterial infection [1]. If the diagnosis cannot be established by gram stain, empiric treatment needs to be continued until CSF culture results are available, which often takes 1–3 days in clinical practice. To detect the most common viral meningitis and encephalitis pathogens in Europe, namely, enterovirus (EV), herpes simplex virus (HSV), and varicella zoster virus (VZV), single polymerase chain reaction (PCR) is usually used. Unfortunately, this method is often not available at night and on weekends as it is time-consuming and requires skilled staff. Meningoencephalitis (ME) PCR assays aim to detect the most frequent bacterial and viral pathogens of the central nervous system (CNS) within a short time, usually in less than 2 h. The BioFire FilmArray® is a ME panel which covers 14 pathogens that commonly cause CNS infections, including six bacteria (*Escherichia coli K1*, *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Streptococcus agalactiae* and *Streptococcus pneumoniae*), seven viruses (cytomegalovirus [CMV], EV, HSV type 1 and 2, human herpesvirus (HHV) 6, VZV and human parechovirus), and *Cryptococcus neoformans/gattii* [2]. Previous studies mainly evaluated the array's accuracy in asservated samples and compared this to a reference standard [2, 3]. One meta-analysis of eight studies including a total of 3059 patients reported a high sensitivity of 90% (95% confidence interval [CI] 86%–93%) and a specificity of 97% (95% CI 94%–99%) [4]. The false-positive rate was 11% and the false-negative rate 4%. However, as mentioned by the authors, data on the array's accuracy are still limited, which is reflected in the small number of studies retrieved for their meta-analysis. Also, the included studies were heterogeneous, subgroup analyses were often not possible and, most importantly, clinical data are rare. In consequence, it remains unclear whether samples that tested positive only by the PCR array but not by other methods (usually termed "false-positive" results) really reflect a wrong PCR test result or whether the method is superior to the previously used tests and, thus, allows the detection of clinically relevant pathogens that could not be found otherwise. This problem was recently addressed in a meta-analysis which tried to correct for clinical significance, but the risk of bias, especially for the bacterial reference standard, was high in almost all studies that were included [5]. Thus, further reporting of the array's performance with inclusion of detailed clinical data for discordant results of various microbiological tests is crucial. Here, we report on the clinical impact, benefits and limitations of the ME FilmArray® in emergency patients with suspected CNS infection and include clinical data for relevant patients.

METHODS

This study was conducted at the emergency department of a tertiary care university hospital in Munich, Germany. All patients attending the emergency department who underwent lumbar puncture for clinically suspected CNS infection between October 2017 and October 2019 were prospectively enrolled, and both clinical data and laboratory results were evaluated. All CSF samples were routinely tested for cell count, protein and glucose levels at the Institute of Laboratory Medicine. Microbiologic CSF assessment followed only if the suspicion for a CNS infection persisted thereafter, based on a combination of clinical features (symptoms such as fever or headache) and standard CSF measures (increased cell count, increased protein and/or decreased glucose). Owing to the clinical approach used in the study, the final decision for microbiologic CSF diagnostics such as the PCR array was made by the attending physician. All microbiologic tests were performed at our Institute for Microbiology according to the manufacturer's instructions. The BioFire FilmArray® ME Panel (BioFire Diagnostics, LLC) was used for multiplex testing of CSF samples. Data are illustrated in numbers (n) and/or percentages. For statistical analyses IBM SPSS Statistics 28.0 was used. The Mann–Whitney U-test was chosen in case of abnormal distribution and p values < 0.05 were taken to indicate statistical significance. The study was approved by the Ethics Committee of the University of Munich (project number 12-409).

RESULTS

During the study period of 25 months (October 2017 until October 2019), 1578 patients presenting to the interdisciplinary emergency department underwent a lumbar puncture as part of the standardized emergency workup of their complaints (Figure 1). Forty-three percent ($n = 681$) of all lumbar punctures were performed because a CNS infection was suspected. Reasons for lumbar punctures in the other 57% ($n = 897$) included clinical suspicion for an autoimmune disease of the CNS, suspected meningeosis carcinomatosa or lymphomatosa, pseudotumor cerebri or subarachnoid haemorrhage.

