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ORIGINAL PAPER

Clinical benefits of introducing real-time multiplex PCR for cerebrospinal fluid as routine diagnostic at a tertiary care pediatric center

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Abstract

Background Sepsis-like illness with suspected meningitis or encephalitis is a common reason for using empiric antimicrobial therapy in infants and children. However, in cases of viral meningitis not covered by these antimicrobials, this management is ineffective and due to side effects potentially harmful.

Methods A retrospective analysis of cerebrospinal fluid (CSF) multiplex PCRs (Biofire FilmArray®) in children with clinical suspicion of meningitis, encephalitis or sepsis-like illness was performed over the period of 1 year. Subsequently, a subgroup of children (age of 8–84 days of life) diagnosed with viral meningitis (enterovirus, HHV-6, human parechovirus) was compared to an age-matched control group.

Results During the study period, the multiplex PCR panel was performed on 187 individual CSF samples that met the inclusion criteria. About half of the patients (92/187) were less than 1 year of age. In 27 cases (14.4%), the PCR yielded a positive result with the majority (12/27) being indicative of an enteroviral infection. In the age group of 8–84 days of life, 36.4% of the patients had a positive result. When the patients with a PCR positive for a viral agent were compared to an age-matched group of patients, no differences were observed regarding symptoms and laboratory parameters. However, the duration of antimicrobial therapy could be significantly reduced through the use of multiplex PCR.

Conclusion The use of on-site diagnostic multiplex PCR was able to reduce the use of antimicrobials in selected cases. This test can guide clinical decisions earlier during the course of medical care compared to standard diagnostics.

Keywords Antimicrobial stewardship · Point-of-care test · FilmArray · Meningitis · Encephalitis

Introduction

Lumbar puncture (LP) is a routinely performed diagnostic test in the pediatric clinical setting. Usually the examination is performed to test for infectious diseases (meningitis, meningoencephalitis, encephalitis), but also for a variety of other entities, e.g., autoimmune or metabolic disease. In the pediatric emergency room setting fever, seizures (febrile, non-febrile) and altered mental status are the most common symptoms leading to lumbar puncture.

The incidence of meningitis is higher in children below the age of 5 years with the highest proportion of cases being found in children below the age of 6 months [1, 2]. In many hospitals or outpatient clinics, infants with fever, especially within the first 3 months of life—having a higher proportion of bacterial infection—will receive a thorough work-up including urine analysis, blood culture and lumbar puncture, if no other primary site of infection can be identified [1]. Obvious clinical signs, such as a bulging fontanel, nuchal rigidity and convulsions, are often only present in the later course of disease and are associated with poor outcome [3]. Often, and especially in small infants, empiric antibiotic and/or antiviral therapy is initiated without appropriate diagnostics or awaiting the results from the microbiology laboratory. This might lead to side effects of the medication, e.g., ototoxicity and

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antibiotic-associated diarrhea [4] and often results in a considerable amount of stress for the patients and their parents. Moreover, the change in the child's microbiome, especially in little children, may have longer lasting negative effects (e.g., increase in allergic diseases, obesity, and neurocognitive changes [5–7]). Eventually, over use of antibiotics has been shown to lead to an increase of multidrug resistant bacteria.

New strategies need to be implemented that help physicians with the initial therapeutic decisions, possibly sparing children from unnecessary antiinfective treatment. One strategy is the multiplex PCR test which—with pre-manufactured kits—facilitate the rapid identification of a variety of infectious agents. This might also lead to faster identification of highly aggressive disease-causing agents, e.g., HSV and *Streptococcus pneumonia* [8]. Here we present our experience after 1 year of usage of novel multiplex PCR in the routine clinical setting of suspected central nervous system (CNS) infection. The aim of the study was to assess changed diagnostic and therapeutic procedures and evaluate the consequences of the introduction of Film Array® multiplex PCR. In addition, we wanted to identify the most effective way to use this new technique in the routine patient management.

Materials and methods

Study population

A retrospective analysis of the lumbar puncture multiplex PCR diagnostics in a single tertiary care center for pediatrics was performed between 27th of June 2016 and 26th of June 2017. Children who underwent spinal tap for unclear primary site of infection or for suspected meningitis or meningoencephalitis were included in the analysis. The identification of patients and the performance of the test were at the discretion of the responsible physician. As there were no strict primary symptoms defined, the testing was performed in presence of a variety of symptoms (fever without focus, febrile and non-febrile seizures, circumscribed neurological deficits, sepsis-like illness). Only patients below 19 years and only the first lumbar puncture during a course of disease were included in the analysis.

Additional information was obtained from the medical records including age, preexisting conditions, symptoms at presentation, laboratory results (blood, CSF), antimicrobial therapy, length of hospital stay and reasoning for therapeutic decisions. The CSF samples were analyzed for glucose, protein, cell count and differentiation. Blood cultures and CSF cultures were performed using standard microbiological procedures [9].

