Sudanese Journal of Paediatrics 1984; 3: 10-19

Review Article

LABORATORY DIAGNOSIS OF MENINGITIS: AN OVERVIEW IN RELATION TO THE SUDAN.

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INTRODUCTION

Sudan lies within the meningitis belt and meningococcal disease has been one of its major problems throughout the twentieth century. Over 12,000 and 55,000 cases were recorded in the years 1935 and 1950 respectively; and during the period 1968-1980, over forty thousands (40,513) were inflicted with the disease of whom 1,220 have died[1]. The magnitude of the problem in the paediatric age group could easily be imagined in a country where almost half of its population (≅ 20 millions) are below 16 years of age. During the first 6 months of the 1980 outbreak, 1,643 children (age group 1-14 years) had meningitis from a total of 3,800[1]. Over 60% of those cases occurred in Khartoum Province which has the largest crowded residential areas in the country.

The establishment of the diagnosis early in the course of the disease is not only important from an epidemiological point of view - through highlighting the need for specific immunization for prevention as it is the case in meningococcal meningitis - but also matters for every patient in such a life-threatening infection. The clinical signs and symptoms of meningeal disease - though of great help are nonspecific. Fever, bulging fontanell, nuckal rigidity, Kerning's sign and Brudzniski's sign only suggest an intracranial pathology. They can result from tuberculous, fungal and aseptic meningitis and can be the manifestation of a brain abscess or a tumour[2]. In the tropics, other diseases like malaria may

closely simulate the disease. In a study of 117 patients with meningitis admitted to the medical wards of Muhimbili Medical Centre at Dar es Salaam, the disease was considered initially in only 40 (34%) patients. Twenty-nine (25%) cases were labelled as malaria while the remaining patients either had no referral diagnosis or were thought to have other problems. The impact of such a delay in diagnosis is even more harmful in children with meningitis. It was found to influence the frequency of neurological sequelae in Ethiopian children and the presence of neurological abnormality at the time of diagnosis was found to increase the case fatality rate significantly[4].

LABORATORY DIAGNOSIS

The diagnosis of meningitis depends upon careful examination of cerebrospinal fluid (SCF) obtained by lumbar puncture. The SCF specimen is probably one of the most critical specimens processed by a laboratory since it is obtained throught a potentially - though negligibly - hazardous procedure, requires skill to undertake and might lead the way to the correct management of a life-threatening disease. So the chance should be taken to extract maximum information when undertaking a lumbar puncture.

CSF pressure, proteins and glucose

Measurement of CSF pressure is an important component to examine since it reflects the degree of intracranial pressure. When found to be considerably high, care should be taken not to draw too much of the fluid. The colour of CSF is also a helpful guide, whether turbulent as it is in the usual case of pyogenic meningitis or xanthochromic following subarachnoid haemorrhage or when pus is present in considerable amounts.

CSF proteins are moderately increased in bacterial meningitis usually to a level below 0.2 g/100 ml. However, such an increase may accompany other inflammatory diseases of the nervous system such as encephalitis and poliomyelitis. On the other hand, remarkable depression of CSF glucose and the ratio of CSF to blood glucose

levels (normally about 66%) is highly indicative of bacterial meningitis. CSF glucose might even be absent in some cases.

CSF lactate

The increase in CSF lactate concentration in patients with bacterial meningitis was noticed as early as in the 1920's[5] and was found later to be associated with anaerobic glycolysis of cerebral tissue[6] possibly due to decreased cerebral blood flow and cerebral hypoxia[7]. The value of this investigation was emphasized in earlier studies and was reported to be helpful in the early and differential diagnosis of bacterial meningitis[8,9]. However, subsequent investigations observed the occasional occurrence of normal lactate level in the presence of septic meningitis[10] whereas others[11] completely doubted its use. In a study of 185 specimens of CSF including those of 29 patients with culture-proven bacterial meningitis, Rutledge et al[11] reported the predicitive value of increased CSF lactate concentration (i.e. greater than 3 mMol/l) to be 26.7%, the remainder being false positives. They also observed in an experimental model that only after 3 days of meningitis did the CSF lactate concentration increase above 3 mMol/l suggesting that the lactate measurement has no role in early diagnosis of bacterial meningitis.