After the analysis of standard CSF variables (cell count, protein and CSF/serum glucose), 45% ($n = 307$) of patients with clinically suspected CNS infection were still considered likely to have a CNS infection. These patients underwent further microbiologic CSF diagnostics (Figure 1). Among these, 75% ($n = 230$) of the samples were also analysed by PCR array. Pathogens were found in 33% ($n = 102$) of the CSF samples from patients with persistent suspicion for a CNS infection (Table 1) and detection was either by conventional diagnostics and PCR array ($n = 47$), conventional diagnostics only ($n = 36$) or PCR array only ($n = 19$). Concerning the detection of some pathogens, CSF contamination could not be excluded: One CSF sample found to be positive for *Staphylococcus capitis* was from an immunocompromised 47-year-old male with facial palsy and untreated HIV infection (14 cells/µl, protein 85 mg/dl, glucose 62 mg/dl, 3500 copies HIV1/ml CSF). Another sample positive for *Staphylococcus haemolyticus* was from a 77-year-old female with recent history of ependymoma resection, CSF drainage and CSF leakage (2017 cells/µl, glucose 34 mg/dl, protein 138 mg/dl) and there was one sample with *Streptococcus pyogenes* from an 84-year-old female with fever, headache, meningism, mastoiditis and otitis media (6506 cells/µl, glucose 36 mg/dl, protein 667 mg/dl).

From all samples analysed by PCR array, 29% ($n = 66/230$) had a positive test result: $n = 12$ for bacteria, $n = 53$ for viruses, and

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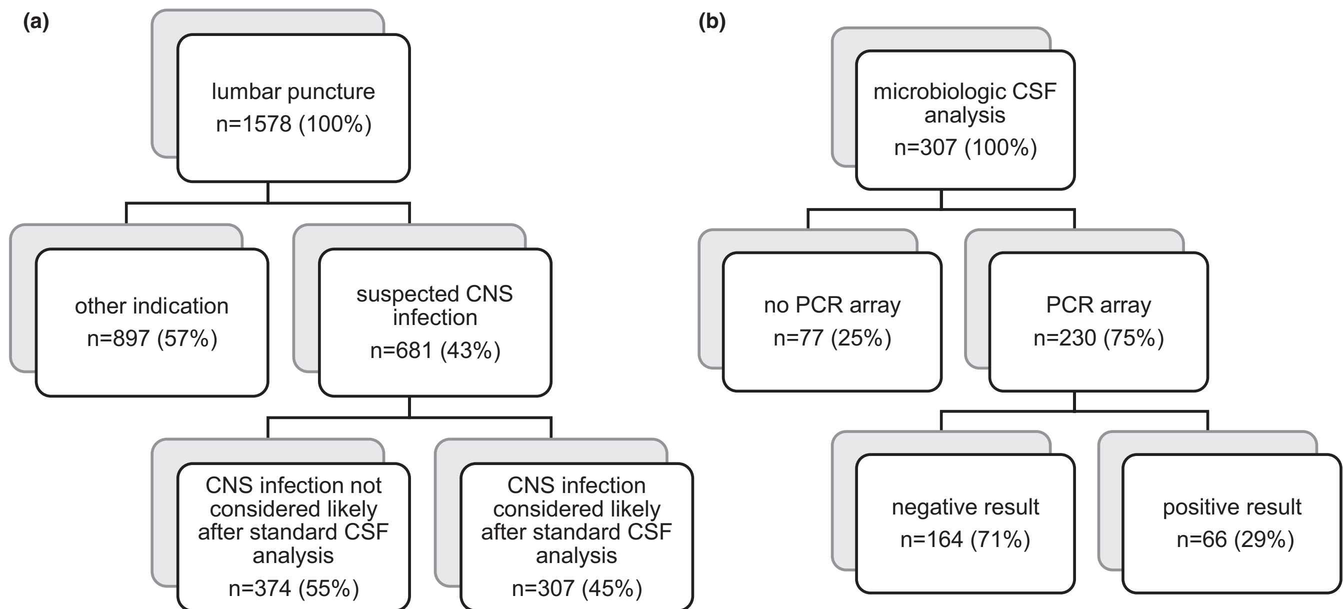