Multiplex PCR

Multiplex PCR on all samples was performed according to the manufacturer's protocol (BioFire; FilmArray® Meningitis/Encephalitis (ME) PCR Panel) [8]. The CSF panel was used to detect 14 pathogens: CMV, enterovirus, HSV 1/2, HHV-6, human parechovirus, VZV, *Cryptococcus neoformans/gattii, Escherichia coli* K1, *Haemophilus influenzae, Listeria monocytogenes, Neisseria meningitidis, Streptococcus agalactiae* (GBS) and *Streptococcus pneumoniae*. The results were available approximately 75 min after the start of the assay in the bacterial laboratory. The transport of the samples from the clinical wards to the laboratory usually took about 15 min. Testing was available during the opening hours of our on-site bacteriological laboratory (Monday to Friday 8 a.m. to 4 p.m. and on weekends 10 a.m. to 12 a.m.).

Statistical analysis

Statistical analysis was performed using Prism5 5.0a for Mac OS-X. Chi square test was applied to assess statistically significant differences in qualitative variables (gender, fever, symptoms and seasons); student's *t* tests and one-way ANOVA were used for quantitative variables (age; laboratory parameters, duration of therapy). In all the analyses, $p < 0.05$ (*) was considered statistically significant. The data were expressed as the mean ± the standard deviation.

Results

During the 12-month study period, a total of 187 lumbar punctures were performed that met the inclusion criteria. Three children had two spinal taps on separate occasions; 2, 3 and 5 months apart. Two of these children had preexisting hemato-oncological conditions, while one child had no underlying preexisting disease.

Of these 187 lumbar punctures, 92 were performed in infants, while the remaining 95 were done in older children. 70% of all lumbar punctures were performed in children up to the age of 5 years (Online Resource Fig. S1A). Most infants tested were at the age group up to three months, comprising approximately 80% of all infants (Online Resource Fig. S1B).

The majority of children had no preexisting conditions: 77% in the infant group and 69% in the older children (Online Resource Fig. S1C). In children above the age of 1 year the most common preexisting conditions were oncological (15%) and neurological diseases (8%), while in younger children premature birth (10%) and metabolic

diseases (4%) were predominant. In both groups one child had two preexisting conditions (syndromal and oncological disease in one and premature birth and oncological disease in the other patient).

However, the main reason for lumbar puncture differed between the two groups. While most infants had a spinal tap due to fever without focus (53%) or suspicion of early-onset sepsis (29%), older children showed a variety of symptoms: focal neurological deficit (e.g., 26% with facial nerve palsy), febrile seizure (24%), fever without focus or suspected meningitis/meningoencephalitis (23%) and non-febrile seizures (9%) (Online Resource Fig. S1D).

Of the 187 cerebrospinal fluids tested, only 27 (14.4%) yielded a positive result by multiplex PCR. A virus was identified in the majority (88.9%) of the 27 positive PCR results: twelve cases of enterovirus, ten cases of HHV-6 and two cases of human parechovirus. We subdivided the children into four age groups: in the oldest group (1–18 years of life) 6.3% had a positive PCR result; in the first week of life (day 0 to day 7) zero of the 30 patients had an infective agent identified by PCR. The highest number of positive PCR results was seen in patients at the age of 8–84 days (equivalent to 12 weeks) with 36.4% (Fig. 1a). 27.8% of infants in between 13th to 52th weeks of life had a positive result.

In the age group of 1–18 years of life, one HHV-6 positive PCR result and in the group of the infants of 13th to 52th weeks, three HHV-6 positive PCR results were—due to accompanying clinical, morphological and laboratory findings—considered not to be disease causing. This resulted in lower percentage of clinically relevant multiplex PCR results: 5.3% (age 1–18 years) and 11.1% (13–52 weeks). In the primary diagnostic work-up, three of these children were diagnosed with bacterial disease which were not detected in the CSF (culture and multiplex PCR): one infant had bacterial bilateral otitis media (28 weeks of age), one infant (49 weeks of age) had sepsis caused by *Streptococcus pneumoniae* (blood culture positive, CSF PCR negative and CSF culture negative) and one child (14 months of age) had pyelonephritis caused by *Escherichia coli*. One infant (10 months of life) presented with a severely altered mental status and no accompanying exanthema or fever. The consecutive work-up led to the suspicion of an abusive head trauma, and therefore the role of the identified HHV-6 in the LP remains unclear.

In the remaining three multiplex PCR positive cases (11.1%), bacteria were identified: *Streptococcus pneumoniae* ($n=2$; 11 months and 1.4 years) and *Escherichia coli* K1 ($n=1$; one month) (Fig. 1b). All of these were considered disease causing and the CSF multiplex-PCR results were subsequently confirmed by bacterial culture results. Additionally, during the time period evaluated, one case of systemic neonatal listeria infection (CSF day 1 of life) with intracranial abscess was neither detected by CSF multiplex-PCR nor by CSF culture, but only by the simultaneously performed blood culture. In addition, one case of high clinical suspicion of GBS sepsis was neither identified by PCR nor by bacterial cultures from the CSF and the blood, but only through the bacterial swab cultures (nasal, throat, ear canal, perianal swab and gastric acid culture: all GBS positive). No fungal infection was identified during the study period.

