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ORIGINAL ARTICLE

Multicenter Evaluation of the Implementation Status of Laboratory Tests in Korea and the Potential Usefulness of a Multiplex PCR Assay in Patients with Suspected Central Nervous System Infections

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SUMMARY

Background: **The rapid diagnosis and treatment of central nervous system (CNS) infections are critical to minimizing morbidity and mortality. We evaluated the implementation status of laboratory tests in patients with suspected CNS infection, and the potential usefulness of a multiplex PCR assay for rapid and simultaneous detection in cerebrospinal fluid (CSF) of 14 targets capable of causing CNS infections.**
Methods: **The study was conducted at 5 hospitals located in Daegu and Gyeongju over a period of 6 months. A total of 140 patients with suspected CNS infection were included. CSF samples were tested for 6 bacteria, 7 viruses, and 1 yeast using multiplex PCR (FilmArray Meningitis/Encephalitis Panel, BioFire Diagnostics/Biomerieux, Salt Lake City, UT, USA) and conventional diagnostic testing including chemistry tests, cell count, bacterial culture, antigen detection assay, and pathogen-specific PCR.**
Results: **The five conventional tests most commonly performed were the chemistry and cell count (100%), bacterial culture (94.3%), enterovirus PCR (52.9%), and herpes simplex virus PCR (25.7%). Among the 140 CSF samples, 27 (19.3%) and 42 (30.0%) tested positive by conventional and the FilmArray ME panel testing, respectively.**
Conclusions: **The detection rate of pathogens for CNS infections increased using only one FilmArray test compared to all of the conventional methods actually performed in patients with suspected CNS infection.**
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INTRODUCTION

Central nervous system (CNS) infections, such as meningitis and encephalitis, are serious clinical conditions. The morbidity and mortality of these infections can be high, particularly with bacterial meningitis. Therefore, the rapid and accurate detection of pathogens in cerebrospinal fluid (CSF) is important for the most effective

and appropriate management of patients [1-3].
Conventional microbiological approaches including polymerase chain reaction (PCR) tests are currently implemented [4]. Routine chemistry and cellular analyses of CSF may allow differentiation between bacterial and viral infections; however, these methods are not specific. The culture method is highly specific, but takes several days and may produce false negative results [3]. Pathogen-specific PCR tests are selectively ordered upon clinical suspicion and, thus, have low diagnostic yields. Moreover, most of the pathogen-specific PCR tests are performed in reference laboratories. This leads to a slow turnaround time (TAT), resulting in the delayed or unnecessary administration of antimicrobials.
Many of these limitations can be overcome with multiplex molecular tests, which are currently available as a point-of-care testing (POCT) concept [5-10]. The FilmArray Meningitis/Encephalitis (ME) panel is a multiplexed diagnostic PCR test for the simultaneous and rapid detection of 14 pathogens directly from CSF samples. In this study, we investigated the current status of conventional tests for CNS infection and evaluated the potential usefulness of multiplex PCR testing for the direct detection of pathogens from the CSF samples of patients with suspected CNS infection.

MATERIALS AND METHODS

We enrolled 140 patients who were suspected of having meningitis or encephalitis, and who had undergone culture or individual PCR tests for pathogen detection in CSF at 5 hospitals (Table 1). After performing routine analysis for microbiological investigation, residual CSF sample was immediately analyzed using the multiplex PCR.
Routine CSF analysis, such as glucose and albumin levels or cell count, bacterial culture, and antigen assays, were carried out at each hospital. Depending on the clinical characteristics of the patient or the available facilities at each institution, pathogen-specific PCR tests were performed either within the respective institution or at an external referral laboratory (Table 2). FilmArray ME testing was performed independently at each institution.