FIGURE 1 Flow chart of the study population illustrating patients who had a lumbar puncture (a) and those with microbiologic cerebrospinal fluid (CSF) analysis in addition to standard diagnostics for cell count, protein and glucose (b). Lumbar puncture was either indicated due to clinically suspected central nervous system (CNS) infection or for other reasons, such as autoimmune disease of the CNS, meningeosis, pseudotumor cerebri or subarachnoid haemorrhage (a). Based on CSF standard measures, CNS infection was either ruled out (no further CSF diagnostics) or additional microbiologic CSF analysis was performed (b). Not all samples with further CSF diagnostics were analysed by polymerase chain reaction (PCR) array.

n = 1 for *C. neoformans*. Specifically, the pathogens detected by PCR array were EV (n = 23), VZV (n = 17), *S. pneumoniae* (n = 10), HSV2 (n = 8), human herpesvirus 6 (HHV6; n = 5), *L. monocytogenes* (n = 2) and *C. neoformans* (n = 1). The standard microbiologic CSF diagnostics used in our study were: CSF culture and gram stain for *S. pneumoniae*; CSF culture and single PCR for *L. monocytogenes*; single PCR and serology for HSV and VZV; single PCR for EV and HHV6; and antigen testing for *C. neoformans*. A comparison between the PCR array and reference method was available for n = 59 samples (Table 2), which demonstrated an overall agreement of 80% (n = 47/59). The agreement was 58% for bacteria (n = 7/12), 85% for viruses (n = 39/46) and 100% for *C. neoformans*/*C. gattii* (n = 1/1). The samples that were only found to be positive by PCR array identifying *S. pneumoniae* as a causative pathogen are of particular interest and need further attention: In four of the five patients with a positive array result for *S. pneumoniae* and a negative CSF culture, treatment with antibiotics had already been started before the lumbar puncture: A 37-year-old female was referred from another hospital with acute bacterial meningitis secondary to mastoiditis and had already been treated with cefuroxime for 2 days (7863 cells/µl, protein 218 mg/dl, 34/94 CSF/serum glucose, blood culture negative, gram stain negative). A 53-year-old man with recent sinusitis and acute bacterial meningitis had received ceftriaxone from the paramedics prior to hospital admission (1064 cells/µl, protein 762 mg/dl, 10/101 CSF/serum glucose, blood culture negative, gram stain negative). A 58-year-old female was referred from another hospital, where sinusitis and acute bacterial meningitis had been treated with ampicillin/sulbactam and additionally ceftriaxone prior to transfer (667 cells/µl, protein 800 mg/dl, 10/148 CSF/serum glucose, blood culture negative, gram stain negative). A 30-year-old intubated female was referred from another hospital where mastoiditis was treated with penicillin, ampicillin/sulbactam and ceftriaxone (1983 cells/µl, protein >1000 mg/dl, 10/157 CSF/serum glucose, blood culture negative, gram stain positive). A 93-year-old female with sinusitis and acute bacterial meningitis had a negative CSF culture and a positive PCR array but had not received antibiotics before the lumbar puncture (664 cells/µl, granulocytic, protein >1000 mg/dl, 10/148 CSF/serum glucose, blood culture negative, gram stain negative).

