Diagnostic accuracy of a commercial multiplex PCR for the diagnosis of meningitis and encephalitis in an Italian general hospital

Christian Leli[1], Luigi Di Matteo[1], Franca Gotta[1], Daria Vay[2], Lara Calcagno[3], Tiziana Callegari[3], Maurizio Cassinari[3], Elena Cattana[3], Maria Matilde Ciriello[3], Valeria Copponi[3], Maria Cristina Sacchi[3], Daniele Zambon[3], Roberto Guaschino[3], Andrea Rocchetti[1]

[1] Microbiology Laboratory, SS. Antonio e Biagio e C. Arrigo Hospital, Alessandria, Italy;
[2] Pathology Unit, SS. Antonio e Biagio e C. Arrigo Hospital, Alessandria, Italy;
[3] Clinical Pathology Laboratory, SS. Antonio e Biagio e C. Arrigo Hospital, Alessandria, Italy

SUMMARY

Background: Infectious meningitis and encephalitis are potentially life-threatening conditions caused mostly by bacterial and viral agents. A rapid diagnosis and prompt treatment are associated with a favourable outcome. In recent years nucleic acid amplification tests have been developed to speed detection and identification of pathogens directly from cerebrospinal fluid (CSF). Aim of this study was to compare the diagnostic accuracy of a commercially available multiplex PCR assay for etiological diagnosis of infectious meningitis directly from CSF samples to that obtained after CSF cultures. A secondary endpoint was to look for a possible screening threshold based on main CSF indices and urgent blood tests results, to define CSF samples with low pre-test probability of PCR and/or culture positive result.

Methods: we performed a retrospective analysis of results of CSF samples already processed as part of routine clinical care in the period February 2016 through December 2018.

Results: 109 CSF samples were included in the study and a total of 14 bacteria were identified by either PCR, culture or both methods, along with nine samples positive for viruses. After exclusion of the isolates not detectable by the multiplex PCR panel, the diagnostic accuracy was: 100% (95% confidence interval (CI): 54.1% to 100%) sensitivity; 98.9% (95% CI: 93.5% to 99.9%) specificity; 85.7% (95% CI: 42% to 99.2%) positive predictive value; 100% (95% CI: 95.1% to 100%) negative predictive value; 96 (95% CI: 13.6 to 674.6) positive likelihood ratio; Zero negative likelihood ratio; Cohen's kappa: 0.918, $p<0.0001$. CSF proteins value ≤ 28 mg/dl and CSF glucose/blood glucose ratio ≥ 0.78 were associated with both PCR negative result for bacteria or viruses and culture negative result.

Conclusions: even if on a limited sample of patients, the multiplex PCR evaluated in this study showed a very good diagnostic performance compared to culture and the thresholds found can be a useful tool to better choose which samples should be tested.

Keywords: Meningitis; Encephalitis; Multiplex Polymerase Chain Reaction; Bacteria; Virus; ROC analysis.

INTRODUCTION

Infectious meningitis and encephalitis are potentially life-threatening inflammatory diseases of the central nervous system caused by bacterial, fungal, viral and seldom parasites agents [1-3]. They represent medical emergencies in all age groups worldwide [4], being associated with very high mortality [5] with an early clinical picture that sometimes can be aspecific, such as headache and fever, not always associated with neck stiffness and altered mental state [6, 7]. Are therefore mandatory a rapid diagnosis and subsequent prompt treatment, associated with a favourable outcome [8].

Corresponding author
Christian Leli
E-mail: christian.leli@libero.it

Cerebrospinal fluid (CSF) indices, CSF Gram stain and culture are necessary for an etiological diagnosis and culture is still considered the gold standard [9]. Nevertheless, recovery of bacterial or fungal agents from CSF may take several days and is affected by previous antimicrobial treatment [9, 10]. To aid in the sometimes challenging clinical diagnosis of infectious meningitis and encephalitis, in recent years nucleic acid amplification tests (NAATs) have been developed to speed detection and identification of pathogens directly from CSF [10-20].

The main aim of this study was to compare the diagnostic accuracy of a commercially available multiplex PCR assay for etiological diagnosis of infectious meningitis directly from CSF samples to that obtained after CSF cultures, used as gold standard. The secondary endpoint was to look for a possible screening threshold based on main CSF indices and urgent blood tests results, to define CSF samples with low pre-test probability of PCR and/or culture positive result.