In a next step we further looked into the group of infants between day 8 and 84 of life ($n=44$). This group did not only have the highest yield of positive PCR results, they also were the subgroup of children in which most lumbar punctures were performed. They also were the group who posed the biggest dilemma for the clinicians, as most of these children presented with fever without focus in the emergency room. For a more detailed analysis, we compared patients with positive viral results in the multiplex-PCR (virus PCR positive group total of 15: 10 with enteroviral infection, 3 with HHV-6 infection; 2 with parechovirus infection) with a subgroup of patients with similar clinical and laboratory results ($n=8$; control group) but without detection of pathogens. All the following results correspond to the comparison of these two groups.

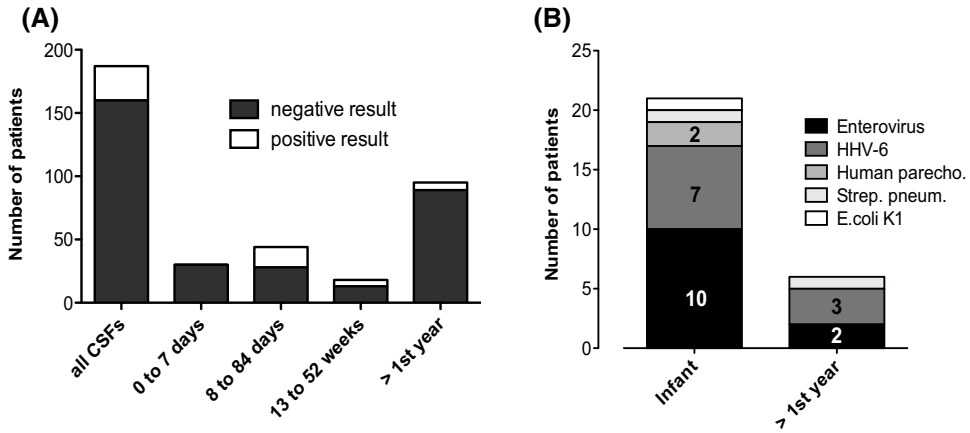

Fig. 1 CSF multiplex PCR results. **a** Positive (white) and negative (dark gray) results sorted by different age groups. **b** Different isolated infectious agents in infants (left) and children above the age of 1 year (right): enterovirus (black; *n* 12), HHV-6 (dark gray; *n* 10), human parechovirus (gray; *n* 2), *Streptococcus pneumoniae* (light gray; *n* 2), *Escherichia coli* K1 (white; *n* 1)

These two groups, did not significantly differ regarding gender, age (median control group 47.5 years; median virus PCR positive group 37.0 years) or fever at presentation (Table 1).

Other symptoms, which were routinely mentioned in the medical history—floppiness (less activity than usual, sucking weakness), agitation (inquietude, high-pitched screaming), gastrointestinal symptoms (diarrhea, vomiting, foul-smelling stools) and history of acute illness in the patient's siblings—showed no differences between the control group and the virus PCR positive group (Table 1). All patients with enteroviral and with human parechoviral meningitis presented during warmer months (May–September), all HHV-6 patients presented during winter (December–February), while the control group was scattered throughout the year (Chi square test of enteroviral positive patients and control group: $p < 0.01$).

Regarding laboratory values (Table 2), patients in the subgroups showed high-normal or only slightly elevated inflammatory markers (C-reactive protein up to mean of 1.2 mg/ml, interleukin-6 up to mean of 138 pg/ml, leukocytes up to mean of 12 667/µl). The liver values (e.g., GOT) were normal and were only mildly elelvated in one patient with enterovirus positive PCR (GOT 162 U/l). Patients with enterovirus detected in the cerebrospinal fluid showed elevated cell counts (179 ± 213/µl) and protein (108.0 ± 159.4 mg/

Table 1 Subgroup analysis of patient features and symptoms

	Control group	Virus PCR positive group	*p* value
Age at primary presentation in (days)	48.0 ± 21.5	38.1 ± 20.5	0.29
Male gender in (%)	50.0%	60.0%	0.65
Fever at primary presentation in (%)	87.5%	93.3%	0.64
Agitation at primary presentation in (%)	25.0%	46.7%	0.40
Floppiness at primary presentation in (%)	87.5%	60.0%	0.35
Gastrointestinal symptoms at primary presentation in (%)	25.0%	40.0%	0.66
Sick sibling in patient's history in (%)	12.5%	46.7%	0.18

When indicated the mean ± standard deviation was given. The *p* values were calculated with Fisher's exact test for the dichotomous measures. For the statistic evaluation of "days of life" a *t* test was performed. None of the compared parameters reached a statistically significant level, that is none of them was $p \leq 0.05$