Cerebrospinal fluid cell count and protein levels were significantly higher in patients who received CSF testing by PCR array compared to those who did not (p <0.001 and p = 0.007), and glucose levels were correspondingly lower (p = 0.014). No differences were observed for serum C-reactive protein (p = 0.606) and serum leucocyte count (p = 0.877). Also, patients whose samples revealed a positive PCR array result had significantly higher CSF cell counts (p <0.001) and protein levels (p = 0.003), and lower glucose levels (p = 0.001) than those in whom the array was negative. For positive versus negative array results, differences for serum C-reactive protein (p = 0.478) were also observed, but serum leucocyte counts were similar (p = 0.657). The likelihood of obtaining a positive PCR array result differed among the clinical syndromes that triggered the lumbar puncture; in patients with suspected meningitis 42% (n = 50/119) of PCR arrays were positive, followed by facial palsy 23% (n = 6/26), encephalitis 11% (n = 8/70) and radiculopathy 8% (n = 1/13).

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TABLE 1 List of all detected pathogens in cerebrospinal fluid samples from patients with suspected central nervous system infection

Detected pathogen		Number of positive samples
Included in the PCR array		73
Bacteria	*Listeria monocytogenes*	3
	Streptococcus pneumoniae	10
Viruses	EV	22
	EV and HHV6	1
	HHV6	4
	HSV2	9
	VZV	23
Yeast	*Cryptococcus neoformans*	1
Not included in the PCR array		29
Bacteria	*Borrelia burgdorferi*	12
	Corynebacterium kroppenstedtii and *Cutibacterium acnes*	1
	Cutibacterium acnes	1
	Staphylococcus aureus	1
	Staphylococcus capitis[a]	1
	Staphylococcus haemolyticus[a]	1
	Streptococcus anginosus, Fusobacterium nucleatum, Actinomyces species and Staphylococcus warneri	1
	Streptococcus pyogenes[a]	1
	Treponema pallidum	1
Viruses	EBV	1
	HHV7	1
	HIV	3
	HIV and *Toxoplasma gondii*	1
	TBEV	3
Total		102

Abbreviations: EBV, Epstein–Barr virus; EV, enterovirus; HHV6, human herpesvirus 6; HHV7, human herpesvirus 7; HIV, human immunodeficiency virus; HSV2, herpes simplex virus type 2; PCR, polymerase chain reaction; TBEV, tick-borne encephalitis virus; VZV, varicella zoster virus.

[a]Contamination cannot be completely excluded: for details, see text.

Polymerase chain reaction arrays in patients with suspected CNS infection revealed a negative result in 71% (*n* = 164/230). The most frequent final clinical diagnoses associated with CNS infection in these patients were viral meningitis and encephalitis (*n* = 31) and, in *n* = 5 of these, a pathogen was detected (tick-borne encephalitis virus [TBEV] *n* = 3, VZV *n* = 1, HSV2 *n* = 1). In the remaining *n* = 26 samples with negative PCR array and a final clinical diagnosis of viral meningitis or encephalitis, no pathogen was identified. Further diagnoses in patients with a negative PCR array result were systemic infections (*n* = 24), neuroborreliosis (*n* = 10; *Borrelia burgdorferi n* = 8), facial palsy (*n* = 15; VZV *n* = 1), seizures (*n* = 11) and bacterial meningitis (*n* = 4, with a pathogen detected in three of these samples: *S. capitis n* = 1, *S. pyogenes n* = 1, *Streptococcus anginosus* in combination with other bacteria *n* = 1). Further pathogens found in samples with a negative PCR array were HIV (*n* = 4, one of which was additionally infected with*Toxoplasma gondii*), Epstein–Barr virus (*n* = 1), and HHV7 (*n* = 1). Concerning HSV, there was only one sample, which was found to be exclusively positive by serology: in CSF from a 31-year-old male with conus-cauda-syndrome, standard PCR and PCR array were both negative (CSF 60 cells/µl, glucose 66 mg/dl, protein 61 mg/dl; increasing HSV antibody CSF/serum-index from 2.04 to 2.96 in a consecutive CSF analysis). Concerning VZV, one sample from a 58-year-old male with headache, ear pain and facial palsy and another from a 61-year-old male with headache and recent history of herpes zoster opthalmicus were defined as positive based on clinical presentation in combination with serology only.