MATERIALS AND METHODS

Design of the study

We performed a retrospective analysis of results of CSF samples already processed as part of routine clinical care in the interval between February 2016 to December 2018. Inclusion criteria: multiplex PCR and culture results of CSF samples collected by lumbar puncture requested for diagnostic purposes along with CSF indices such as glucose, proteins and white blood cell count and with blood tests such as complete blood count, C-reactive protein and blood glucose levels, all processed simultaneously. Exclusion criteria: all CSF samples drawn from lumbar puncture without CSF indices or complete blood count, C-reactive protein and blood glucose drawn at the same time, were excluded from the study. Likewise were excluded all CSF samples drawn from shunt systems.

Clinical samples

All CSF samples were collected by lumbar puncture and processed immediately. The first CSF sample was processed for laboratory testing: white blood cell count, proteins and glucose; the second sample for microbiologic tests, such as Gram staining, culture and multiplex PCR testing. The blood samples were drawn by venipuncture at the same time.

PCR processing

An aliquot of 200 µl of CSF was analyzed using FilmArray Meningitis/Encephalitis panel test (BioFire Diagnostics LLC, Salt Lake City, UT, USA) according to the manufacturer's instructions. In about one hour the assay simultaneously detects and identifies a panel of six bacteria: *Escherichia coli* K1, *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Streptococcus agalactiae*, and *Streptococcus pneumoniae*; seven viruses: cytomegalovirus (CMV), enterovirus (EV), herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), human herpesvirus 6 (HHV-6), human parechovirus (PeV) and varicella-zoster virus (VZV); two yeasts: *Cryptococcus neoformans/Cryptococcus gattii* (the assay does not differentiate *C. neoformans* from *C. gattii*). The system is composed of four automated stages: a nucleic acid extraction, a reverse transcription, a nucleic acid amplification and a results analysis [21].

CSF bacterial culture

Each CSF sample was inoculated onto: Columbia sheep blood agar and chocolate agar, incubated at 35-37°C, with 5% CO_2 for up to 5 days; MacConkey agar and Sabouraud agar, incubated under aerobic conditions at 35-37°C for up to 5 days; Schaedler agar and vancomycin-kanamycin Schaedler agar, incubated in an anaerobic atmosphere at 35-37°C for up to 5 days (all media from Becton Dickinson, Sparks, MD, USA). An aliquot (if available) of 1 to 1.5 ml was also inoculated into a BACTEC Peds Plus/F bottle, which was loaded into the BACTEC 9240 instrument (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD, USA) and incubated for 5 days. Identification and antibiotic susceptibility of the isolates was carried out using the VITEK 2 system (bioMérieux, Marcy l'Etoile, France).

CSF mycobacterial culture

When also mycobacterial culture was requested, one ml aliquot of the CSF sample was processed for direct detection of mycobacteria and possible rifampicin resistance by means of Xpert® MTB/RIF assay (Cepheid Inc., Sunnyvale, CA, USA). A second aliquot of CSF was centrifugated, the supernatant discarded and the pellet resuspend-

ed with sterile phosphate buffer for both smear and culture. An aliquot of 500 µl was mixed with 800 µl of growth supplement (Becton Dickinson, Sparks, MD, USA), inoculated into one BACTEC Mycobacteria Growth Indicator Tube (MGIT) 960 tube (Becton Dickinson, Sparks, MD, USA) and incubated at 37°C in the BACTEC MGIT 960 instrument for 42 days. Two aliquots of 250 µl each were inoculated onto two Lowenstein-Jensen slants and incubated at 37°C for 8 weeks. Identification of mycobacteria grown in culture was confirmed by means of Xpert® MTB/RIF assay.