Cytology

The normal CSF contains a small number of cells, usually not exceeding 5 lymphocytes. To examine for the number and identify the different types of cells that reflect the pathological state, the fluid should be examined immediately. After centrifugation, a counter chamber is used to define the total number of white blood cells (WBCs) and a differential cell count is performed on a Wright-stained smear of the sediment. Pyogenic organisms excite mainly polymorphonuclear leucocytosis. Infections with neurotropic viruses present with mononuclear pleocytosis although polymorphonuclear cells might be observed during the acute stage of the latter disease. The number of polymorphonuclear cells present in pyogenic meningitis are usually very large; but this is not always the case. Children admitted rather early in

the course of the disease may have a normal CSF initially, which if repeated later due to deterioration of the patient would reveal meningitis[12]. Moreover, cases of bacterial meningitis have been reported who showed no meningeal signs and had relatively low SCF pleocytosis. Sixteen (1.5%) of the 1,064 cases of bacterial meningitis beyond the neonatal period - reviewed by Gieseler and Nelson[13] - had no meningeal signs during their entire hospitalization. In nine of those (56%), the first lumbar puncture disclosed fewer than 1,000 WBCs whereas in seven (44%) the CSF contained < 500 WBCs/mm^3.

Direct microscopy

Direct microscopy of stained CSF, although not specific, is a sinsitive method that guides treatment and could give a rapid answer. The yield obtained by Gram stain in cases of pyogenic meningitis was reported to be about 75%[11,14]. However, its interpretation might prove difficult in mixed infections[15].

Cultural methods

The culture technique in meningitis is a well-established diagnostic tool. A specimen of CSF fluid should be cultured on a blood agar plate, a chocolate agar plate, on Fildes or Leventhal media and in broth. However, pretreatment tends to render sterile the CSF fluid; and this was mostly found in pneumococcal and meningococcal disease[2]. This presents a significant problem in the Sudan; since inadvertent intake of antibiotics by the patients before reporting to the hospital was found to be a major handicap in identifying the organisms during epidemics of infections diseases in Sudanese children. During an outbreak of diphtheria in Khartoum that involved 107 children, only 41 cases (38.3%) could be bacteriologically proven. Cultures were negative in the remaining 66 patinets of whom 43 (40.3%) had received antibiotics before reporting to hospital[16]. In the study of Sippel et al[1] of the 1980 cerebrospinal meningitis (CSM) outbreak in Khartoum Province, only 25% of 114 CSF specimens of patients with probable CSM gave a positive yield.

Specific serologic tests

Recently, several rapid and specific serological methods were found to be of great use in demonstrating bacteria in the CSF. These were immunofluorescence (IFL)[17], immunoelectroosmophoresis (IEOP)[18] and radioimmunoassay[19]. IFL detects cell-bound antigens in CSF whereas IEOP demonstrates soluble antigens. The former technique was found to have higher sensitivity when compared to the other serological methods and gave a correct diagnosis in 84% of 75 patients with meningitis in one of Olcén's series[20]. In this study, IEOP yielded correct results in 55% of cases and was comparable to the results reported by others[21]. However, the latter three methods became less popular because they require high-powered experience for interpretation, are laborious to do and necessitate the use of comparatively expensive equipment.

In 1973, Dr Göran Kronvall[22] from the University of Lund, Sweden, introduced the term "co-agglutination" as a new serological method for typing pneumococci. The principle of this new technique was based on the phenomenon that when adding a specific antipneumococcal antibodies to a stabilised suspension of Cowan 1 strain of *Staphylococcus aureus*, the antibodies bind with the Fc parts to protein A on the cell wall, leaving their Fab parts free for reactions with the corresponding antigen. The resulting staphylococcal-bound antibodies readily co-agglutinate when coming in contact with a specific pneumococcal capsular antigen; and the result of the reaction is readily visible. This technique has been used since then for identification of other bacteria including streptococci and meningococci and in the latter case it was found to detect both cell-bound and soluble bacterial antigens which are present in the CSF[20]. In 1974, Pharmacia Diagnostics in Sweden obtained the commercial rights for the co-agglutination technique and produced later Phadebact CSF Test which contains 4 different reagents for the identification of the commonest organisms causing bacterial meningitis i.e. *H influenzae* type b, *S Pneumoniae* and *N meningitidis*. The reagents are capable of detecting all clinically relevant serotypes of *S Pneumoniae* and can detect groups A, B, C, Y and W-135 of *N meningitidis*. Before performing the test, the CSF