Interestingly, in a significant number of patients with additional microbiologic analysis, CSF cell count was normal (*n* = 119/307). Also, in a significant number of patients, CSF was analysed by PCR array despite a normal CSF cell count (*n* = 63/230). The main clinical indications for use of the PCR array despite a normal cell count were continued suspicion for HSV encephalitis or facial palsy. From all samples analysed by PCR array despite a normal cell count, only two samples revealed a positive test result, with both testing positive for HHV6. A 97-year-old man with disorientation, headache and ear pain had a negative standard HHV6 PCR despite a positive array result and a 60-year-old man with seizures had positive HHV6 results both by PCR array and conventional PCR. In both cases the detection of HHV6 was not clinically relevant and, based on additional HHV6 positive blood samples, was most likely attributable to an inherited chromosomally integrated HHV6. Among all patients with suspected CNS infection despite normal cell count who underwent a PCR array, a relevant causative pathogen could only be detected in one: a 28-year-old patient with impaired consciousness who was found to have HIV with a significant HIV viral load in the CSF. The most frequent final diagnoses in patients with PCR array analysis despite a normal CSF cell count were non-CNS infections (*n* = 25), seizures (*n* = 10), psychiatric disorders (*n* = 4) and idiopathic facial palsy (*n* = 4; Table 3).

Further microbiologic tests but no PCR arrays were performed in 25% (*n* = 77/307) of CSF samples from patients with persistent suspicion for a CNS infection. Of the patients with further CSF diagnostics but no PCR array, 47% were male (*n* = 36/77) and their average age was 56 years (in the array group 51% [*n* = 118/230] were male and the average age was 47 years). The clinical presentation of patients with further diagnostics but no PCR array (*n* = 77) was suggestive of meningitis (*n* = 21), facial palsy (*n* = 19), encephalitis (*n* = 18), polyradiculitis (*n* = 15) or other diseases (*n* = 4). Syndromes in patients with PCR array (*n* = 230) were suggestive of meningitis (*n* = 119), encephalitis (*n* = 70), facial palsy (*n* = 26), polyradiculitis (*n* = 13) or other diseases (*n* = 2). In patients with further microbiologic testing but no PCR array, a CNS infection was found in 23%

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TABLE 2 List of pathogens covered by the PCR array and compared to standard diagnostics as reference

Detected pathogens	Number of positive samples (array or standard method)	PCR array positive	Standard method positive	Agree-ment of positive results	PCR array sensitivity TP/(TP + FN)	PCR array specificity TN/(TN + FP)
Bacteria						
Listeria monocytogenes	2	2	1	1	1/1 (100%)	101/102 (99%)
Streptococcus pneumoniae	10	10	6	6	6/6 (100%)	92/96 (96%)
Viruses						
EV	15	15	14	14	14/14 (100%)	19/20 (95%)
HSV2	9*	8	8	7	7/8 (88%)	201/202 (100%)
HHV6	5	5	4	4	4/4 (100%)	10/11 (91%)
VZV	17*	15	16	14	14/16 (88%)	191/192 (99%)
Yeast						
Cryptococcus neoformans	1	1	1	1	1/1 (100%)	9/9 (100%)
Total	59	56	50	47	47/50 (94%)	623/632 (99%)

Note: Only results of samples analysed both by PCR array and at least one standard method are shown (for details, see text). Positivity for standard method is defined by one or more positive standard method results. "FP" results are defined as "only array positive". One sample was positive both for EV and HHV6 by PCR array. *For HSV2 in one patient and for VZV in two patients, diagnosis was based on clinical presentation in combination with positive serology.

Abbreviations: EV, enterovirus; FN, false negative; FP, false positive; HHV6, human herpesvirus 6; HSV, herpes simplex virus; TN, true negative; TP, true positive; VZV, varicella zoster virus.