Table 2 Laboratory values (blood, CSF) of the analyzed patients (enterovirus, HHV-6, human parechovirus and comparison group) in the age group 8–84 days of life

Evaluated laboratory value	Norm	Control group	Virus PCR positive group	Enterovirus	HHV-6	Human parechovirus	*p* value
CrP in (mg/dl)	≤ 0.5 mg/dl	0.86 ± 1.03	0.89 ± 0.88	0.91 ± 0.95	1.21 ± 0.90	0.36 ± 0.33	ns
Interleukin 6/IL-6 in (pg/ml)	≤ 15 pg/ml	53.5 ± 29.2[a]	58.7 ± 68.6[b]	46.0 ± 36.0[c]	138 ± 172.5	36.6 ± 37.6	ns
GOT in (U/l)	≤ 77 U/l	35 ± 9[a]	44 ± 38[d]	49 ± 46[e]	33 ± 13[f]	40 ± 5	ns
Leukocytes in [/µl]	age-dependent	7888 ± 3835	9070 ± 4684	9135 ± 3567	12,667 ± 6643	3350 ± 354	ns
Amount of cells (CSF) in (/µl)		36 ± 87[g]	118 ± 188[b]	179 ± 213[a]	5 ± 2	10 ± 4	ns
Protein amount (CSF) in (mg/dl)	≤ 50 mg/dl	55.6 ± 45.3[g]	85.4 ± 129.1[h]	108.0 ± 159.4[a]	42.0 ± 19.0	49.0[i]	ns

All values given as mean ± SD

The statistic evaluation was performed with *t* tests; *p* values above 0.05 were considered not significant (ns)

[a]Only measured in 7/8 patients

[b]Only measured in 14/15 patients

[c]Only measured in 9/10 patients

[d]Only measured in 12/15 patients

[e]Only measured in 8/10 patients

[f]Only measured in 2/3 patients

[g]Only measured in 6/8 patients

[h]Only measured in 13/15 patients

[i]Only measured in 1/2 patients

dl). However, this did not reach a statistical significance. Taken together, none of the routinely performed medical history and laboratory values showed a statistically significant difference between the control group and the virus PCR positive group.

Most patients at the age of 8–84 days were started on an empiric antimicrobial therapy consisting of antibiotics and/or aciclovir. Of the 23 patients in our subgroup, 20 were started on an antibiotic combination therapy consisting of ampicillin and cefotaxime (three times daily), one patient was started on ceftriaxone (once daily; enterovirus group); two patients received no antibiotics at all (1 with enterovirus, 1 with HHV-6). 17 of the 23 patients received aciclovir (three times daily); 6 in the control group and 11 in the virus PCR positive group. Five of the six patients that did not receive aciclovir had their lumbar puncture performed during the working hours of the in-house bacteriological laboratory and therefor the clinician in charge received the multiplex PCR result (including HSV-PCR) within about 90 min and was able to avoid empirical treatment.

Patients of the control group, which had negative CSF multiplex PCR results, were treated with antibiotics for 4.4 ± 2.3 days, while patients with a virus positive multiplex PCR (HHV-6, enterovirus, human parechovirus) were only treated for 1.3 ± 0.9 days (t test; $p \leq 0.001$; Fig. 2a). In a separate analysis for the different viruses, the two subgroups with the detection of enterovirus or HHV-6 in the CSF received significantly less antibiotics than the control group.

To analyze if clinicians received the multiplex PCR results in a comparable amount of time in the different groups, we compared the duration of aciclovir treatment, as this was usually stopped, right after getting the HSV-PCR result (with the whole multiplex PCR panel). The amount of days patients were treated was clearly comparable (t test; $p = 0.96$), with 0.5 ± 0.5 days for patients with negative multiplex PCR results and 0.5 ± 0.5 for patients with positive multiplex PCR results (Fig. 2b).

The control group stayed in hospital for 5.1 ± 2.5 days and we saw no statistically significant difference (t test; $p = 0.66$)