Routine CSF analysis and pathogen-specific PCR

All 140 CSF samples were routinely tested to determine the glucose and protein levels and white blood cell count. Bacterial cultures were performed at each hospital using their respective laboratory standard procedures. Solid agar plates and broth were incubated for 2 to 5 days at 35 - 37°C in 5% CO_2. For positive cultures, isolate identification was performed using standard procedures including biochemical, phenotypic, and matrix-assisted laser desorption ionization-time of flight mass spectroscopy (MALDI-TOF MS) analysis. The cryptococcal antigen assay and bacterial antigen screening assay (for detection of *Haemophilus influenzae*, *Streptococcus agalactiae*, *Neisseria meningitidis*, and *Streptococcus pneumoniae*) were performed using the latex agglutination method.
Pathogen-specific PCRs were performed on CSF to identify herpes simplex virus (HSV-1 and HSV-2), enterovirus (EV), varicella-zoster virus (VZV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), human herpesvirus 6 (HHV-6), and JC virus. Bacterial multiplex PCR was used for the detection of *S. pneumoniae*, *H. influenzae*, *N. meningitidis*, *S. agalactiae*, and *Listeria monocytogenes.* All of these PCRs were performed at an external referral laboratory depending on the circumstances of each hospital, except for EV identification using real-time PCR (GeneXpert EV assay, Cepheid; Sunnyvale, CA, USA) that was conducted in hospital A (Table 2).

FilmArray ME panel testing

A total of 200 μL of each CSF sample was analyzed using the multiplex PCR test (FilmArray ME Panel, BioFire Diagnostics/Biomerieux, Salt Lake City, UT, USA) according to manufacturer's instructions. The assay included 14 targets: 6 bacteria (*E. coli* K1, *H. influenzae*, *L. monocytogenes, N. meningitides, S. agalactiae,* and *S. pneumoniae*), 7 viruses (CMV, EV, HSV-1, HSV-2, VZV, HHV-6, and Human parechovirus) and 1 yeast (*Cryptococcus neoformans/C. gattii*).

RESULTS

Implementation rates of conventional tests for CNS infection

Routine chemistry and cellular analyses of CSF were performed on every sample enrolled (100%), and the bacterial culture method was used for the majority of the samples (94.3%). Cryptococcal antigen testing was selectively performed in only 2.1% (n = 3) of the samples. At two institutions (hospital A and C), the samples (n = 20) were analyzed with the bacterial antigen screening assay. Among all the pathogen-specific molecular tests, the performance rate of EV PCR testing was the highest (52.9%), and the second most commonly performed molecular test was HSV PCR (25.7%). Only one institution (hospital B) performed bacterial multiplex PCR testing for four bacteria. All molecular testing including bacterial multiplex PCR was carried out at an external referral laboratory except in one institution (Enterovirus PCR in hospital B) (Table 2).

Comparison of the FilmArray ME panel and conventional tests results

Among the 140 CSF samples, positive results were obtained in 27 (19.3%) and 42 (30.0%) by conventional testing and FilmArray ME panel testing, respectively. Co-detection (2 pathogens in the same sample) were observed in 3 (2.1%) samples by FilmArray ME Panel testing (Table 3).

Table 1. Characteristics of study subjects and basic laboratory CSF results.

	Mean ± SD
Age groups (n = 140)	
< 1 year	**39 (27.9%)**
1 - 17 years	**39 (27.9%)**
18 - 55 years	**36 (25.7%)**
> 56 years	**26 (18.6%)**
Gender	
Male:Female	**4:3**
CSF chemistry	
Glucose (mg/dL)	**61.7 ± 16.7**
Protein (mg/dL)	**130.5 ± 274.5**
Cell count	
White blood cell (/μL)	**478.2 ± 1709.0**
Segmented neutrophil (/μL)	**380.7 ± 1626.9**
Lymphocyte (/μL)	**160.5 ± 281.7**

Bacterial targets

A total of six samples tested positive for a bacterial pathogen using the routine culture method. Among these pathogens, *S. pneumoniae* was accurately detected in two samples using the FilmArray ME panel. Three bacteria *(Stenotrophomonas maltophilia*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*) that were not included in the FilmArray ME panel were detected in three samples using the conventional culture method. True discordant results were obtained for two CSF samples; one of those tested positive for *H. influenza*e with the FilmArray ME panel but negative by the conventional methods (antigen assay and culture). The other sample was positive for *E. coli* by the culture method but negative by FilmArray ME testing (Table 4).