($n = 18/77$). Infectious diagnoses were neuroborreliosis ($n = 4$), infectious facial palsy ($n = 4$), bacterial meningitis ($n = 3$), viral meningitis ($n = 2$), shunt infection ($n = 2$), herpes zoster ($n = 2$) and neurosyphilis ($n = 1$). Detected pathogens in the patients with bacterial meningitis were *S. haemolyticus* ($n = 1$) and *L. monocytogenes* ($n = 1$). In one patient post neurosurgery (due to glioblastoma), the pathogen could not be detected but CSF variables (14,839 cells/μl) and the clinical course made a bacterial meningitis diagnosis very likely. The most frequent diagnoses without CNS infection in the remaining $n = 59$ patients were other infections ($n = 14$), idiopathic facial palsy ($n = 11$), ischaemic stroke ($n = 3$), seizures ($n = 3$), neuralgic shoulder amyotrophy ($n = 2$), Miller Fisher syndrome ($n = 2$) and headache ($n = 2$). In $n = 4$ patients, neuroborreliosis was ruled out but a final diagnosis could not be established.

DISCUSSION

Our data show that the use of the PCR array in the CSF of emergency patients with suspected CNS infection seems helpful in the diagnostic workflow of an emergency department. The majority of CNS pathogens in our study population were covered by the array. However, a significant number of relevant pathogens (e.g., *Borrelia* species, TBEV and several bacteria) were not included in the array. Application of the array was most promising in samples from patients with CSF pleocytosis. In patients with acute bacterial meningitis who had been treated with empiric antibiotics, the PCR array was able to identify pathogens that would have otherwise remained undetected. In patients without pleocytosis, the PCR array did not help to detect any clinically relevant pathogen in our patients.

As mentioned above, in our study population, the majority of detected CNS pathogens were covered by the PCR array. This is important because limitations of the array in regions with high prevalence of microorganisms that are not covered by the array are reported [6]. In general, data on clinical performance of the array in comparable settings are limited and, in contrast to our data, many of the available studies in patients with suspected CNS infection did not report on pathogens that were not covered by the array. There is one study with prospectively collected CSF specimens ($n = 1560$) from 11 centers that also checked for bacteria detected by culture that were not targeted by the FilmArray® (*Staphylococcus epidermidis*, *Salmonella* sp. isolate, *Propionibacterium/Cutibacterium* sp. isolates and *Nocardia* sp. isolates), but the authors did not define specific clinical inclusion criteria [2]. A monocentric study using a similar multiplex array system (LightMix®) in a Swiss cohort of patients with symptoms of meningitis ($n = 220$) also reported on pathogens that were not targeted by their array, namely, *E. coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, *S. epidermidis* and *Staphylococcus hominis* [7], and in a small cohort of patients in the United States with community-acquired meningitis ($n = 48$ samples with CSF pleocytosis and negative CSF gram stain), 15% ($n = 5$) of pathogens such as West Nile virus and Histoplasma were only identified by standard techniques [8]. In our study, important pathogens not covered by the array were *Borrelia burgdorferi*, TBEV, HIV and some bacteria. *Borrelia* species and TBEV are not commonly tested by conventional PCR in clinical routine as sensitivities of PCR are low in these

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TABLE 3 List of all clinical final diagnoses in patients who had a PCR array of their cerebrospinal fluid (CSF) despite a normal CSF cell count and numbers of positive array results

