

Aseptic Meningitis in Children—The Singapore Experience

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Abstract

Objective: To study the incidence, aetiology, clinical characteristics and management of paediatric aseptic meningitis in a paediatric hospital in Singapore. **Materials and Methods:** Patients with cerebrospinal fluid (CSF) pleocytosis, a negative Gram stain and bacterial culture were reviewed retrospectively from 1 January to 31 December 2000. Eighty-seven patients who fulfilled the criteria for aseptic meningitis and without neurological deficits were studied. In addition, reverse transcriptase polymerase chain reaction (RT-PCR) using pan enterovirus primers was subsequently performed on 73 of these CSF specimens which were available for storage. **Results:** The incidence of aseptic meningitis was approximately 37 cases per 10,000 admissions. Non-polio enteroviruses were isolated from 29 of 64 (45.3%) CSF and 38 of 52 (73.1%) stool samples. RT-PCR was positive in 43 (58.9%) of the archived CSF specimens. The aetiologies of the remaining cases were mostly unidentified. Their ages ranged from 5 days to 12 years (median, 2 months). All patients except 1 had fever. Vomiting or poor feeding occurred in 44.7%, cough or running nose in 35.3%, irritability was observed in 35.3%, seizures in 7.1%, a rash in 10.6% and diarrhoea in 5.9%. All patients recovered without sequelae. The median CSF white cell count was 212 cells/mm³ (range, 7 to 12000). The median glucose concentration was 2.7 mmol/L (range, 1.6 to 4.4). The median CSF/ blood glucose ratio was 0.52 (range, 0.23 to 0.73). Median length of stay was 7 days (range, 4 to 17). Eighty-four patients (96.6%) received antibiotics for a median of 5.5 days (range, 2 to 14). **Conclusion:** Enteroviruses were the most common aetiological agent identified. A method of early diagnosis using RT-PCR for enteroviruses is necessary to reduce the current duration of antibiotic usage and to decrease the length of hospital stay.

Ann Acad Med Singapore 2002; 31:756-60

Key words: Enteroviruses, Infants, RT-PCR, Viral culture

Introduction

Aseptic meningitis is a common diagnosis among infants admitted for fever without an obvious source.^{1,2} In cases where the aetiological agent could be identified, more than 90% were due to enteroviruses.^{3,4} Due to the relative lack of data from Southeast Asia and the increasing use of reverse transcriptase polymerase chain reaction (RT-PCR) to detect enteroviral ribonucleic acid (RNA), this study was undertaken to examine the incidence, aetiology, clinical characteristics and management of paediatric aseptic meningitis in KK Women's and Children's Hospital (KKWCH) when RT-PCR was not available as a routine service. KKWCH is an 898-bedded hospital with services in obstetrics, gynaecology, neonatology and paediatrics. It has 182 paediatric beds. The majority of admissions to the paediatric unit were from the emergency department.

The demographic profile of the patients, the clinical

presentation, common associated symptoms, disease outcome and the length of hospital stay were collated. The type of laboratory investigations commonly requested and viral cultures, if any, were recorded and their results discussed. Specific antimicrobial or antiviral therapy if any was noted.

Materials and Methods

Patients with cerebrospinal fluid (CSF) pleocytosis but with a negative Gram stain and bacterial culture were reviewed retrospectively from 1 January to 31 December 2000. CSF pleocytosis was defined as follows.^{2,5} The upper limit for neonates was 22 white cells/mm³. For infants up to 2 months of age, the limit was 15 cells/mm³. For infants older than 8 weeks, the limit was 5 white cells/mm³.

A diagnosis of aseptic meningitis was made in patients with CSF pleocytosis as given above with a negative

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bacterial culture of the CSF. Patients with symptoms of encephalitis or neurological deficits were excluded. Infections were categorised as community acquired if disease signs and symptoms were present upon or within 48 hours of admission.