Viral targets

The FilmArray ME assay identified 17 samples as positive for EV and the pathogen-specific PCR was positive in 16 samples. Discordant results for EV were observed in five samples. Among these five samples, three were negative by pathogen-specific PCR and positive by FilmArray ME assay. The other two samples showed opposite results. The FilmArray assay was uniquely positive (i.e., pathogen-specific PCR was not performed) for HHV-6 (n = 8), VZV (n = 6), HSV-2 (n = 1), CMV (n = 1), HSV-1 (n = 1), and human parechovirus (n = 1) (Table 4).

Total Agreement of FilmArray ME panel and pathogen-specific PCR

A total of 74 CSF samples were tested by both the pathogen-specific PCR for EV and the FilmArray assay. The 14 positive results and 55 negative results were consistent in both tests, and 5 test results were discordant, resulting in an overall agreement of 93.2%. The overall agreement of both tests for the presence of HSV (n = 36), VZV (n = 12), and CMV (n = 10) was 100% each (Table 5).

DISCUSSION

Laboratory testing is essential for the diagnosis of CNS infection, and the culture of CSF is considered the gold standard for the diagnosis of bacterial meningitis [1]. Since the culture results are often not obtained for more than 48 hours after sampling, various rapid antigen assays have been used to facilitate the diagnosis of acute bacterial meningitis [12,13]. However, since the overall predictive value of the CSF antigen assay is low, its use was significantly reduced by favoring more sensitive molecular techniques [4,5]. Currently, molecular diagnostics are being used more actively for non-bacterial infections, and multiplex platforms have been introduced for the diagnosis of CNS infection [14].

In this study, antigen tests were used in a limited number of institutions, and the PCR method was used mainly for the detection of viruses. Several viruses, such as HHV-6, VZV, HSV-2, CMV, HSV-1, and parechovirus were detected by the FilmArray PCR method in 18 samples that were not detected by conventional PCR testing (Table 4). This is unavoidable with the current methods of selectively performing pathogen-specific PCR. In addition, the greater the number of targets detected by the PCR method, the higher the probability of detecting pathogens. Since the PCR method has an inevitable limitation in that it cannot detect a non-target pathogen, it is not possible to completely eliminate the negative consequences of not performing the tests. Although both multiplex PCR and conventional PCR have the same limitations, multiplex PCR has the advantage of having more targets to detect, and fewer samples are needed. Therefore, the false negative rate caused by not performing the test could be relatively small compared to that of conventional PCR. According to the results of this study, the FilmArray assay alone could raise the detection rate of pathogens from 19.3% to 30.0% (Table 3). In particular, it was possible to carry out more tests for viruses, which could have increased the positive rate.

For bacterial targets, one discrepant result of *H. influenzae* was detected in the FilmArray assay but not in culture (Table 6, Case 1). Since *H. influenzae* is normally present in the respiratory tract, it was reasonable to consider the false positive result. However, the CSF sample with this inconsistent result was collected on the seventh day of patient admission. The results of the CSF analysis on the day of admission were as follows: glucose 52 mg/dL, protein 90.7 mg/dL, cell count 1,280/μL, and culture negative. Considering the results of CSF analysis on the day of admission and the medication used by the patient during hospitalization, it was

Table 2. Implementation rates of routine and selective CSF tests.