Diagnosis	Number of patients	Number of positive PCR array results
Non-CNS infection	25	1
Systemic infection	13	1
Pneumonia	3	0
Herpes zoster oticus	2	0
Pharyngitis	1	0
Tonsillitis	2	0
Urinary tract infection	1	0
Cytomegalovirus infection	1	0
Endocarditis	1	0
Leptospirosis	1	0
Seizure	10	1
Psychiatric disorder	4	0
Psychosis	2	0
Adjustment disorder	1	0
Depression	1	0
Idiopathic facial palsy	4	0
Impaired consciousness of unknown aetiology	4	0
Malignancy	3	0
Glioblastoma	1	0
Oligodendroglioma	1	0
Cerebral tumour not further specified	1	0
Vascular disease	3	0
Cerebral ischaemia	2	0
Cerebral haemorrhage of a prolactinoma	1	0
Others	10	0
Transient neurological deficit not further specified	2	0
Dementia	1	0
Posterior reversible encephalopathy syndrome	1	0
HIV encephalopathy	1	0
Serotonergic syndrome	1	0
Brain stem lesion of unknown aetiology	1	0
Guillain--Barré syndrome	1	0
Gamma-hydroxybutyrate intoxication	1	0
Monoclonal gammopathy	1	0
Total	63	2

Note: Both samples that were positive by PCR array were positive for clinically not relevant human herpesvirus 6.

Abbreviations: CNS, central nervous system; HIV, human immunodeficiency virus; PCR, polymerase chain reaction.

pathogens. For *Streptococcus* and *Staphylococcus* species, however, it would be desirable to broaden the multiplex PCR for these clinically relevant targets. In some patients in our study cohort with a clinical diagnosis of viral meningitis, no pathogen was detectable by all applied methods.

In a recent study from Lindström et al. [9] 4199 CSF samples were consecutively tested by the PCR array, thereby detecting 315 pathogens in 309 CSF samples from 303 patients. Thirty-four viral targets were identified by the panel that could not be confirmed with routine diagnostics and 21 viral targets were identified using standard PCR but not the array. That study was the largest conducted to date on the PCR array including clinical correlation of discrepant results. However, the analysis was focused on viral targets and there were no clinical inclusion criteria as used in our study. All CSF samples sent for viral diagnostics to the microbiological laboratory over 34 months were analysed both by PCR array and standard PCR for HSV1, HSV2, VZV and EVs. Interestingly, in cases where the array failed to detect EV, viral load was significantly lower [9].

In our study population, the sensitivity and specificity of the array compared to standard methods were high. The good performance of the array in comparison to other methods is in accordance with previous data on asservated standardized or preselected positive samples [10, 11]. For example, the multicenter prospective study from Leber et al. mentioned above, compared array results to standard PCR or culture, finding a 100% positive agreement rate and a 99% negative agreement rate for most pathogens [2]. Another study with n = 291 samples that tested positive in routine diagnostics found a positive agreement rate of 98% (78/80) for bacterial pathogens, 90% (145/161) for viruses and 52% (26/50) for *C. neoformans/gattii* [3]. Recent meta-analyses also calculated high overall diagnostic accuracy of the PCR array, with both sensitivity and specificity of 90% or above [4, 5]. Concerns about false-negative and false-positive results are primarily raised for HSV1 and HSV2 [12, 13], which was also an issue in our study as we found one sample to be positive for HSV2 only by serology (array false-negative) and another sample to be only positive for HSV2 by PCR array (standard PCR and serology false-negative). For VZV we found two samples to be positive only by serology and one sample positive only by PCR array. Therefore, we agree that, particularly for HSV and VZV, it could be helpful to perform the PCR array in combination with single PCR and serology [14]. In our study cohort, we did not observe the high rate of false-negative results reported for EV. The highest proportion of so-called false-positive results is described in the literature for *S. pneumoniae*, followed by *S. agalactiae* [4]. Here, we instead saw a particular advantage of the array in patients with meningitis under empiric antibiotic treatment as it allowed the identification of clinically relevant bacteria despite negative results in conventional tests. This suggests that the so-called false-positive results from previous studies might simply be "true positives". The explanation could be that the conventional standard methods might not have been effective because vital pneumococci were simply no longer present after antibiotic challenge, whereas remnants of the bacterial genome could still be detected by PCR. This interpretation of the data is in line with the

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detection of single positive array results in culture-negative samples of other reports [7, 15–17]. Thus, the array might indeed show a benefit in patients with likely bacterial meningitis according to CSF cell count, CSF protein, and CSF/serum glucose ratio despite negative gram stain and culture results as is often the case in antibiotically pre-treated patients [18, 19].