Statistics

Values are expressed as median and interquartile range (IQR). Comparisons of median values were performed by means of Mann-Whitney U test or Kruskal-Wallis H test, as appropriate. Agreement between PCR and culture was evaluated by Cohen's kappa coefficient. With respect to bacteria detectable by the multiplex panel, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+) and negative likelihood ratio (LR-) of the assay, compared with culture used as gold standard, were calculated as described by Hazra and Gogtay [22]. A receiver operator characteristic (ROC) analysis was performed to find a possible threshold among the variables, to be used as screening value to define CSF samples with a low pre-test probability of a positive result by both multiplex PCR and culture. SPSS statistical package, release 17.0 (SPSS Inc, Chicago, IL, USA) was used for all statistical analyses. The significance level was ≤0.05.

Ethical considerations

Ethical approval was not needed because this is a retrospective analysis of data from samples collected as part of standard care and those included in the database were deidentified before access. No personal information was stored in the study database. No patient intervention occurred with the obtained results.

RESULTS

During the interval from February 2016 to December 2018 a total of 109 CSF samples fulfilled the inclusion criteria and were included in the study. Samples come from patients hospitalized in the following wards: Emergency Department 24/109 (22%), Neurology 22/109 (20.1%), Infectious Diseases 16/109 (14.7%), Intensive Care Unit 15/109 (13.8%), Hematology 10/109 (9.2%), Internal Medicine 7/109 (6.4%), Neurosurgery 5/109 (4.6%), Pediatrics 4/109 (3.7%), Oncology 3/109 (2.8%), Psychiatry 1/109 (0.9%), Ophthalmology 1/109 (0.9%), Heart Surgery 1/109 (0.9%). We had a multiplex PCR positive result for bacteria or viruses in 16/109 (14.7%) of the samples. The characteristics of the patients included in the study and the comparison of median values according to multiplex PCR results are described in Table 1; 58/109 (53.2%) were females. The comparison of median values of blood neutrophil count, C-reactive protein, CSF glucose, CSF proteins, CSF white blood cell count and CSF glucose/blood glucose ratio between CSF samples PCR positive for bacteria vs CSF samples PCR positive for viruses (Table 1) showed a significant difference (p=0.039; p=0.023; p=0.001; p=0.01; p=0.05; p=0.001, respectively). The same analysis performed between CSF samples PCR positive for viruses vs PCR negative (Table 1) showed no significant difference but a significantly higher number of CSF white blood cell count in CSF samples positive for viruses (p=0.029). A total of 14 bacteria were identified by either PCR, culture or both methods (Table 2). One sample positive by PCR was negative by culture, and conversely, seven samples positive by culture were negative by PCR. The crude agreement between PCR and culture was moderate: k=0.564, p<0.0001. Three of the seven CSF samples positive only by culture (one for *Staphylococcus aureus*, one for *Staphylococcus schleiferi* and one for *Pseudomonas aeruginosa*) were from patients who have undergone intracranial neurosurgical procedures. Two patients were admitted to Infectious Diseases ward (one positive for *Mycobacterium tuberculosis* complex and one for *Kingella spp.*), one from a patient admitted to Heart Surgery (*Staphylococcus aureus*) and one patient was hospitalized in Oncology (*Turicella otitidis*). After exclusion of the seven samples positive for the isolates not detectable by the multiplex PCR panel, the diagnostic accuracy of the multiplex PCR was: 100% (95% confidence interval (CI): 54.1% to 100%) sensitivity; 98.9% (95% CI: 93.5% to 99.9%) specificity; 85.7% (95% CI: 42% to 99.2%) positive predictive value; 100% (95% CI: 95.1% to 100%) negative predictive val-

Table 1 - Characteristics of the patients included in the study and comparison of median values according to PCR results.

