New methods in meningitis diagnosis

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Summary

Meningitis remains a worldwide problem and central nervous system (CNS) infections are associated with devastating sequelae, including cognitive deficits, vision and hearing impairment, motor and sensory deficits and epilepsy in over one half of survivors. Rapid diagnosis of meningitis is essential to improve chances at survival and minimize unnecessary healthcare costs related to isolation procedures and empiric treatment. Multiplex molecular assays are an attractive option for the simultaneous detection of several microbial targets. Currently, several assays are marketed. The aim of our review is to comprehensively evaluate the molecular available systems of using a new multiplex polymerase chain reaction (PCR) panel in determining the microbiologic etiologies of meningitis.

Introduction

Meningitis remains a worldwide problem and central nervous system (CNS) infections are associated with devastating sequelae, including cognitive deficits, vision and hearing impairment, motor and sensory deficits and epilepsy in over one-half of survivors (25,17,14). The evaluation of suspected CNS infections is complex, as clinical signs and symptoms are often not specific to the causative pathogen; several criteria must be considered: clinical-radiographic evidence of meningitis includes at least two of the following signs: fever, headache, vomiting, nuchal rigidity, and bulging fontanelle as well as cerebro-spinal fluid (CSF) pleocytosis (5 or greater white cells/μL) or evidence of meningeal enhancement on brain imaging. In many suspected CNS infections, large volumes of CSF are required for diagnostic testing in according with national guidelines (12,18). In the African meningitis belt, bacterial pathogens such as *Neisseria meningitidis* and *Streptococcus pneumoniae* have historically been among the most common etiologies, while in Europe several pathogens are accountable for the disease; recurrence and increase of cases are reported in several European places. It is estimated that in up to 50% of encephalitis cases and up to 60% of meningitis cases, the etiology remains unidentified. Enteroviruses (EVs) and herpes simplex virus (HSV) are the most common causes of infectious meningitis and encephalitis; with HSV meningoencephalitis being a cause of high morbidity and mortality (12,7,3,1,22,24,21). The rates of bacterial meningitis have significantly declined since the widespread implementation of immunization programs. Bacterial meningitis is most commonly caused by *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae* type B. For some pathogens like as *Streptococcus pneumoniae* the introduction of pneumococcal conjugate vaccines has universally resulted in a decline in vaccine-serotype pneumococcal meningitis incidence throughout Europe and North America. However, serotype replacement by non-vaccine serotypes has been reported (13). In an immuno-compromised host, the broad spectrum of differential diagnoses creates the need for multiplex assays to streamline the diagnosis process. Rapid diagnosis of meningitis is essential to improve chances at survival and minimize unnecessary healthcare costs related to isolation procedures and empiric treatment. Multiplex molecular assays are an attractive option for the simultaneous detection of several microbial targets. Currently, several assays are marketed. The aim of our review is to comprehensively evaluate the molecular available systems of using a new multiplex PCR panel in determining the microbiologic etiologies of meningitis.

Systems overview

Nucleic acid tests (NAT) such as laboratory-based PCR are highly sensitive but require sophisticated laboratory infrastructure including biosafety cabinets to avoid contamination issues, as well as highly skilled laboratory technicians. Multiplex PCR panels have been developed for meningitis pathogens and syndromic diseases using PCR kits for array-based platforms (14-20 species per array); these platforms require the highest level of skill and laboratory

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infrastructure. In PCR, the DNA target is exponentially amplified through repeating three major steps: 1) denaturation of double-strand (ds) DNA into single-strand (ss) DNA; 2) annealing of primers to the complementary ss target sequences; and 3) extension of the primers in the 5' to 3' direction by heat-stable DNA polymerase to produce dsDNA molecules. The copy number of DNA molecules is doubled in each extension step, generating millions of copies of the original DNA molecules when PCR is completed. Because the method does not require live or intact cells, PCR is a valuable tool for detecting bacterial pathogenic agents from clinical specimens (19). At the other end of the spectrum, point-of-care (POC) nucleic acid tests are fully automated and can be run in a near-patient laboratory by clinic staff after moderate training, though POC meningitis assays are currently available only for tuberculosis meningitis. Latex agglutination tests (LATs) are available in most countries at district level laboratories (level 2), as they require laboratory equipment (refrigerator, centrifuge) and cold storage, but in several countries these tests are not recommended for diagnostic use (4).

Immuno-chromatographic test

For the diagnosis of meningococcal meningitis, duplex dipsticks have been developed in order to enable identification of four different serogroups of *N. meningitidis (CERMES)* using a cerebrospinal fluid (CSF) sample obtained by lumbar puncture. A similar technique has been developed for *S. pneumoniae* with BinaxNOW immunochromatographic test. This is an in vitro rapid immunochromatographic assay for the detection of S. *pneumoniae* antigen in the urine of patients with pneumonia and in the cerebral spinal fluid (CSF) of patients with meningitis (11).

