LABORATORY INVESTIGATION AND DIAGNOSIS OF MENINGITIS AND MENINGOCOCCAL SEPTICAEMIA

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Though overall numbers of cases of meningitis and meningococcal septicaemia have been rising in recent years, there has been a dramatic fall in cases of Hib meningitis following the successful introduction in 1992 of conjugated Hib vaccines into the UK childhood immunization schedule. The major UK bacterial meningitis pathogens are now the meningococcus and the pneumococcus. The high incidence of meningococcal infection is due to the simultaneous occurrence of high-rates of hyperendemic serogroup B disease, on which is superimposed a historically high level of serogroup C infections, the latter especially marked in teenagers and adults. The burden of meningococcal disease is expected to fall by about a third, as new serogroup C conjugated vaccines which were introduced into the UK childhood immunization schedule in the autumn of 1999 have their desired effect. Meningococcal disease case fatality rates are falling, in contrast with those of pneumococcal and other types of bacterial meningitis. Other bacteria causing appreciable numbers of cases of meningitis include *M. tuberculosis*, group B streptococci, *Esch. coli*, *Listeria monocytogenes* and staphylococci; the latter associated particularly with post-surgical infection.

Meningococci retain almost universally sensitivity to benzylpenicillin, chloramphenicol and third generation cephalosporins; however a small, but rising, percentage of UK invasive pneumococcal isolates show either reduced sensitivity to benzylpenicillin, or even outright resistance.[1] This trend has worrying implications, not only for the management of culture-confirmed pneumococcal meningitis, but also for the choice of empirical antibiotic therapy when bacterial meningitis is clinically likely but a specific pathogen has not been incriminated.[2]

Most patients with meningococcal meningitis have evidence of blood-borne infection – a positive blood culture, a vasculitic rash or both – but a minority of patients with invasive meningococcal infection present with septicaemia unaccompanied by meningitis. The illnesses form a clinical continuum. The highest attack rates are in young children, especially infants, with a second peak of infections occurring in late teenage and early adulthood. In contrast, pneumococcal meningitis may occur at any age, though the highest age-specific attack rates are also found in the first years of life. Though absolute numbers of cases are small, the peak attack rate is seen in the first few weeks of life, and is associated with infection acquired from the maternal birth canal.

Meningococci, which colonise only Man, are carried in the nasopharynx by about 10% of individuals. Carriage rates are low in young children, peak at about 30% in the late teenage years, then fall again with age. The peak prevalence of carriage of pneumococci is in young children but adults are also frequently colonised. Though there are 13 serogroups of meningococci, only two (serogroups B and C) are commonly associated with invasive disease in this country. There are more than 80 serotypes of pneumococci but, similarly, a much smaller number of serotypes are associated with invasive disease and meningitis.

Patients with fever and a vasculitic rash should be treated as cases of suspected meningococcal infection until proved otherwise. The first doctor to suspect bacterial meningitis or meningococcal septicaemia should give an immediate parenteral dose of benzylpenicillin, or other suitable antibiotic, and arrange immediate admission to hospital. Management of these conditions is a medical emergency. The outcome is critically dependant on rapidity of diagnosis and management. It has been suggested that GPs should take a throat swab and / or a set of blood cultures at this time, but this presents some logistic difficulties and is probably not necessary. Diagnosis of meningitis and meningococcal infection can be extremely difficult and about 30% of patients are seen by their GP on more than one occasion before the diagnosis is suspected and the patient admitted to hospital.

Once in hospital, urgent evaluation is required. Intravenous access should be established and parenteral antibiotic treatment commenced as quickly as possible. These steps should not be deferred until after the results of initial investigations are available, nor should they await lumbar puncture.

RECENT CHANGES IN THE INVESTIGATION OF PATIENTS WITH SUSPECTED BACTERIAL MENINGITIS OR MENINGOCOCCAL SEPTICAEMIA

Following repeated advice from the Chief Medical Officer[3,4] and demonstration of significantly reduced mortality, not only in meningococcal infection[5] but probably also in other types of bacterial meningitis,[6] increasing proportions of patients with suspected meningitis or meningococcal infection are now being treated by their GP with a pre-hospital dose of benzylpenicillin or other parenteral antibiotic.[7] Although this is good for patient care, it has had the additional effect of reducing the chances of recovering the causative pathogen particularly from blood, though also to a lesser extent from CSF.[8] Although some 30% of patients with suspected meningococcal infection now receive a dose of benzylpenicillin prior to hospital admission, this proportion is too low. Further education of patients and professionals is an urgent priority to improve on this figure.

In hospital, there has been a trend away from CSF sampling through the use of lumbar puncture, particularly by paediatricians.[7] This is probably because of an increased appreciation of the risk of brainstem herniation through the foramen magnum ('coning'), with frequently fatal results. No prospective studies have been carried out to determine whether the risk of coning is increased by lumbar puncture (nor would such studies be likely to receive ethical approval), but the strong temporal association between lumbar puncture is well recognised and is persuasive of a causal

association. The risk is highest when there is raised intracranial pressure. The absence of papilloedema does not exclude the possibility of raised intracranial pressure, present for only a short duration prior to the opthalmologic examination.