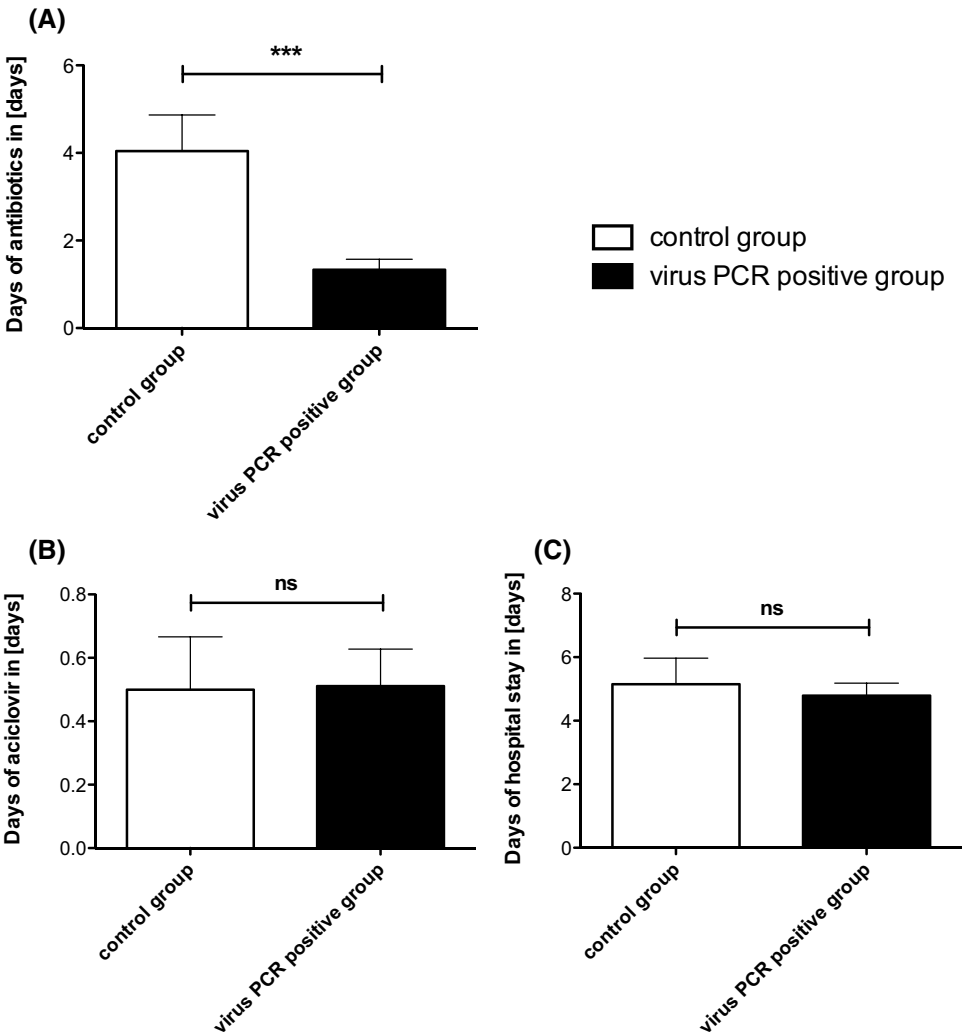

Fig. 2 Treatment of different groups (control group white; virus positive PCR group black; $p^{***} \leq 0.001$; $p^{ns} > 0.05$). **a** Days of treatment with antibiotics. **b** Days of treatment with aciclovir. **c** Days of hospital stay. All values were given as mean ± standard deviation

to the virus PCR positive group with 4.8 ± 1.6 days (Fig. 2c). The infants remained in the hospital either for on-going antibiotic therapy (mostly in the control groups) or for intravenous hydration and recurring febrile episodes.

Discussion

Here we describe our experience after the introduction of a multiplex PCR cerebrospinal fluid panel in the routine clinical setting of a tertiary pediatric hospital. We could show that—even with our relatively small number of patients—after a year we could significantly reduce the usage of antibiotics in infants at the age of 2–12 weeks. The most commonly detected infectious agents were enterovirus, human parechovirus and HHV-6, as expected from the literature [10]. As shown before [11], with routine diagnostic measures the responsible physicians could not reliably differentiate between infants with a viral infection and infants with bacterial infections or inflammatory symptoms without a pathogen.

The highest number of positive PCR results was found in a subgroup of infants (day 8–84 of life). These patients typically present with fever and or change in the behavior in the emergency room and are often difficult to assess for the clinicians. Reliable biomarkers to safely identify the children with bacterial infection are lacking [12]. Especially in young infants, first clinical symptoms of meningitis are not very specific and medical history usually does not offer a lot of help. In this age group we could demonstrate, that the amount of antibiotic usage could be significantly reduced in the group of patients with the detection of a viral cause of the disease and no signs of concomitant bacterial disease. The constant overuse of antibiotics has led to the rise of multidrug resistant bacteria and in addition, antibiotics have also well-known side effects in the individual patient (e.g., antibiotic-associated diarrhea, ototoxicity, allergic reactions). Other sequelae probably triggered by the changes in the young microbiome are only being discovered lately [13]. After the repeated administration of antibiotics before the age of 2 years, the risk of developing early childhood obesity was elevated in a cohort of 1306 children [14]. Moreover, there seems to be an association to asthma, atopic dermatitis and allergic rhinitis in children [5].

The multiplex-PCR allowed us identifying infectious agents previously not tested on a regular basis. Before using the multiplex-PCR in our clinical setting, HSV-PCR was routinely performed for infants undergoing lumbar puncture and a clinical picture compatible with this disease. However, in our specific setting of a University Hospital with an Institute for Microbiology located in a separate building complex (i.e., transport, laboratory turnaround time, reporting, no weekend routine laboratory working hours) for virology it usually took between 2 and 5 days to receive results. With the FilmArray® available on-site we were able to stop aciclovir treatment faster in comparison to the historical cohort (data not shown). Additional viral PCRs were only rarely ordered when it seemed indicated; e.g., enterovirus PCR in patients with high CSF cell counts and suspicion of non-bacterial disease, HHV-6 PCR in post-bone marrow-transplant patients. Therefore, the introduction of a new on-site technique allowed us to test a variety of pathogens at once, but also to significantly reduce the turnaround time. Even hospitals with preexisting on-site CSF PCR testing, could see a significant improvement of the diagnostic time management after the introduction of CSF multiplex PCR [15].