Variable	*Whole sample (n=109)*		*Samples positive for bacteria (n=7)*		*Samples positive for viruses (n=9)*		*Samples negative (n=93)*		
	Median	*IQR*	*Median*	*IQR*	*Median*	*IQR*	*Median*	*IQR*	*p*
Age (Years)	60	41.5-71	59	21-66	54	35.5-76	60	42-71	0.878
White blood cell count (cells/mm^3)	8440	6635.5-11250	12230	8750-23860	7750	6420-8825	8320	6580-11035	0.151
Haemoglobin (g/dL)	12.3	10.9-13.9	13.4	12.6-14.3	11.8	9.9-13.4	12.3	10.9-13.9	0.261
Haematocrit (%)	38.3	33.9-41.9	40.7	37.1-44.1	35.4	29.9-41.2	38.3	33.9-41.8	0.172
Platelets (cells/µL)	232000	159000-271000	191000	144000-344000	232000	156500-315000	233000	172000-270000	0.844
Neutrophils (cells/mm^3)	5940	4120-9195	8220	7970-21830	5480	4200-6810	5870	4120-9195	0.101
Eosinophils (cells/mm^3)	40	15-110	30	20-30	50	10-105	50	10-120	0.371
Basophils (cells/mm^3)	30	20-50	20	20-30	30	20-45	30	20-55	0.756
Lymphocytes (cells/mm^3)	1180	770-1760	1050	510-1480	1210	1025-1790	1190	770-1790	0.546
Monocytes (cells/mm^3)	420	270-630	350	40-790	440	280-560	420	270-630	0.871
Neutrophils/ lymphocytes ratio	4.9	2.5-10.4	14.6	3-21.2	4.3	3.1-6.6	4.8	2.3-10.4	0.126
C-reactive protein (mg/dL)	1.6	0.2-8.8	16.4	11.6-29.3	0.5	0.06-5.0	1.5	0.2-6.3	0.006
Blood glucose (mg/dL)	108	93-131.5	122	92-148	99	94.5-117.5	111	92-132.5	0.705
CSF glucose (mg/dL)	64	50-75.5	10	0-14	65	49-72.5	65	52.5-78	<0.0001
CSF proteins (mg/dL)	55.9	32.6-85	251.7	156.2-371.8	55.9	39.4-170.3	50.5	29.1-76.6	<0.0001
CSF white blood cell count (cells/mm^3)	3	1-42	485	25-10000	33	3-355	3	1-17	<0.0001
CSF glucose/blood glucose ratio	0.57	0.47-0.70	0.10	0.00-0.15	0.53	0.49-0.66	0.58	0.51-0.71	<0.0001

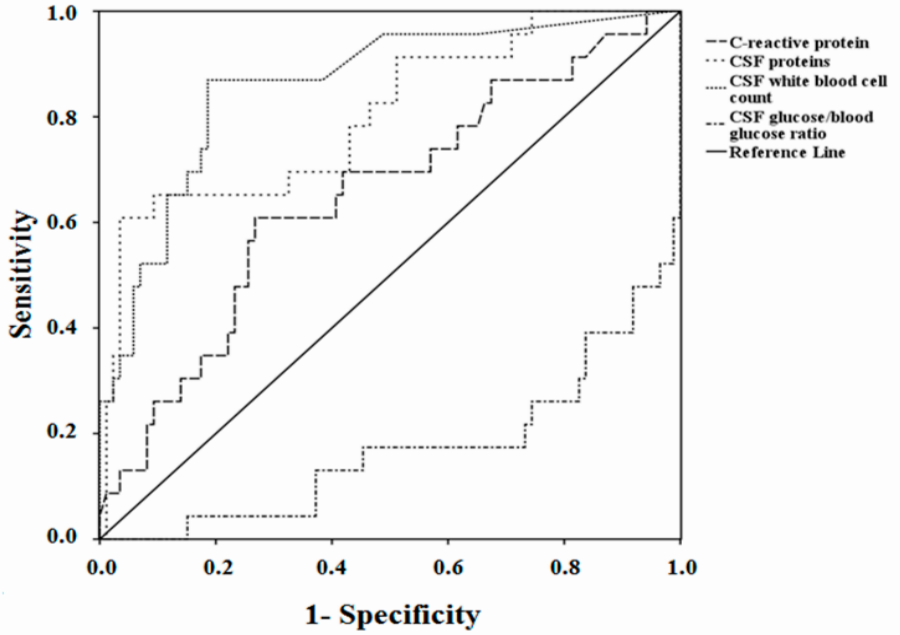
ue; 96 (95% CI: 13.6 to 674.6) LR+; Zero LR-; 99% (95% CI 94.6% to 99.9%) overall accuracy. Also the agreement evaluated by means of Cohen's kappa between PCR and culture after exclusion of the isolates not included in the panel was almost perfect: k=0.918, $p<0.0001$.