There are, in addition, diagnostic pitfalls associated with the interpretation of the findings from an examination of CSF, especially with regard to apparently 'negative' specimens. In about 7% of cases of suspected meningococcal meningitis in which a lumbar puncture is undertaken, and from which a meningococcus is subsequently grown from the CSF, the initial examination of the CSF reveals absolutely no abnormality: no cells, no organisms seen, normal glucose and normal protein. It is assumed that in these cases meningococci have by chance arrived in the subarachnoid space only for a few minutes prior to lumbar puncture, and that there has been insufficient time for the body to mount a cellular immune response. Thus a 'negative' lumbar puncture in the presence of symptoms and signs suggestive of meningitis should never be used as a reason to justify withholding antibiotic and other appropriate treatment.[7]

With the increasing emphasis on pre-hospital antibiotic treatment of cases of suspected meningococcal infection, the need for improved non-culture diagnostic methods became apparent in the late 1980s and PCR diagnostic tests were developed at that time.[9,10] They have subsequently been refined and are now widely available to clinicians throughout the UK. Meningococcal PCR diagnostic services provided by national reference laboratories have become heavily used;[11] last year they accounted for about a third of all microbiologically confirmed meningococcal disease cases. The proportion of meningococcal disease cases confirmed by PCR is rising rapidly each year. Pneumococcal PCR tests are less well developed but are now close to the point at which they may also become widely available.

The technique of antigen detection by latex agglutination has also been re-examined and sensitivity has been greatly enhanced by the use of ultrasound to stimulate better and faster agglutination.[12]

WHY ATTEMPT TO MAKE A SPECIFIC BACTERIOLOGICAL DIAGNOSIS IN MENINGITIS?

Identification of the specific cause of infection greatly aids patient and contact management by:

- excluding other possible pathogens;
- (often) providing a bacterial isolate for antimicrobial sensitivity testing;
- identification of the bacterial aetiology, allowing an educated guess on antimicrobial sensitivity;
- assisting in the management of contacts;
- providing important epidemiological information that contributes to the national database.

If a lumbar puncture is not undertaken, it is especially important that clinicians let their microbiological and public health medicine colleagues know of the admission of any patient with suspected bacterial meningitis or meningococcal septicaemia.

All significant isolates from cases of bacterial meningitis and meningococcal septicaemia should be sent to a national reference laboratory for more detailed characterisation.

MAKING A DIAGNOSIS

Blood microscopy

Examination of plain blood films or buffy coats will often reveal intracellular bacteria in septicaemic patients.[13] This technique is not routinely used today, although there is no good clinical reason for omitting this simple, but diagnostically helpful, investigation.

Blood cultures

A peripheral white blood cell (total and differential) count can be obtained rapidly. The total count is usually high, due to large numbers of circulating polymorphonuclear leucocytes. Blood cultures should be collected, and will be positive in 50% or more of patients who have not already received a dose of parenteral benzylpenicillin. If a dose of parenteral antibiotic has been given, then blood cultures are usually sterile. Blood cultures should be incubated for several days, though most positives will have declared themselves within the first 48 hours. Pneumococci sometimes produce a characteristic haemolysed appearance in blood culture bottles but then cannot be recovered on sub-culture. This haemolysed appearance is sufficiently characteristic to give a strong indication of the likely bacterial aetiology. It should be possible to confirm a pneumococcal diagnosis in such circumstances by antigen or DNA methods.

Blood or serum PCR

Specific and sensitive tests for the detection of meningococcal DNA are now widely available throughout the UK. The UK Meningococcal Reference Laboratories offer a routine PCR service with a rapid turn-around time. The Manchester Public Health Laboratory serving England and Wales carries out a screening PCR based on a unique DNA sequence within the *ctrA* gene, with all positive samples subjected to a second test based on the *saiD* gene. The latter target, though less sensitive than the *ctrA* PCR, if positive, provides serogroup identification of the causative meningococcus.[14] Use of the PCR test has grown tremendously in the last four years with 16,000 samples anticipated per annum by the Manchester laboratory.

The best blood specimen is the first EDTA haematology sample following admission of the patient to hospital. Meningococcal DNA is cleared rapidly from the circulation, and specimens more than 6-12 hours after commencement of effective antibiotic treatment are much less likely to yield a positive result. Currently, pneumococcal PCR is confounded by the occurrence of false positive results, especially in children who are colonised with pneumococci,[15] though it may prove possible to overcome this problem. Most patients with pneumococcal meningitis are also bacteraemic.

Examination of cerebrospinal fluid (CSF)

Despite the hazards of lumbar puncture, examination of CSF offers the best chance of confirming the diagnosis and establishing the microbial aetiology in suspected bacterial meningitis. Its use should be considered in all cases of suspected bacterial meningitis. Lumbar puncture should not be undertaken in the presence of raised intracranial pressure, or in meningococcal septicaemia where it may provoke or exacerbate instability. Fairly obviously, the CSF shows no abnormality in meningococcal septicaemia unaccompanied by meningitis, a presentation encountered

in 10-20% of patients suffering from meningococcal disease.