LATs

Pastorex™ LAT is available as a kit which consists of colored latex particles coated with mouse monoclonal or rabbit polyclonal antibodies for the direct qualitative detection and identification of *Neisseria meningitidis a/b/c/y/w135; E. coli* K1; *Haemophilus influenzae* type B; *Streptococcus pneumoniae*; and group B S*treptococci*. *Meningococcus* group B antigen, being structurally and immunologically related to *E. coli* K1 antigen, is provided as a single test latex reagent. Depending on the age of the child, a positive reaction suggests a different diagnosis. If found positive on a sample taken from a newborn, an *E. coli* K1 infection would be more probable, while in older children *Meningococcus* group B is a more likely infection. Most of the commercially available LAT kits include reagents to test the most common bacteria causing meningitis. A wide range of sensitivity and specificity has been reported in various studies from 60% to 93% for the various organisms (2).

Molecular assays

BioFire Diagnostics FilmArray™ meningitis/encephalitis panel

Multiplex molecular assays are an attractive option for the simultaneous detection of several microbial targets. They are now routinely used for bloodstream, respiratory, and gastrointestinal infections. In October 2015, the U.S. Food and Drug Administration approved the FilmArray™ meningitis/encephalitis (ME) panel (BioFire® Diagnostics, Salt Lake City, USA), the first multiplex PCR panel for the detection of CNS infections. This assay test the presence of 14 pathogens known to cause meningoencephalitis, including six bacteria, seven viruses, and one yeast (*Escherichia coli* K1, *H. influenzae*, *Listeria monocytogenes*, *N. meningitidis*, *Streptococcus agalactiae*, *S. pneumoniae*, cytomegalovirus (CMV) enterovirus (EV), herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), human herpesvirus 6 (HHV-6), human parechovirus (HPeV), varicella-zoster virus (VZV), and *Cryptococcus neoformans/C. gattii*) (3,8,9,16,23,15). The ME panel requires 200 microliters (µL) of CSF and has a running time of ~1 h. To briefly explain the method, 200 µL of uncentrifuged CSF and hydration solution are drawn into the FilmArray ME reagent pouch by vacuum. The reagent pouch is then placed in a FilmArray instrument and tested. According to the manufacturer, the FilmArray™ ME panel has been tested and cleared for the examination of CSF specimens stored under refrigeration (4°C) for up to 7 days. Several multicentric studies reported 84.4% positive agreement and >99.9% negative agreement between the ME panel and routine methods. Therefore, the FilmArray™ ME panel tests for 14 of the most common causes of infectious meningitis/encephalitis and may provide a rapid, front-line diagnostic option for laboratories that do not have the capacity to perform routine real-time PCR and currently rely on send-out testing (21,10)

Seegene Allplex™ meningitis panel

Simultaneous detection and identification of 18 meningitis pathogens including 6 bacteria (*Escherichia coli* K1, *Streptococcus pneumoniae, Streptococcus agalactiae, Neisseria meningitidis, Haemophilus influenzae* type B*, Listeria monocytogenes*) and 12 viruses (HSV1, HSV2, HHV7, VZV, EBV, CMV, HHV6, enterovirus, human parechovirus, Mumps virus, parvovirus B19, adenovirus,) using multiplex one-step real-time RT-PCR. The system is based on three different panels that the technicians choose for the detection of different pathogens. The ME panel requires 300 uncentrifuged microliters µL) of CSF with a run time of ~2.5 h. The lowest detection limits ranged from 10^1 copies/µL to 5×10^1 copies/µL. In conclusion, the Seegene Allplex Meningitis detection kit showed high sensitivity and specificity for the most common bacterial and viral agents causing acute meningitis. A high detection rate was observed in the CSF samples obtained from patients clinically diagnosed with acute meningitis (20,6).

Conclusions

CSF culture is crucial to the diagnosis of meningitis in all microbiology labor worldwide. Rapid molecular diagnostic systems were successfully implemented with a decrement of cost for new tests. Viral meningitis can be now diagnosed during routine clinical practice, within optimal times. To conclude, inclusion of molecular test for the detection of bacterial and viral in the routine diagnosis is a valuable adjunct in the rapid and accurate diagnosis of meningitis and direct detection of nucleic acids from CNS samples. Moreover, it is less prone to common causes of false-positive.

References

1. Bilal A, Taha MK, Caeymaex L,et al. Neonatal meningococcal meningitis in France from 2001 to 2013. Pediatr Infect Dis. J 2016;35:1270-2.
2. Brigtsen AK, Dedi L, Melby KK, et al. Comparison of PCR and serotyping of group B Streptococcus in pregnant women: the Oslo GBS-study. J Microbiol Methods. 2015;108:31-5.
3. Cailleaux M, Pilmis B, Mizrahi A, et al. Impact of a multiplex PCR assay (FilmArray®) on the management of patients with suspected central nervous system infections. Eur J Clin Microbiol Infect Dis. 2020;39:293-7.