The largest number of patients was diagnosed with enteroviral meningitis. For enteroviral cases, as described before, higher cell counts were seen in the CSF [16] and cases exclusively occurred in summer and early autumn [17]; however, this did not reach a statistical significant level in the analysis of cases. In patients with a marked elevation of CSF leucocyte count and/or a high proportion of mononuclear cells, a specific enterovirus PCR may be sufficient to reach the same result as the multiplex PCR. However, usually clinicians did not decide to discontinue antibiotics based upon the slightly elevated CSF cell counts and the presentation of cases within the summer months in our cohort, but only stopped antibiotics when the results of the multiplex PCR were back. A positive PCR result for enterovirus, HHV-6 or parechovirus—together with supporting clinical signs and laboratory results—was considered sufficient to withhold antibiotics and aciclovir leading to the above-mentioned reduction in antimicrobial therapy. Since enterovirus meningitis is by itself a rather serious disease, overall hospital stay and its associated costs were not reduced since these infants required supervision, intravenous infusion, and monitoring. As some enteroviral infections are associated with severe acute complication, e.g., brain stem encephalitis, and neurological sequelae [18], a special follow-up for these patients might be beneficial. In a selected subgroup of patient therapy with immunoglobulins might be warranted [19].

Because we detected parechovirus in only two patients, no association with clinical or laboratory signs could be detected due to the small numbers. In human parechovirus infections seizures seem to be quite common [20] and white matter lesions leading to long-lasting damage can develop [21]. A specific antiviral therapy is not available and therefore antibiotics and aciclovir can be discontinued once this pathogen is detected. In severe cases, i.v. immunoglobulin has been used with success [22].

While we have found HHV-6 in ten patients, the clinical significance of these results is somewhat unclear [23]. HHV-6 is a common infectious agent in childhood and leads to a variety of symptoms, mostly leading to fever and exanthema subitum, with encephalitis and meningitis being less

likely [24]. HHV-6 has also been demonstrated to be chromosomally integrated and inherited [25]. The significance of identification of HHV-6 in healthy children is unclear, as it seems to be able to persist in CSF without leading to any symptoms [26]. In our patient group, as also seen in previously published data [27], HHV-6 has been detected in the CSF, while there was another infection identified—in our case three concurrent bacterial infections. None of these cases was detected in our analyzed subgroup of day 8–84 of life. Still the patients with sepsis-like illness have to be thoroughly worked-up and diagnostics and clinical reevaluations in the patients have to be continued, even if a pathogen has been identified, in order not to miss coinciding bacterial infection or false-negative cases. Especially in HSV infections, detectability of the virus in the CSF may be delayed. Therefor, if there is suspicion of HSV infection, aciclovir therapy should be started immediately and continued until it has been definitively excluded (e.g., by repeating lumbar puncture).

In comparison to other centers [8], using the same multiplex-PCR panel, we did not find any group *B Streptococcus* and only one case of *Escherichia coli* infection. This is probably also due to the fact that in our neonatal unit, we have in general a low rate of invasive infections. Moreover, some neonates did not get lumbar punctures performed before starting antibiotic therapy due to their unstable condition. One patient was clinically diagnosed with GBS sepsis, but we were not able to identify GBS by CSF PCR or CSF culture or by blood culture. In addition, during the studied period, we identified a child with invasive CNS listeriosis, although, microbiological testing was not positive in CNS culture or CNS multiplex-PCR. However, in this patient a blood culture was positive for listeria and there was subsequent abscess formation seen in the brain. In studies comparing culture with multiplex-PCR, the specificity was usually high (up to 100%), but sensitivity was as low as 50% [28]. This was mostly a problem in patients with a low load of the infectious agent. As the multiplex PCR test covers several different pathogens, the range of sensitivity must be taken into account by the clinicians, before making clinical decisions.

Although this is a rather expensive test, in this initial phase the indication for the usage of this new diagnostic tool was at the discretion of the clinicians and did not need to be approved by the Division of Pediatric Infectious Diseases. This approach probably also is connected to the relatively low amount of positive testing results. This was also seen in Naccache et al. where in 265 samples 87% of the CSF multiplex panels yielded a negative result [29].