CSF should be examined immediately for the presence of cells and bacteria. In bacterial meningitis, polymorphs usually dominate the cellular response. In occasional cases of bacterial meningitis polymorphs are seen in the CSF, and a purely lymphocytic cellular response is observed. Acridine orange staining may offer advantages of sensitivity over Gram staining.[16] Glucose and protein levels should be determined, and the CSF glucose level compared with the blood glucose level in a sample taken at about the same time. In bacterial meningitis, the CSF glucose is usually less than 70% of the blood glucose level and the CSF protein level is normally raised. The CSF lactate level distinguishes viral from bacterial meningitis, but may not be any more sensitive than other methods.[17]

Antigen testing can be a useful adjunct to microscopy and culture, though antigen tests for serogroup B meningococci, the commonest cause of bacterial meningitis, are unfortunately the least sensitive; results of antigen testing do not often affect clinical management.[18] Studies of the use of ultrasound enhanced latex agglutination tests on CSF have yet to be reported, but this technique holds scope for improvement in the sensitivity of the test.[12]

CSF should be cultured on blood and chocolate agar plates incubated in 5% CO_2 for at least 48 hours. If any bacteria are visible on microscopy, direct antibiotic sensitivity tests should be carried out. If tuberculosis is suspected, additional appropriate culture media should be inoculated. Visualisation and culture of tubercle bacilli from CSF can be very difficult.

CSF is the best and easiest specimen to use for PCR testing. Fewer PCR inhibitors are found than in blood, and the DNA copy number is often high. Although meningococcal DNA is cleared quickly from the bloodstream, it persists for some days in the CSF. Therefore, late lumbar puncture and examination of CSF by PCR is worth considering in patients suspected of meningococcal infection in whom a diagnosis has not been confirmed after the first 48 hours. By this stage of the illness, the clinical condition of most of the patients will be stable, intracranial pressure will be normal or near normal, and lumbar puncture is a safer procedure.

Swabs of the posterior pharyngeal wall

Young children rarely carry meningococci in their throat, and thus the isolation of a well-capsulated serogroup B or C meningococcus from the posterior nasopharyngeal wall of an infant or a young child with symptoms and signs of invasive meningococcal infection is highly significant. Careful swabbing of the posterior pharyngeal wall, directly behind the uvula, followed by immediate plating out of the swab (even at night) should be part of the diagnostic routine in all young children with suspected meningococcal infection. Highest yields are obtained from swabs in which the posterior pharyngeal wall is accessed via the mouth, but if the patient is too young, or too confused to co-operate, the per-nasal route is a very acceptable access. The positivity rate is close to 50% if swabbing is carried out carefully and isolation rates are essentially unaffected by prior antibiotic treatment.[8]

Meningococcal nasopharyngeal colonisation is common in teenagers and young adults (20-30%) and therefore the significance of a meningococcus in the nasopharynx is more difficult to determine. However, because carriage of capsulated, disease-causing strains of meningococci is still infrequent (serogroup B) or rare (serogroup C), the finding of such a capsulated strain is important and is likely to be significant in a patient with clinical signs and symptoms suggestive of invasive meningococcal infection. Meningococci isolated from the posterior pharyngeal wall are phenotypically indistinguishable from the invading strain in more than 95% of cases.

Swabbing the posterior pharyngeal wall is of much less value if pneumococcal meningitis is suspected. Carriage is very common in the early years of life and is still frequent in older children and adults. Isolation of a pneumococcus from the nasopharynx does not provide helpful information in the investigation of suspected meningitis at any age except within the first month of life.

The parents and siblings of young children with meningococcal infection are often colonised with meningitis.[19] Such meningococcal strains are usually, though not invariably, phenotypically indistinguishable from the disease-causing strain. Though not recommended routinely, swabbing first-degree relatives of an infant or young child with suspected meningococcal disease is worthy of serious consideration if the child has already received a pre-admission dose of benzylpenicillin and if lumbar puncture is not contemplated. If swabbing of relatives is undertaken, careful counselling of any identified meningococcal carriers should be undertaken, to avoid inducing feelings of guilt and responsibility for the child's illness.

Aspiration of skin rash in meningococcal infection

This technique of needle aspiration of a haemorrhage spot in the skin appears to have been used more widely in the past. Though there is comparatively little contemporary experience, there seems no doubt that if this is done carefully and practiced regularly, it adds significantly to the chance of confirming a diagnosis of invasive meningococcal infection.[20,21] Purpuric skin lesions should be aspirated, not scraped. Meningococci may be detected by microscopy (only intracellular diplococci should be considered significant), by culture, or (probably) by PCR.

Urine

Urine antigen detection tests for meningococci and pneumococci have not achieved popularity to date. Ultrasound enhanced latex tests and PCR tests also require further evaluation.

Near patient testing

There is some commercial interest in developing a dipstick test that would detect reliably the presence of meningococci in peripheral blood. Such a test would make life easier for the GP when faced with a child most probably suffering from a viral respiratory infection but in whom meningococcal infection cannot be excluded. In the future such tests may be based on DNA chip technology.

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