4. Campbell S, Landry ML. Rapid antigen tests, p. 23-41. In: Advanced techniques in diagnostic microbiology. Springer, Boston, USA, 2014, 2nd edition.
5. Camuset G, Picot S, Jaubert J, et al. Invasive group B streptococcal disease in non-pregnant adults, Réunion Island, 2011. Int J Infect Dis. 2015;35:46-50.
6. Chan M, Koo SH, Jiang B, et al. Comparison of the Biofire FilmArray Respiratory panel, Seegene AnyplexII RV16, and argene for the detection of respiratory viruses. J Clin Virol. 2018;106:13-7.
7. D'Ovidio C, Pompilio A, Crocetta V,et al. Fatal sepsis by Klebsiella pneumoniae in a patient with systemic lupus erythematosus: the importance of postmortem microbiological examination for the ex post diagnosis of infection. Int J Legal Med. 2015;129:1097-1101.
8. Du B, Hua C, Xia Y, et al. Evaluation of the BioFire FilmArray meningitis/encephalitis panel for the detection of bacteria and yeast in Chinese children. Ann Transl Med. 2019;7:437.
9. Garcia-Clemente P, Menendez-Suso JJ, Falces-Romero I, et al. Utility of FilmArray ME panel for prompt Neisseria meningitidis detection in non-cerebrospinal fluid samples - a case report. J Infect. 2020;80:121-42.
10. Gonzalez-Donapetry P, Garcia-Rodriguez J, Cendejas-Bueno E. A case of a FilmArray ME false negative in meningococcal meningitis. J Infect. 2019;79:277-87.
11. Haddar CH, Terrade A, Verhoeven P, et al. Validation of a new rapid detection test for the detection of Neisseria meningitidis A/C/W/X/Y antigens in cerebrospinal fluid. J Clin Microbiol. 2020;58:e01699-19.
12. Hodeib S, Herberg JA, Levin M, Sancho-Shimizu V. Human genetics of meningococcal infections. Hum Genet. 2020;139:961-80.
13. Kalenahalli KJ, Kumar NA, Chowdary KVA, Sumana MS. Fatal swine influenza A H1N1 and mycoplasma pneumoniae co-infection in a child. Tuberk Toraks. 2016;64:246-9.
14. Lucas MJ, Brouwer MC, van de Beek D. Neurological sequelae of bacterial meningitis. J Infect. 2016;73:18-27.
15. Mina Y, Schechner V, Savion M, et al. Clinical benefits of FilmArray meningitis-encephalitis PCR assay in partially-treated bacterial meningitis in Israel. BMC Infect Dis. 2019;19:713.
16. Nabower AM, Miller S, Biewen B, et al. Association of the FilmArray meningitis/encephalitis panel with clinical management. Hosp Pediatr. 2019;9:763-9.
17. Piccini G, Torelli A, Gianchecchi E, et al. Fighting Neisseria meningitidis: past and current vaccination strategies. Expert Rev Vaccines. 2016;15:1393-407.
18. Putz K, Hayani K, Zar FA. Meningitis. Prim Care. 2013;40:707-26.
19. Satilmis S, Ulger Toprak N, Ilgin C, Soyletir G. Evaluation of direct 16S rRNA PCR from clinical samples for bacterial detection in normally sterile body sites. J Infect Dev Ctries. 2019; 13:978-83.
20. Shin SY, Kwon KC, Park JW, et al. Evaluation of the Seeplex meningitis ACE detection kit for the detection of 12 common bacterial and viral pathogens of acute meningitis. Ann Lab Med. 2012;32:44-9.
21. Sorek N, Ashkenazi S, Livni G, Ben-Zvi H. Neisseria meningitidis and cytomegalovirus simultaneous detection in the filmarray meningitis/encephalitis panel and its clinical relevance. IDCases. 2019;17:e00516.
22. Strelow VL, de Miranda E, Kolbe KR, et al. Meningococcal meningitis: clinical and laboratorial characteristics, fatality rate and variables associated with in-hospital mortality. Arq Neuropsiquiatr. 2016;74:875-80.
23. Suarez-Fernandez S, Iturzaeta A, Rodriguez-Lucas C. Usefulness of FilmArray meningitis/encephalitis panel in the management of an uncommon case of herpes simplex virus type 2 meningitis. Rev Esp Quimioter. 2019;32:477-8.
24. Tang LM, Chen ST. Klebsiella pneumoniae meningitis: Prognostic factors. Scand J Infect Dis. 1994;26:95-102.
25. Tian R, Hao S, Hou Z, et al.. The characteristics of post-neurosurgical bacterial meningitis in elective neurosurgery in 2012: a single-institute study. Clin Neurol Neurosurg. 2015;139:41-5.