With respect to viruses and fungi, a total of nine samples were positive: CMV: 1/109 (0.9%); HSV-1: 2/109 (1.8%); HSV-2: 1/109 (0.9%); HHV-6: 2/109 (1.8%); VZV: 3/109 (2.7%). No EV nor PeV were identified. No sample was positive for *Cryptococcus neoformans/Cryptococcus gattii*.

The ROC analysis is described in Figure 1. In our series, CSF proteins value ≤ 28 mg/dl and CSF glucose/blood glucose ratio ≥ 0.78 were associated with both PCR negative result for bacteria or viruses and culture negative result. On the other hand, either C-reactive protein values ≥ 66 mg/dl or CSF white blood cell count ≥ 1200/mm^3 or CSF glucose/blood glucose ratio values ≤ 0.19 corre-

Table 2 - Bacteria identified from CSF according to method.

Bacteria	*Identified by both methods*	*Identified only by PCR*	*Identified only by culture*	*Total*
Gram positive				
Streptococcus pneumoniae	3	0	0	3
Streptococcus agalactiae	1	0	0	1
Staphylococcus aureus	0	0	2	2
Staphylococcus schleiferi	0	0	1	1
Listeria monocytogenes	1	0	0	1
Mycobacterium tuberculosis complex	0	0	1	1
Turicella otitidis	0	0	1	1
Gram negative				
Neisseria meningitidis	1	1	0	2
Pseudomonas aeruginosa	0	0	1	1
Kingella spp.	0	0	1	1
Total	6	1	7	14



Variable	Area under the curve	95% Confidence interval	*p*
C-reactive protein (mg/dl)	0.651	0.522-0.780	0.027
CSF proteins (mg/dl)	0.802	0.694-0.911	<0.0001
CSF white blood cell count (cells/mm^3)	0.860	0.772-0.947	<0.0001
CSF glucose/blood glucose ratio	0.169*	0.064-0.275	<0.0001

* Equivalent to 0.831 (1-0.169)

Figure 1 - Receiver Operating Characteristic curves of C-reactive protein, CSF proteins, CSF white blood cell count, CSF glucose/blood glucose ratio in predicting eliminare PCR positive result for bacteria or viruses and/or culture positive result.

sponded to CSF samples PCR positive for bacteria and culture positive. The best diagnostic cut-offs found for the variables with the greatest areas under the curve were: CSF white blood cell count: 17 cells/mm^3 with 87% sensitivity, 81% specificity; CSF glucose/blood glucose ratio: 0.51 with 73.9% sensitivity, 82.6% specificity; CSF proteins: 126.3 mg/dl with 60.9% sensitivity, 96.5% specificity.

DISCUSSION

Infectious meningitis and encephalitis are potentially life-threatening syndromes, that may be due to bacteria, fungi, viruses and rarely parasites [1-3]. Since time is a critical factor in meningitis care, NAATs are more and more frequently used to speed identification of pathogens directly from CSF samples [7,11-14]. Indeed, the risk for specific pathogens is related to immunity and age [23], such for example in patients affected by liver cirrhosis [24] or in the elderly population [25,26] and in patients with these conditions, NAATs permit a rapid etiological diagnosis of meningitis and a prompt treatment. Therefore, multiplex PCR for detection of pathogens directly in CSF samples can drive first-line therapy.