Virus investigations were sent to the Department of Pathology, Singapore General Hospital. In addition, remaining CSF specimens which were not used for culture were stored in -70°C . RT-PCR using pan enterovirus primers and previously published methods⁶ was subsequently performed on 73 archived CSF specimens.

Results

There were 87 patients who fulfilled the criteria for aseptic meningitis. There was incomplete data for 2 patients. There were 51 (58.6%) male and 36 (41.4%) female patients. Fifty-eight (66.7%) patients were Chinese, 25 (28.7%) were Malay, 2 (2.3%) were Indian and 2 (2.3%) of other races. Their ages ranged from 5 days to 12 years. The median age was 2 months. Sixty-nine per cent were younger than 4 months.

During the period of study, there were 23,290 admissions into the paediatric wards. Therefore, the incidence of aseptic meningitis was approximately 37 cases per 10,000 admissions. Cases of aseptic meningitis averaged 7 per month (Fig. 1).

The bulk of infections were community acquired (95.4%)

except for 3 term neonates and a 3-month-old premature infant. These 4 patients had been hospitalised since delivery. The 3 neonates had various initial problems including intrauterine growth retardation with hypoglycaemia, respiratory distress as a result of abruptio placentae and presumed sepsis as a result of prolonged premature rupture of membranes. Infection occurred between the 6th and 7th days of life for the 3 neonates. Two cases occurred in the month of May and one each in June and September. Coxsackievirus B5 was isolated in all cases.

Patients were admitted for a median of 7 days (range, 4 to 17 days). The length of stay for 1 patient was excluded as it was the premature infant mentioned above who was hospitalised for problems of prematurity.

Eighty-four patients (84/87, 96.6%) received empiric antibiotics for a median of 5.5 days (range, 2 to 14 days). The most common combination of antibiotics given was ampicillin and gentamicin (37 cases), followed by ceftriaxone (26 cases). The combination of ampicillin and ceftriaxone was administered for 17 cases. The 4 infants with nosocomial infections received empiric cefotaxime and cloxacillin. In addition, 2 of these infants with onset of disease on days 6 and 7 of life, also received acyclovir empirically before test results were back.

Among the 85 patients with complete data, all but 1 patient had fever. The majority (81.2%) were febrile for 2 days or less before admission (range, 0-7; median, 1).

Fig. 1. Monthly distribution of aseptic meningitis and corresponding enterovirus serotypes isolated* in KKWCH in 2000.

TABLE I: SIGNS AND SYMPTOMS ASSOCIATED WITH ASEPTIC MENINGITIS IN 85 PATIENTS WITH COMPLETE DATA

Signs and symptoms	No. of patients
Febrile 2 days or less before admission	69 (81.2%)
Febrile 4 days or less during admission	72 (84.7%)
Temperature	Mean 38.9°C
	Range 37.0°C-40.5°C
Vomiting or poor feeding	38 (44.7%)
Cough or running nose	30 (35.3%)
Irritability	30 (35.3%)
Seizures	6 (7.1%)
Rash	9 (10.6%)
Diarrhoea	5 (5.9%)
Neck rigidity	26 (30.6%)
Headache +/- photophobia	22 (25.9%)

84.7% were febrile for 4 days or less whilst in the ward (range, 0 to 8; median, 3). The mean peak temperature was 38.9°C (range, 37.0°C-40.5°C). Vomiting or poor feeding occurred in 38 (44.7%) patients, cough or running nose in 30 (35.3%) patients, irritability in 30 (35.3%) patients and seizures in 6 (7.1%) patients. Headache with or without photophobia was present in all patients 3 years and above, who were old enough to complain of the symptoms. Clinically, neck rigidity was detected in 26 patients, all older than 1 year old. No infant presented with a bulging frontanelle. A rash was found in 9 (10.6%) patients. Only 5 (5.9%) patients had diarrhoea (Table I).

All patients recovered without sequelae. There was no hearing deficit among 11 patients who had oto-acoustic emission screening.