	No. (%) of cases						No. of positive cases (% of total cases)
	Hospital A (n = 30)	Hospital B (n = 26)	Hospital C (n = 17)	Hospital D (n = 29)	Hospital E (n = 38)	Total (n = 140)	
Routine test							
Chemistry	30 (100.0)	26 (100.0)	17 (100.0)	29 (100.0)	38 (100.0)	140 (100.0)	NA
Cell count	30 (100.0)	26 (100.0)	17 (100.0)	29 (100.0)	38 (100.0)	140 (100.0)	NA
Bacterial culture	30 (100.0)	25 (96.2)	17 (100.0)	23 (79.3)	37 (97.4)	132 (94.3)	6 (4.3)
Antigen test							
Cryptococcus	0	1 (3.9)	0	0	2 (5.3)	3 (2.1)	0
HI/SA/NM/SP	3 (10.0)	0	17 (100.0)	0	0	20 (14.3)	0
Selective PCR test							
HSV 1 and 2	22 (73.3) [a]	0	0	11 (37.9) [a]	3 (7.9) [a]	36 (25.7)	4 (2.9)
EV	21 (70.0)	23 (88.5) [a]	17 (100.0) [a]	11 (37.9) [a]	2 (5.3) [a]	74 (52.9)	16 (11.4)
VZV	1 (3.3) [a]	0	0	11 (37.9) [a]	1 (2.6) [a]	12 (8.6)	1 (0.7)
CMV	0	0	0	10 (34.5) [a]	0	10 (7.1)	0
EBV	0	0	0	0	1 (2.6) [a]	1 (0.7)	0
HHV-6	0	0	0	0	1 (2.6) [a]	1 (0.7)	0
JC virus	0	0	0	0	1 (2.6) [a]	1 (0.7)	0
Bacterial Multiplex PCR [b]	0	23 (88.5) [a]	0	0	0	23 (16.4)	0

[a] - Referral laboratory test
[b] - Detection of *Streptococcus pneumoniae, Haemophilus influenzae, Neisseria meningitides, Streptococcus agalactiae*, and Listeria monocytogenes.
Abbreviations: HI - *Haemophilus influenzae*, SA - *Streptococcus agalactiae*, NM - *Neisseria meningitides*, SP - *Streptococcus pneumoniae*, HSV - Herpes simplex virus, EV - Enterovirus, VZV - Varicella-zoster virus, CMV - Cytomegalovirus, EBV - Epstein-Barr virus, HHV-6 - Human herpes virus 6.

Table 3. Results obtained with the FilmArray ME panel and conventional microbiological tests.

	Conventional microbiological tests [a]		FilmArray PCR		Total	
	No.	% of total	No.	% of total	No.	% of total
All (n = 140)						
negative	113	80.7	98	70.0	93	66.4
positive	27	19.3	42	30.0	47	33.6
single	27	19.3	39	27.9	42	30.0
co-detection	0	0.0	3 [b]	2.1	4 [c]	2.9
Age group (n)						
< 2 years (39)	3	2.1	7	5.0	7	5.0
1 - 17 years (39)	15	10.7	20	14.3	22	15.7
18 - 55 years (36)	7	5.0	10	7.1	12	8.6
> 56 years (26)	2	1.4	5	3.6	6	4.3

[a] - Culture, direct antigen test, and pathogen-specific PCR.
[b] - *Streptococcus pneumoniae* and HHV-6; HSV-2 and HHV-6; HSV-1 and HHV-6.
[c] - 3 cases of FilmArray co-detection, 1 case of Stenortophomonas maltophilia (culture) and CMV (FilmArray).
Abbreviations: HHV-6 - Human herpes virus 6, HSV - Herpes simplex virus, CMV - Cytomegalovirus.

Table 4. Comparison of the results for individual pathogens obtained by FilmArray ME panel and conventional testing.

	FilmArray PCR	Conventional test		No. of same result	Discrepant results	
	No. of positive results	No. of positive results	Method		No. of discrepant results	Remarks (n)
Bacteria						
S. pneumoniae	2	2	culture	2	0	Antigen NT/bacterial PCR NT (2)
H. influenzae	1	0	-	0	1	FilmArray positive/culture negative /antigen negative/bacterial PCR NT (1)
E. coli	0	1	culture	0	1	FilmArray negative/culture positive/bacterial PCR NA (1)
S. maltophilia	0	1	culture	0	1	FilmArray NA (1)
A. baumannii	0	1	culture	0	1	FilmArray NA (1)
K. pneumoniae	0	1	culture	0	1	FilmArray NA (1)
Virus						
EV	17	16	PCR	14	5	FilmArray positive/EV PCR negative (3), FilmArray negative/EV PCR positive (2)
HHV-6	8	0	-	0	8	HHV-6 PCR NT (8)
VZV	7	1	PCR	1	6	VZV PCR NT (6)
HSV-2	5	4	PCR	4	1	HSV-2 PCR NT (1)
CMV	1	0	-	0	1	CMV PCR NT (1)
HSV-1	1	0	-	0	1	HSV-1 PCR NT (1)
Human parechovirus	1	0	-	0	1	Human parechovirus PCR NT (1)

Abbreviations: NT - not tested, NA - not available, EV - Enterovirus, HHV-6 - Human herpes virus 6, VZV - Varicella-zoster virus, HSV - Herpes simplex virus, CMV - Cytomegalovirus.