The comparison of diagnostic accuracy of the multiplex PCR with that of culture on CSF samples was the first aim of the study. Sensitivity, specificity and overall accuracy of the multiplex PCR in detecting bacterial agents included in the panel compared with culture are equal to the ones found by Leber et al. [21] in a multicenter evaluation of 1560 prospectively collected CSF specimens. Similarly, the overall agreement between multiplex PCR and culture found in this study is similar to the ones reported by other studies on the same multiplex PCR [12,27-29]. The only CSF sample positive by multiplex PCR and not confirmed by culture was for *N. meningitidis*. We considered that a true positive result, indeed, it is more probable that an antimicrobial therapy administered to the patient before lumbar puncture prevented the bacterium from growing and the multiplex PCR detected the DNA because of the high sensitivity of the test, as suggested by the CSF indices of this sample, markedly pathologic: CSF glucose/blood glucose ratio: 0.15; CSF proteins: 190.3 mg/dl and CSF white blood cell: 1730/mm^3. The moderate agreement found considering the pathogens not included in the panel was expected and showed how this test is not a replacement of CSF culture, but an adjunctive tool to be used in suspected meningitis or encephalitis. The etiologic agents of community-acquired bacterial meningitis found in the present study, are the same described in the systematic review and meta-analysis on the global etiology of bacterial meningitis by Oordt-Speets et al. [30]. Similarly, with regard to the recovered bacteria from the patients who have undergone intracranial neurosurgical procedures, *P. aeruginosa* and *Staphylococcus* spp. are the main etiologic agents of healthcare-associated ventriculitis and meningitis, as reported in the 2017 Infectious Diseases Society of America's Clinical Practice Guidelines by Tunkel et al. [31]. With respect to the two cases caused by *Kingella spp.* and *T. otitidis*, case reports of clusters of serious infections by *Kingella denitrificans* and *Kingella kingae* such as bacteremia, endocarditis, meningitis and others have already been described in several countries [32,33]. The patient suffering from meningitis caused by *T. otitidis* was hospitalized in Oncology and the CSF indices were pathologic: CSF glucose/blood glucose ratio: 0.18; CSF proteins: 728 mg/dl and CSF white blood cell count: 257/mm^3.

With respect to the second endpoint of the study, the significant difference in the median values of CSF indices between CSF samples positive for bacteria and viruses matches with the results of Gowin et al. [34]. The positivity rate found in this study (14.7%) is similar to that reported by Tarai and Das in a study on 969 CSF samples [35]. Likewise, the positivity rate and the finding of no significant difference in the comparison of median values of C-reactive protein between CSF samples positive by multiplex PCR for viruses and multiplex PCR negative are in line with the results of Eichinger et al. [36] in a study on 187 CSF samples. The scoring system "bacterial meningitis-Careggi score", based on biochemical and cytological CSF and blood parameters proposed by Lagi et al. [37] considered cut-offs of CSF white blood cell count > 50/mm^3, CSF protein concentration > 80 mg/dl and CSF glucose/blood glucose ratio < 45%. Nevertheless, in that study the outcome evaluated was acute bacterial meningitis, whereas in the present study also PCR positive results for viruses were considered, therefore it is conceivable how in this study the best cut-offs of CSF white blood cell count could be lower as well as CSF glucose/

blood glucose ratio values higher. The same applies to the finding of a CSF/blood glucose ratio threshold of 0.36 by Tamune et al. [38], indeed also in that study they evaluated only bacterial meningitis as outcome.

To minimize the risk of a possible false-negative PCR result for bacteria (Leber et al. [21] described a single false-negative result with *S. agalactiae*) we decided to select as outcome of the ROC analysis the sum of results of both multiplex PCR and culture and to consider also the possibility of an encephalitis, we included multiplex PCR positive results for viruses. Therefore, conservative cutoffs of CSF proteins value ≤ 28 mg/dl and CSF glucose/blood glucose ratio ≥ 0.78 can be considered good screening values.

This study has several limitations. This is a single centre study, thus our findings may differ from those of other institutions. If the strict including criteria ensured reliability of the results, they allowed only a small number of samples to be evaluated. Unfortunately, multiplex PCR results positive for viruses could not be matched with a comparator assay.

CONCLUSIONS

In conclusion, even if on a limited sample of patients, the multiplex PCR evaluated in this study showed a very good diagnostic performance compared to culture and the thresholds found can be a useful tool to better choose which samples should be tested.

REFERENCES

[1] Richie M.B., Josephson S.A. A Practical Approach to Meningitis and Encephalitis. *Semin. Neurol.* 35, 611-620, 2015.

[2] Hoffman O., Weber R.J. Pathophysiology and treatment of bacterial meningitis. *Ther. Disord.* 2, 1-7, 2009.

[3] Venkatesan A., Geocadin R.G. Diagnosis and management of acute encephalitis: A practical approach. *Neurol. Clin. Pract.* 4, 206-215, 2014.

[4] Costerus J.M., Brouwer M.C., Bijlsma M.W., van de Beek D. Community-acquired bacterial meningitis. *Curr. Opin. Infect. Dis.* 30, 135-141, 2017.