One of the common causes that led to a cerebrospinal fluid examination in older children was an isolated facial palsy. Not surprisingly, none of these patients had a positive result in the multiplex-PCR (which does not include *Borrelia burgdorferi*). To optimize costs and expected results, a more targeted approach may be advisable. Therefore, it seems warranted to restrict the more liberal usage of the multiplex PCR—that is without prior consultation of the pediatric infection team—to patients 1–12 weeks of age, presenting with fever to the emergency room. While the multiplex PCR results cover a wide range of possible CNS pathogens and the results are available very fast, the major disadvantage is the relatively high cost of the test (around 200–250 €/test run). Different strategies using specific PCR tests for single pathogens may be more cost-effective, but more difficult to implement.

There are several limitations to our study. Data were collected retrospectively from the medical records of the patients which may result in loss of information and a certain bias in the interpretation of results. Only a single center was involved and only a relatively low number of patients could be analyzed. In addition, before introduction of multiplex PCR, we did not have any PCR diagnostic on-site, but had to send samples to a laboratory off-site, resulting often in a long delay.

Taken together, our study shows that a point-of care rapid CSF diagnostic test can help guide the clinicians and reduce antibiotic treatment in children with suspected meningitis or encephalitis. Critical evaluation of the patient, its course of disease and all other accompanying symptoms can use the CSF panel in a sensible and cost-effective way. However, with this caveat, the multiplex CSF panel is able to offer a way towards faster diagnosis and therefore faster treatment (or discontinuation) of patients with infectious CNS disease [30].

There is disagreement on how new and expensive point-of-care tests should be used in children [31, 32]. While the availability of a rapid test may help clinicians with treatment decisions, the cost of these tests and the interpretation of results require a structured approach. Based on our results we suggest performing multiplex-PCR only on CSF from young infants with suspected sepsis, meningitis or encephalitis and in older children with suspected meningitis or encephalitis.

Compliance with ethical standards

Conflict of interest JH has received speaker fees and MMB has been invited to a seminar by BioMerieux, and the other authors declare that they have no conflict of interest.

References

1. Aronson PL. Evaluation of the febrile young infant: an update. Pediatr Emerg Med Pract. 2013;10:1–17.
2. Franco-Paredes C, Lammoglia L, Hernandez I, Santos-Preciado JI. Epidemiology and outcomes of bacterial meningitis in Mexican