should be heated for 5 minutes at 80°C to destroy the proteins which were found to cause autoagglutination yielding a noninterpretable result. Prior centrifugation of the specimen may also be required in cases of the presence of excessive amounts of protein or blood. The test only needs one drop of CSF to be added to one drop of each reagent and the result is read within 2 minutes. The sensitivity and specificity of the test is well-established now. In a study by Drow et al[23] where a total of 2,817 individual tests were performed on 577 CSF specimens, 74% of the positive results could be detected using Phadebact CSF Test whereas counterimmunoelectrophoresis detected 65%. No falso-positive results were obtained when using the co-agglutination method.

On the other hand, latex agglutination technique is similar to co-agglutination, the reagent being in this case latex particles sensitised with specific antisera. However, the antibodies are randomly adsorbed onto latex particles and some of the antibodies will be attached via their Fab portion rendering them unable to react with antigen. The test is now available commercially in a kit form (Slidex meningite-Kit; bio Mérieux). It requires one drop of CSF (preferably using the supernatant after centrifugation for 10 minutes) to be added to one drop of each of latex suspensions and the reaction (agglutination) is read after 2 minutes. Its 4 reagents can detect *N meningitidis* group A and C, *H influenzae* type b and *S pneumoniae* (83 serotypes). Comparing it to the conventional bacterial cultures and other immunologic techniques in 718 cases of purulent meningitis, Denis et al[24] found it to have the same sensitivity as positive direct examination of CSF (82% and 80.4%, respectively) and it yielded slightly more positive results than the culture technique (73.1%). On the other hand counter-immunoelectrophoresis was positive in 90% of cases.

In comparative studies using reagents sensitised with the same antisera, both co-agglutination and latex tests were found to be about equally sensitive regarding their specificity and sensitivity[25,26]. Both tests were also found to be of special value in areas with limited laboratory facilities. In a study by Whittle et al[27] in Zaria (Nigeria) comparing latex test, counter-current

immunoelectrophoresis, CIE and bacteriological tests in 229 CSF samples from patients with pyogenic meningitis, latex agglutination was reported to give a higher yield than routine bacteriology (Gram stain or culture) and identified respectively 88%, 82% and 94% of cases of meningococcal, pneumococcal and *H influenzae* meningitis. It showed about equal sensitivity to CIE in detecting meningococcal and *H influenzae* but was less sensitive in revealing pneumococcal meiningitis (respectively 82% versus 98%). The latex reagents were found to be stable and not adversely affected if left inadvertently at a high room remperature for a few days. On the other hand, in a recent study by Sanborn and Toure[28], co-agglutination technique proved its adaptability for use in rural Africa. Designing simple, easily operated diagnostic kit they found that West African medical attendats, after only 6 teaching hours, could perform the tests independently and with high degree of accuracy. The co-agglutination reagents which had been prepared in California and shipped to Upper Volta showed no loss of reactivity when exposed to a temperature of 30-40°C at the field for 8 weeks. The authors drew the attention to the fact that the co-agglutination reagents can easily be prepared locally using the existing laboratory facilities. The success of implementing this method in other developing countries would mean a great boost to the primary health care in rural areas where 70-80% of the population live.

ACKNOWLEDGMENT

I am indebted to Professor Yngve Hofvander of the International Child Health Unit at the University Hospital of Uppsala for his deep interest and support throughout the period of this study; and to Dr Gunnar Ahlsten of the Paediatric Department for placing facilities at my disposal which formed the background to this work. My sincere thanks are due to Dr Per Olcén and Dr Dan Danielsson from the Departments of Infectious Diseases and Clinical Bacterology at Örebro Medical Centre Hospital for their stimulating discussions and writings which helped me laying the foundations for this article. I am also grateful to Iva Kulhanek for preparing the manuscript. This work is supported by the Swedish Commission for Technical Co-operation and the Medical Research Council.

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