Table 5. Agreement of the FilmArray ME panel and conventional PCR methods.

	EV PCR (n = 74)		HSV PCR (n = 36)		VZV PCR (n = 12)		CMV PCR (n = 10)	
	positive	negative	positive	negative	positive	negative	positive	negative
FilmArray								
positive	14	3	4	0	1	0	0	0
negative	2	55	0	32	0	11	0	10
Overall rates of agreement	93.2% (69/74)		100% (36/36)		100% (12/12)		100% (10/10)	

Abbreviations: EV - Enterovirus, HSV - Herpes simplex virus, VZV - Varicella-zoster virus, CMV - Cytomegalovirus.

more appropriate to regard the culture result as a false negative. Another discordant result was that *E. coli* was isolated only in the culture method but not detected by FilmArray testing. The FilmArray assay only detects *E. coli* strains possessing the K1 capsular antigen, so no other *E. coli* strains can be detected. Therefore, this inconsistent result may have been due to the presence of an *E. coli* strain without K1. However, considering the other laboratory results of the patient, such as normal CSF parameters, the culture result could be regarded as a false positive (Table 6, Case 2). Other discordant cases in which *S. maltophilia*, *A. baumannii*, and *K. pneumoniae* were detected only in culture, indicate that culture is still a gold standard method and could not be

Table 6. Laboratory results of discrepant cases for bacterial pathogens.

Case	Age/ gender	CSF chemistry and cell count					Culture	Antigen for 4 bacteria [a]	Other PCR	FilmArray
		Glucose (mg/dL)	Protein (mg/dL)	Cell count (/μL)	Differential (%)					
					Segmented neutrophil	Lympho-cyte				
1	38/M	75	40.6	120	10	90	(-)	(-)	NT	*H. influenzae*
2	3/M	54	14.8	0	0	0	*E. coli*	NA	Eneterovirus PCR (-)	negative

[a] *- Haemophilus influenzae, Streptococcus agalactiae, Neisseria meningitides and Streptococcus pneumoniae.*

replaced by PCR for bacterial detection.

This study has limitations, in that it did not accurately verify the results of patients. The pathogens detected in the samples were not reconfirmed by other methods and only the results of the conventional and FilmArray tests were compared. In addition, the clinical information about patient pre-admission history or details regarding the medication used during hospitalization was not collected; only the laboratory results were evaluated. Nevertheless, there could be some significance in that this study showed what kind of tests were performed to diagnose CNS infection in a tertiary hospital and demonstrated that the FilmArray assay alone could increase the positive detection rate in CSF samples.

Since the FilmArray assay is a method that can be performed by the POCT concept, it has the advantage of dramatically reducing TAT by use as an in-hospital examination method. In the previous study, we investigated the laboratory efficacy of POCT multiplex PCR for the detection of respiratory viruses and experienced that the implementation of the POCT PCR improved the laboratory process and greatly reduced the waiting time from prescription to final reporting [13]. Additionally, we are analyzing the clinical effects of the introduction of multiplex testing on the patient prognosis, length of stay, and antibiotic usage rate.

Because CNS infections are associated with significant morbidity and mortality, the rapid detection of the pathogen is critical to appropriate patient care. Therefore, multiplex PCR of the POCT concept will have many effects on the clinical outcomes of patients with CNS infection such as the prognosis, duration of hospitalization, antibiotic usage rate. Further studies will be needed.

CONCLUSION

The detection rate of pathogens in CNS infections could be increased with only the FilmArray assay compared to using the entire conventional tests actually performed in patients with suspected CNS infection.

Declaration of Interest:
No conflict of interest declared.

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