All patients had full blood counts, serum urea and electrolytes, blood and CSF cultures performed. Sixty-seven patients (77%) had in addition, urinalysis and urine cultures. The test for complement fixing antibodies (CFT) in CSF to a panel of neurotropic viruses - herpes simplex (HSV), measles, mumps and flaviviruses (Japanese encephalitis and dengue viruses) was requested for 37 patients (42.5%). Of these, only 2 patients had corresponding serum specimens. The latex agglutination tests for antigens to common bacterial pathogens causing meningitis were performed for 74 (85.1%) patients. Seventy-two patients (82.8%) had serum C-reactive protein estimated.

The median blood total white cell count was 11.8×10^9 cells/L, which was well within normal limits. However, the results ranged from 4.59 to 25.9×10^9 cells/L. The median proportion of polymorphs was 47% (range, 16%-91%). All bacterial cultures of blood, urine and CSF were negative for significant bacterial pathogens. All CSF cultures which were negative at 24 hours remained negative after further reincubation. The results of the CFT for antibodies to neurotropic viruses and latex agglutination test for bacterial pathogens in the CSF were all negative.

TABLE II: THE CSF PROFILE OF PATIENTS WITH ASEPTIC MENINGITIS

n = 87	Median	Range
Red cell count ($\times 10^9$ cells/L)	10	0 - >20,000
No. of traumatic taps = 12		
White cell count ($\times 10^9$ cells/L)	212	7 - 12,000
Glucose (mmol/L)	2.7	1.6 - 4.4
Protein (g/L)	0.75	0.1 - 6.53
Chloride (mmol/L)	121	50 - 129
CSF/blood glucose ratio (n = 55)	0.52	0.23-0.73

CSF: cerebrospinal fluid

TABLE III: RESULTS OF RT-PCR AND ENTEROVIRUS CULTURE OF CSF FROM PATIENTS WITH ASEPTIC MENINGITIS (n = 87)

PCR	Culture			Total
	Positive	Negative	Not done	
Positive	24	6	13	43
Negative	3	22	5	30
Not done	2	7	5	14
Total	29	35	23	87

CSF: cerebrospinal fluid; PCR: polymerase chain reaction

The median CRP value was 5.9 mg/L (range, 0.4-185 mg/L). Eighty-six per cent of the CRP results were less than 30 mg/L.

The median CSF red cell count was 10 and there were 12 traumatic taps. The median CSF white cell count was 212 cells per mm³ (range, 7-12000 cells per mm³). The median glucose concentration was 2.7 mmol/L (range, 1.6-4.4 mmol/L). The median CSF protein was 0.75 g/L (range, 0.1-6.53 g/L) which was higher than the normal limit (0.45 g/L) for children a year and older⁵ (Table II). The majority (72.4%) were higher than 0.45 g/L. The median CSF/blood glucose ratio was 0.52 (range, 0.23-0.73).

Sixty-four (64/87 or 73.6%) patients had CSF specimens, 52 (59.8%) had stool specimens and 2 (2.3%) patients had throat specimens for enterovirus culture. Only 2 patients had all CSF, throat and stool cultures. This could possibly be due to cost constraints such that each patient often had just one or less often, two specimens for culture. Fifteen (17.2%) patients were not investigated for enteroviral meningitis. Of the 64 CSF specimens sent for enteroviral isolation, 29 were positive (45.3%) for non-polio enteroviruses. Viral isolation from stool was higher, 38 of 52 samples were positive (73.1%). Both throat cultures were positive. In all cases where both CSF and stool or throat specimens were positive for enteroviruses, the viruses isolated were of the same serotype. The time to a positive result averaged 4 days for both CSF and stool viral cultures. This ranged from 2 to 12 days with a median time of 4 days for CSF and 2 to 21 days with a median time of 3 days for

stool. However, more stool specimens (21.6%) were positive after 2 days compared to CSF specimens (7.4%).

Among the 72 patients who had either CSF or stool tested for enteroviruses, 47 patients (65.3%) were positive in 1 or both specimens.