In our patients, the array was not helpful in detecting pathogens in samples with normal CSF cell count. We suggest that a pre-selection for CSF cell count and clinical presentation needs to be taken into consideration before the array is used. Studies with such a pre-selection (e.g., positive gram stain, detection of leukocytes and/or bacteria, urgent clinical suspicion) show relatively high detection rates of pathogens in comparison to other studies [20]. Use of the array in patients with no suspicion for a CNS infection or in samples with normal CSF cell count could be avoided [21, 22]. Exceptions might be CSF samples from patients with suspected sterile acute bacterial meningitis (in such cases, the CSF/serum glucose ratio is usually low) or patients with suspected early HSV encephalitis (as studies showed that 20% of patients with HSV encephalitis present with an initially normocytic CSF [23]).

A retrospective study in Colombia indicated that implementation of a PCR array reduced time for therapy changes, but had no impact on length of stay or outcome [24]. One promising prospective observational study of 130 patients with suspected meningitis and/or encephalitis and CSF cell count >5 cells/μl also showed that the application of the array could reduce time to microbiological diagnosis and discontinuation of empiric anti-infective drugs, but also resulted in earlier hospital discharge and reduction of treatment costs [25]. Another prospective study in a tertiary paediatric hospital randomized children with possible CNS infection and CSF pleocytosis over a period of 1 year to PCR array or separate molecular CSF microbiological tests, and showed that the use of the array significantly reduced antimicrobials, duration of hospitalization and costs [26]. In our study, we saw early discharges for patients with positive EV array results (in our study n = 17/23 within 1 day and n = 20/23 within 2 days), but our study was not set up to evaluate the impact of the PCR array on length of stay or duration of antibiotic/antiviral treatment.

This study has further limitations. Although we were able to prospectively analyse a large cohort of patients in the routine of an emergency setting, data were acquired from a single center and absolute numbers of samples of certain (rare) disease entities were low. Thus, subgroup analysis was not always possible. Additionally, the diagnostic workflow was not standardized and some patients were not tested by PCR array despite CSF pleocytosis. Likewise, we did not perform parallel diagnostic measurements using all methods available in all samples. In particular, some samples were only tested using one standard method or revealed different results between two standard methods. Numbers were low for direct comparisons of the array and references; thus, reliable statements on sensitivity and specificity of the array from a methodical point of view are not possible. Unfortunately, the setup of the study did not allow a standardized evaluation of a possible overall benefit (e.g., length of anti-infective therapy or length of stay) if PCR array was performed. Owing to clinical decisions, not all samples were analysed both by the conventional method and the PCR array but the majority of the overall identified pathogens were included in the spectrum of the PCR array (74%, $n = 75$ pathogens in $n = 74$ samples were positive by PCR array, thereof one sample was positive both for EV and HHV6).

In conclusion, the use of the PCR array appears to be advantageous in patients with suspected bacterial meningitis under empiric antibiotic treatment as cultures might already be found negative under these circumstances. Our data do not support a general application of the multiplex array in samples with normal CSF cell count in the emergency setting. We suggest use of the PCR array as a fast, additional method to conventional testing in time-critical patients with clinically suspected meningitis/encephalitis, taking standard CSF variables into consideration before testing is performed.

AUTHOR CONTRIBUTIONS

All authors either made substantial contributions to the conception or design of the work, the acquisition, analysis or interpretation of data, drafted the work, or revised it critically for intellectual content.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest regarding the publication of this article.

DATA AVAILABILITY STATEMENT

Original data is available from the authors on reasonable request.

ETHICS APPROVAL

The study was approved by the Ethics Committee of the University of Munich (project number 12–409) and is in accordance with the Declaration of Helsinki. The manuscript does not contain personal information on individual patients and therefore informed patient consent was not obtained.

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