children: 10-year experience (1993–2003). Int J Infect Dis IJID. 2008;12:380–6.
3. Ku LC, Boggess KA, Cohen-Wolkowiez M. Bacterial meningitis in infants. Clin Perinatol. 2015;42:29–45 **(vii–viii)**.
4. Silverman MA, Konnikova L, Gerber JS. Impact of antibiotics on necrotizing enterocolitis and antibiotic-associated diarrhea. Gastroenterol Clin N Am. 2017;46:61–76.
5. Yamamoto-Hanada K, Yang L, Narita M, Saito H, Ohya Y. Influence of antibiotic use in early childhood on asthma and allergic diseases at age 5. Ann Allergy Asthma Immunol. 2017;119:54–8.
6. Korpela K, Zijlmans MA, Kuitunen M, Kukkonen K, Savilahti E, Salonen A, de Weerth C, de Vos WM. Childhood BMI in relation to microbiota in infancy and lifetime antibiotic use. Microbiome. 2017;5:26.
7. Slykerman RF, Thompson J, Waldie KE, Murphy R, Wall C, Mitchell EA. Antibiotics in the first year of life and subsequent neurocognitive outcomes. Acta Paediatr. 2017;106:87–94.
8. Arora HS, Asmar BI, Salimnia H, Agarwal P, Chawla S, Abdel-Haq N. Enhanced identification of group B *Streptococcus* and *Escherichia Coli* in young infants with meningitis using the biofire filmarray meningitis/encephalitis panel. Pediatr Infect Dis J. 2017;36:685–7.
9. Jorgensen MPHJ, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. Manual of clinical microbiology. Washington, DC: ASM Press, 2015.
10. Olijve L, Jennings L, Walls T. Human parechovirus: an increasingly recognized cause of sepsis-like illness in young infants. Clin Microbiol Rev. 2018;31:1–17.
11. Sharp J, Harrison CJ, Puckett K, Selvaraju SB, Penaranda S, Nix WA, Oberste MS, Selvarangan R. Characteristics of young infants in whom human parechovirus, enterovirus or neither were detected in cerebrospinal fluid during sepsis evaluations. Pediatr Infect Dis J. 2013;32:213–6.
12. Diaz MG, Garcia RP, Gamero DB, Gonzalez-Tome MI, Romero PC, Ferrer MM, Contreras JR. Lack of accuracy of biomarkers and physical examination to detect bacterial infection in febrile infants. Pediatr Emerg Care. 2016;32:664–8.
13. Stiemsma LT, Michels KB. The role of the microbiome in the developmental origins of health and disease. Pediatrics. 2018;141:1–22.
14. Scott FI, Horton DB, Mamtani R, Haynes K, Goldberg DS, Lee DY, Lewis JD. Administration of antibiotics to children before age 2 years increases risk for childhood obesity. Gastroenterology. 2016;151:120–9 e125.
15. Boussioux C, Rousseau C, Salmona M, Bourdon N, Durand C, Delaire C, Simon F, Gonzalez R, Lallement J, Legoff J. Comparison of FILMARRAY meningitis/encephalitis (ME) panel assay and conventional techniques on the turnaround time of cerebrospinal fluid microbiological testing., ECCMID 2016, 2016.
16. de Jong EP, van den Beuken MGA, van Elzakker EPM, Wolthers KC, Sprij AJ, Lopriore E, Walther FJ, Brus F. Epidemiology of sepsis-like illness in young infants: major role of enterovirus and human parechovirus. Pediatr Infect Dis J. 2018;37:113–8.
17. Pons-Salort M, Oberste MS, Pallansch MA, Abedi GR, Takahashi S, Grenfell BT, Grassly NC. The seasonality of nonpolio enteroviruses in the United States: patterns and drivers. Proc Natl Acad Sci USA. 2018;115:3078–83.
18. Teoh HL, Mohammad SS, Britton PN, Kandula T, Lorentzos MS, Booy R, Jones CA, Rawlinson W, Ramachandran V, Rodriguez ML, et al. Clinical characteristics and functional motor outcomes of enterovirus 71 neurological disease in children. JAMA Neurol. 2016;73:300–7.
19. Hixon AM, Clarke P, Tyler KL. Evaluating treatment efficacy in a mouse model of enterovirus D68-associated paralytic myelitis. J Infect Dis. 2017;216:1245–53.
20. Bissel SJ, Auer RN, Chiang CH, Kofler J, Murdoch GH, Nix WA, Painter M, Richer M, Sartelet H, Wang G, et al. Human parechovirus 3 meningitis and fatal leukoencephalopathy. J Neuropathol Exp Neurol. 2015;74:767–77.
21. Britton PN, Dale RC, Nissen MD, Crawford N, Elliott E, Macartney K, Khandaker G, Booy R, Jones CA. Parechovirus encephalitis and neurodevelopmental outcomes. Pediatrics. 2016;137:e20152848.
22. Wildenbeest JG, Wolthers KC, Straver B, Pajkrt D. Successful IVIG treatment of human parechovirus-associated dilated cardiomyopathy in an infant. Pediatrics. 2013;132:e243–7.
23. Green DA, Pereira M, Miko B, Radmard S, Whittier S, Thakur K. Clinical significance of human herpesvirus 6 positivity on the FilmArray meningitis/encephalitis multiplex PCR panel. Clin Infect Dis. 2018. https://doi.org/10.1093/cid/ciy288.
24. Asano Y, Yoshikawa T, Suga S, Kobayashi I, Nakashima T, Yazaki T, Kajita Y, Ozaki T. Clinical features of infants with primary human herpesvirus 6 infection (exanthem subitum, roseola infantum). Pediatrics. 1994;93:104–8.
25. Zhang E, Bell AJ, Wilkie GS, Suarez NM, Batini C, Veal CD, Armendariz-Castillo I, Neumann R, Cotton VE, Huang Y, et al. Inherited chromosomally integrated human herpesvirus 6 genomes are ancient, intact, and potentially able to reactivate from telomeres. J Virol. 2017;91:1–19.
26. Caserta MT, Hall CB, Schnabel K, McIntyre K, Long C, Costanzo M, Dewhurst S, Insel R, Epstein LG. Neuroinvasion and persistence of human herpesvirus 6 in children. J Infect Dis. 1994;170:1586–9.
27. Basmaci R, Mariani P, Delacroix G, Azib S, Faye A, Taha MK, Bingen E, Bonacorsi S, Romero JR, Rotbart HA, et al. Enteroviral meningitis does not exclude concurrent bacterial meningitis. J Clin Microbiol. 2011;49:3442–3.
28. Piccirilli G, Chiereghin A, Gabrielli L, Giannella M, Squarzoni D, Turello G, Felici S, Vocale C, Zuntini R, Gibertoni D, et al. Infectious meningitis/encephalitis: evaluation of a rapid and fully automated multiplex PCR in the microbiological diagnostic workup. New Microbiol. 2018;41:118–25.
29. Naccache SN, Lustestica M, Fahit M, Mestas J, Bard JD. One year in the life of a rapid syndromic panel for meningitis/encephalitis: a pediatric tertiary care facility's experience. J Clin Microbiol. 2018;56:1–11.
30. Dien Bard J, Alby K. Point-counterpoint: meningitis/encephalitis syndromic testing in the clinical laboratory. J Clin Microbiol. 2018;56:1–10.
31. Rossi AF, Khan D. Point of care testing: improving pediatric outcomes. Clin Biochem. 2004;37:456–61.
32. Ferreira CES, Guerra JCC, Slhessarenko N, Scartezini M, Franca CN, Colombini MP, Berlitz F, Machado AMO, Campana GA, Faulhaber ACL, et al. Point-of-care testing: general aspects. Clin Lab. 2018;64:1–9.