From the 87 patients, 73 CSF specimens were available for storage. RT-PCR for enterovirus was performed on these archived CSF specimens and were positive in 43 (58.9%). Of these 43, 24 were culture positive. RT-PCR detected 6 CSF specimens which were negative by culture and 13 additional cases which were not cultured. Of the 30 specimens with negative RT-PCR, 3 were culture positive, 22 were culture negative and 5 were not cultured (Table III).

The interpretation of a traumatic tap is difficult at best. If meningitis cannot be excluded when the white cell count is abnormal, it may be worthwhile to investigate for enteroviruses. Among the 12 traumatic taps in this study, 5 were positive by culture or RT-PCR, 4 were negative and 3 were not tested for enteroviruses.

The true number of aseptic meningitis in this study which could be attributed to enteroviruses could not be concluded as culture of all relevant specimens and RT-PCR was not performed for each case. However, if based on limited investigations whereby a *definite* case of enteroviral meningitis was when *culture or RT-PCR of CSF was positive* and a *possible case* where *throat or stool was culture positive* in the absence of other aetiologies, then there were 48 (55.2%) definite and 10 (11.5%) possible cases of aseptic meningitis due to enteroviruses in this study. Of the remaining 29 cases, 26 (29.9%) were negative for enteroviruses either in the CSF (by culture or RT-PCR) or stool (by culture only). Three (3.4%) cases had no specimen for enterovirus culture or RT-PCR. For these 29 cases, the most common diagnosis made was 'aseptic meningitis' or 'viral meningitis', 3 were diagnosed as 'partially treated bacterial meningitis', 1 mumps meningitis and 1 Kawasaki's disease.

The distribution of enterovirus serotypes (Fig. 1) showed that coxsackievirus B5 was the predominant serotype during the study period, followed by coxsackievirus B3 and B4. There were sporadic cases of echovirus 18, 19 and 30. Enterovirus 71 (EV71) was isolated in September and October coinciding with an outbreak of hand, foot and mouth disease due to EV71 in Singapore (unpublished data).

Discussion

Infants, particularly those 3 months and younger, were by far the largest age group with aseptic meningitis in this hospital. Among the infants, the presentation was often a non-specific febrile illness with no obvious source of

infection. More males appear to be affected. The majority (more than 80%) were febrile for 4 days or less during hospitalisation and 2 days or less prior to admission. This compares to a recent report where the mean duration of illness for aseptic meningitis was 5.5 days.¹ Vomiting or poor feeding followed by upper respiratory tract symptoms and irritability were the most commonly associated symptoms.

Based on the results presented, the clinical presentation did not deviate from what was previously reported.³ The most important differential diagnosis to exclude would be bacterial meningitis including partially treated bacterial meningitis or meningitis from a parameningeal focus. However, in very young infants, reaching a diagnosis may often be fraught with difficulties.

Investigations for enteroviral infection were incomplete. However, the majority (73.6%) of CSF obtained were sent for enteroviral culture, 59.8% and 2.3% of patients had stool and throat specimens respectively for enteroviral culture. The average yield from viral culture of the CSF was 45.3% in this population of patients with aseptic meningitis. Therefore, almost half had enteroviral meningitis as proven by culture. However, the sensitivity of tissue culture for enteroviruses generally do not exceed 65 to 75%.³ The use of a nucleic acid amplification method, such RT-PCR for enteroviral RNA, has been proven to be more sensitive and much more rapid than culture.³ The turn-around-time for RT-PCR is 1 day compared to an average of 4 days for a positive viral culture result in this study. Furthermore a negative result for viral culture is only issued after 3 weeks. Here, RT-PCR identified 6 cases of enteroviral aseptic meningitis where CSF viral cultures were negative (Table III). Three false negatives had occurred when testing the archived CSF specimens. These involved serotypes which could be amplified by the system (data not shown).⁶ However, false negatives had been reported due to various factors including degradation of viral RNA.⁷ This factor may be relevant here since RT-PCR was performed on stored specimens.