[5] Hasbun R., Rosenthal N., Balada-Llasat J.M., et al. Epidemiology of Meningitis and Encephalitis in the United States, 2011-2014. *Clin. Infect. Dis.* 65, 359-363, 2017.

[6] Heckenberg S.G., de Gans J., Brouwer M.C., et al. Clinical features, outcome, and meningococcal genotype in 258 adults with meningococcal meningitis: a prospective cohort study. *Medicine (Baltimore).* 87, 185-192, 2008.

[7] van de Beek D., de Gans J., Spanjaard L. Weisfelt M., Reitsma J.B., Vermeulen M. Clinical features and prognostic factors in adults with bacterial meningitis. *N. Engl. J. Med*. 351, 1849-1859, 2004.

[8] Bodilsen J., Dalager-Pedersen M., Schønheyder H.C., Nielsen H. Time to antibiotic therapy and outcome in bacterial meningitis: a Danish population-based cohort study. *BMC Infect. Dis.* 16, 392, 2016.

[9] Brouwer M.C., Tunkel A.R., van de Beek D. Epidemiology, Diagnosis, and Antimicrobial Treatment of Acute Bacterial Meningitis. *Clin. Microbiol. Rev.* 23, 467-492, 2010.

[10] He T., Kaplan S., Kamboj M., Tang Y.W. Laboratory Diagnosis of Central Nervous System Infection. *Curr. Infect. Dis. Rep*. 18, 35, 2016.

[11] Bahr N.C., Boulware D.R. Methods of rapid diagnosis for the etiology of meningitis in adults. *Biomark. Med*. 8, 1085-1103, 2014.

[12] Liesman R.M., Strasburg A.P., Heitman A.K., Theel E.S., Patel R., Binnicker M.J. Evaluation of a commercial multiplex molecular panel for diagnosis of infectious meningitis and encephalitis. *J. Clin. Microbiol*. 56:e01927-17, 2018.

[13] Llano López L.H., Reischl A.T., Gröndahl B., et al. The BioFire FilmArray enables point of care diagnostic in neonatal parechovirus meningitis. *Infect. Dis. (Lond).* 49, 705-707, 2017.

[14] Wootton S.H., Aguilera E., Salazar L., Hemmert A.C., Hasbun R. Enhancing pathogen identification in patients with meningitis and a negative Gram stain using the BioFire FilmArray(®) Meningitis/Encephalitis panel. *Ann. Clin. Microbiol. Antimicrob*. 15, 26, 2016.

[15] Blaschke A.J., Holmberg K.M., Daly J.A., et al. Retrospective evaluation of infants aged 1 to 60 days with residual cerebrospinal fluid (CSF) tested using the FilmArray Meningitis/Encephalitis (ME) Panel. *J. Clin. Microbiol*. 56(7), 2018.

[16] Arora H.S., Asmar B.I., 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.* 36, 685-687, 2017.

[17] Launes C., Casas-Alba D., Fortuny C., Valero-Rello A., Cabrerizo M., Muñoz-Almagro C. Utility of FilmArray Meningitis/Encephalitis Panel during Outbreak of Brainstem Encephalitis Caused by Enterovirus in Catalonia in 2016. *J. Clin. Microbiol*. 55, 336-338, 2016.

[18] Naccache S.N., Lustestica M., Fahit M., Mestas J., Dien Bard J. One Year in the Life of a Rapid Syndromic Panel for Meningitis/Encephalitis: a Pediatric Tertiary Care Facility's Experience. *J. Clin. Microbiol*. 56(5), 2018.

