THE PATHCARE NEWS

Bacterial Meningitis Multiplex PCR

PathCare is introducing a multiplex PCR test for the detection of the 5 most common bacterial causes of meningitis in cerebrospinal fluid (CSF): *Streptococcus pneumoniae, Haemophilus influenzae* type b, *Neisseria meningitidis, Streptococcus agalactiae* (Group B beta-hemolytic streptococcus) and *Listeria monocytogenes*.

Early diagnosis of the causative organism of meningitis is important for appropriate treatment. Patients are however often treated with antibiotics before hospital admission or CSF collection. The early administration of antibiotics is crucial, but inhibits the growth of bacteria in the specimen leading to negative cultures. Antibiotic therapy can sterilize the CSF within a few hours. Similarly the glucose and protein levels can begin to normalise. The CSF white blood cell count does not change significantly within 24hrs. Conventional diagnostic tests, including Gram stain, culture and bacterial antigen tests have limited diagnostic sensitivity and specificity. The bacterial multiplex PCR offers high sensitivity and specificity for identifying bacterial pathogens causing meningitis. According to a validation study by Shin *et al*. this method identified the causative pathogens in 44 of 78 (56.4%) patients who were clinically diagnosed with acute meningitis. The PCR method detected the causative pathogens in 37 cases (47.4%) that tested negative in the conventional diagnostic tests.[1]

The bacterial multiplex PCR will be done on request from 0.5 ml CSF (see revised request form). In order to introduce the test, clinicians will be contacted when the test may be indicated, namely culture-negative CSF samples with a high likelihood of bacterial meningitis based on the following CSF findings:[2,3]

- ≥ 1000 white blood cells (wbc) per µl (non-traumatic tap)
- predominance of neutrophils
- protein ≥ 0.8 g/l

In 99% of patients with bacterial meningitis >100 wbc/µl is present in the CSF. In the majority (87% of patients) very high white cell counts of >1000 wbc/µl is observed. In early bacterial meningitis a pre-dominance of lymphocytes may be seen.
A traumatic tap will lead to an artificial increase in white blood cells by one WBC for every 500 - 1000 red blood cells in the CSF.

Our validation of this new PCR test showed excellent sensitivity and specificity. The limit of detection (minimum number of organisms required for detection) for the most common causes: *S. pneumoniae, H. influenzae and N. meningitidis* is low, but a higher number of organisms is required to detect *S. agalactiae* and *L. monocytogenes*. Specimens containing fewer organisms than the limit of detection may therefore yield a false negative PCR result.

References:

1. 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. Annals of Laboratory Medicine 2012; 32: 44-49.
2. Boyles TH, Bamford C, Bateman K et al. Guidelines for the management of acute meningitis in children and adults in South Africa. South Afr J Epidemiol Infect 2013; 28(1): 5-15.
3. Seehusen DA, Reeves MM and Fomin DA. Cerebrospinal fluid analysis. American Fam Physician 2003; 68: 1103-1108.

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DIE PATHCARE NUUS

Bakteriële Meningitis Multipleks PKR

PathCare stel 'n multipleks-PKR toets vir die aantoon van die 5 algemeenste bakteriële oorsake van meningitis in serebrospinale vog (SSV) bekend: *Streptococcus pneumoniae, Haemophilus influenzae* tipe b, *Neisseria meningitidis, Streptococcus agalactiae* (Groep B beta-hemolitiese streptokokke) en *Listeria monocytogenes*.

Vroeë diagnose van die oorsaak van meningitis is belangrik vir toepaslike behandeling. Pasiënte word egter dikwels voor hospitaaltoelating en die versameling van 'n serebrospinale vog monster met antibiotika behandel. Die vroeë toediening van antibiotika is noodsaaklik, maar inhibeer die groei van bakterieë en dit lei dan tot negatiewe kwekings. Antibiotika terapie kan die SSV binne 'n paar ure steriliseer. Soortgelyk sal die glukose- en proteïenvlakke ook begin normaliseer. Die SSV-witseltelling verander nie binne 24 uur betekenisvol nie. Konvensionele toetse soos die Gram-kleuring, kweking en bakteriële antigeentoetse het beperkte diagnostiese sensitiwiteit en spesifisiteit. Die multipleks-PKR bied 'n hoë sensitiwiteit en spesifisiteit vir die identifisering van bakteriële patogene wat meningitis veroorsaak. Volgens 'n studie deur Shin *et al.* het hierdie metode die veroorsakende patogene in 44 uit 78 (56.4%) pasiënte wat klinies met akute meningitis gediagnoseer is, aangetoon. Die PKR-metode kon die patogeen vir 37 (47.4%) gevalle wat met konvensionele diagnostiese toetse negatief getoets het, aantoon.[1]

Die bakteriële multipleks-PKR sal op aanvraag gedoen word op 0.5 ml SSV (sien gewysigde aanvraagvorm). Met bekendstelling van die toets sal klinici gekontak word wanneer hierdie toets aangedui mag wees, naamlik kweking-negatiewe SSV-monsters met 'n hoë waarskynlikheid van bakteriële meningitis gebaseer op die volgende SSV-bevindings:[2,3]

- ≥ 1000 witbloedselle (wbs) per µl (nie troumatiese tap)
- oorwegend neutrofiele
- proteïen ≥ 0.8 g/l

In 99% van pasiënte met bakteriële meningitis is >100 wbs/µl in die SSV teenwoordig. In die meerderheid, 87% van pasiënte, word baie hoë witseltellings van >1000 wbs/µl aangetoon. In vroeë bakteriële meningitis mag oorwegend limfosiete waargeneem word. 'n Troumatiese tap lei tot 'n kunsmatige verhoging in die witbloedselle: een wbs vir elke 500 - 1000 rooibloedselle in die SSV.

Bevestiging van hierdie nuwe PKR-toets by ons laboratorium toon uitstekende sensitiwiteit en spesifisiteit. Selfs met lae organismetellings kan die algemeenste veroorsakende organismes: *S. pneumoniae, H. influenzae* en *N. meningitidis* aangetoon word, maar 'n hoër organismetelling is nodig vir die aantoon van *S. agalactiae* en *L. monocytogenes*. Monsters met 'n organismetelling laer as die opsporingslimiet kan dus tot 'n vals negatiewe PKR resultaat aanleiding gee.

Bronne:

1. 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. Annals of Laboratory Medicine 2012; 32: 44-49.
2. Boyles TH, Bamford C, Bateman K et al. Guidelines for the management of acute meningitis in children and adults in South Africa. South Afr J Epidemiol Infect 2013; 28(1): 5-15.
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