Despite the increased sensitivity of RT-PCR compared to culture, from 30% to 40% of suspected enteroviral meningitis elude specific diagnosis.⁸ Therefore, a recent study recommended combined pharyngeal swab (culture) and CSF examination to improve the detection rate of enteroviral meningitis.⁸ Stool usually yields the highest titre of enteroviruses. However, excretion from this site may be prolonged.⁴ Therefore, the isolation of enteroviruses from stool should be interpreted carefully.⁴ Viral excretion from the throat is shorter. Therefore, a positive isolation from the throat may be more specific than isolation from the stool.⁸ For some serotypes, isolation from the pharynx may be much higher than the stool as reported recently

during an outbreak of enterovirus 71.⁹

Common neurotropic viruses, such as herpes simplex virus, normally cause an encephalitis with altered levels of consciousness, focal seizures and neurological deficits. The flaviviruses usually produce an encephalitis. Measles encephalitis usually occurs during the period of the measles exanthem. The focal encephalitis and post measles encephalomyelitis is often a post-infectious or auto-immune disease. Often, levels of consciousness are altered and focal signs present. Mumps is the only virus where central nervous system involvement has more features of meningitis than encephalitis.¹⁰ In this study, 1 patient (1.1%) was diagnosed as mumps meningitis. Therefore, the test for complement fixing antibodies to HSV, mumps, measles and flaviviruses should be reserved for those with appropriate signs and symptoms or risks. Other methods of laboratory testing, such as nucleic acid testing, would likely be necessary, if clinical suspicion is high for infections such as herpes simplex encephalitis.

A possible bacterial cause of acute aseptic meningitis would be *Mycoplasma pneumoniae*, especially if associated with prior pneumonia or respiratory disease.¹¹ However, the diagnosis is difficult. Most rely on serology. In this study, no patient was investigated for *Mycoplasma pneumoniae* infection.

Empiric antimicrobial therapy would generally be necessary in the initial management of aseptic meningitis but may be discontinued after 24 to 48 hours when culture results were negative or if an enterovirus was detected in the CSF. In this study, although negative preliminary bacterial culture results of the CSF and blood were available after 24 to 48 hours, 49.4% of patients received 5 days or more of antibiotics possibly due to lack of early results from viral cultures. Therefore, if a viral aetiological agent can be identified early, there may be a positive impact on management as antibiotics can be stopped and the patient discharged earlier.

The CSF profiles in this study are similar to a recent report¹² which compared CSF findings in aseptic versus bacterial meningitis. The median CSF/blood glucose concentration ratio was 0.52. A ratio of less than 0.6 was considered low.⁵ However, only a ratio less or equal to 0.3 was considered highly predictive of bacterial meningitis.¹³ The CSF/blood glucose concentration ratio was often less than 0.6 in this study but the majority (51 of 55 patients/92.7%) were equal to or more than 0.40. Only one patient's ratio was 0.28, the next lowest value was 0.38. The total protein concentration was often raised compared to the normal value (0.45 g/L) reported for children above 1 year. However, in this study, 69% were less than 4 months old and this may have had contributed to the higher value.

In conclusion, aseptic meningitis is fairly common with

37 cases per 10,000 admissions for the year 2000. The outcome was good for all patients in this study. Enteroviruses were the most common aetiological agent identified. However, investigations for enterovirus infection were incomplete and may have led to less cases being identified. The lack of early results from viral cultures may have contributed to increased duration of empiric antibiotic use and long hospital stay. The study underscores a need for a diagnostic test with a relatively quick turnaround time to reliably detect the presence of enteroviruses. RT-PCR for enteroviruses has been determined to positively affect management by reducing the duration of hospitalisation, antibiotic use and saving costs.^{4,14} Our laboratory has now made available RNA amplification for the diagnosis of enterovirus meningitis.

Acknowledgement

We would like to thank Loo Liat Hui (Clinical Microbiology Laboratory, KK Women's and Children's Hospital) for performing the RT-PCR.

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