[19] Hanson K.E. The first fully automated molecular

diagnostic panel for meningitis and encephalitis: how well does it perform, and when should it be used? *J. Clin. Microbiol*. 54, 2222-2224, 2016.
[20] Wilen C.B., Monaco C.L., Hoppe-Bauer J., Jackups R. Jr., Bucelli R.C., Burnham C.A. Criteria for reducing unnecessary testing for herpes simplex virus, varicella-zoster virus, cytomegalovirus, and enterovirus in cerebrospinal fluid samples from adults. *J. Clin. Microbiol.* 53, 887-895, 2015.
[21] Leber A.L., Everhart K., Balada-Llasat J.M., et al. Multicenter Evaluation of BioFire FilmArray Meningitis/Encephalitis Panel for Detection of Bacteria, Viruses, and Yeast in Cerebrospinal Fluid Specimens. *J. Clin. Microbiol*. 54, 2251-2261, 2016.
[22] Hazra A., Gogtay N. Biostatistics Series Module 7: The Statistics of Diagnostic Tests. *Indian. J. Dermatol*. 62, 18-24, 2017.
[23] van de Beek D., Cabellos C., Dzupova O., et al. ESCMID guideline: diagnosis and treatment of acute bacterial meningitis. *Clin. Microbiol. Infect*. 22, S37-62, 2016.
[24] Pagliano P., Boccia G., De Caro F., Esposito S. Bacterial meningitis complicating the course of liver cirrhosis. *Infection*. 45, 795-800, 2017.
[25] Pagliano P., Ascione T., Boccia G., De Caro F., Esposito S. *Listeria monocytogenes* meningitis in the elderly: epidemiological, clinical and therapeutic findings. *Infez. Med.* 24, 105-11, 2016.
[26] Pagliano P., Arslan F., Ascione T. Epidemiology and treatment of the commonest form of listeriosis: meningitis and bacteraemia. *Infez. Med.* 25, 210-216, 2017.
[27] Graf E.H., Farquharson M.V., Cárdenas A.M. Comparative evaluation of the FilmArray meningitis/encephalitis molecular panel in a pediatric population. *Diagn. Microbiol. Infect. Dis*. 87, 92-94, 2017.
[28] Hanson K.E., Slechta E.S., Killpack J.A., et al. Preclinical Assessment of a Fully Automated Multiplex PCR Panel for Detection of Central Nervous System Pathogens. *J. Clin. Microbiol.* 54, 785-787, 2016.
[29] Messacar K., Breazeale G., Robinson C.C., Dominguez S.R. Potential clinical impact of the filmarray meningitis encephalitis panel in children with suspected central nervous system infections. *Diagn. Microbiol. Infect. Dis*. 86, 118-120, 2016.
[30] Oordt-Speets A.M., Bolijn R., van Hoorn R.C., Bhavsar A., Kyaw M.H. Global etiology of bacterial meningitis: A systematic review and meta-analysis. *PLoS One*. 13:e0198772, 2018.
[31] Tunkel A.R., Hasbun R., Bhimraj A., et al. 2017 Infectious Diseases Society of America's Clinical Practice Guidelines for Healthcare-Associated Ventriculitis and Meningitis. *Clin. Infect. Dis*. 64, e34-e65, 2017.
[32] Hassan I.J., Hayek L. Endocarditis caused by *Kingella denitrificans*. *J. Infect*. 27, 291-295, 1993.
[33] Yagupsky P. Outbreaks of *Kingella kingae* Infections in Daycare Facilities. *Emerg. Infect. Dis*. 20, 746-753, 2014.
[34] Gowin E., Januszkiewicz-Lewandowska D., Słowiński R., Błaszczyński J., Michalak M., Wysocki J. With a little help from a computer: discriminating between bacterial and viral meningitis based on dominance-based rough set approach analysis. *Medicine (Baltimore)*. 96, e7635, 2017.
[35] Tarai B., Das P. FilmArray® meningitis/encephalitis (ME) panel, a rapid molecular platform for diagnosis of CNS infections in a tertiary care hospital in North India: one-and-half-year review. *Neurol. Sci.* 40, 81-88, 2019.
[36] Eichinger A., Hagen A., Meyer-Bühn M., Huebner J. Clinical benefits of introducing real-time multiplex PCR for cerebrospinal fluid as routine diagnostic at a tertiary care pediatric center. *Infection.* 47, 51-58, 2019.
[37] Lagi F., Bartalesi F., Pecile P., et al. Proposal for a new score-based approach to improve efficiency of diagnostic laboratory workflow for acute bacterial meningitis in adults. *J. Clin. Microbiol*. 54, 1851-1854, 2016.
[38] Tamune H., Takeya H., Suzuki W., et al. Cerebrospinal fluid/blood glucose ratio as an indicator for bacterial meningitis. *Am. J. Emerg. Med*. 32, 263-266, 2014.