ORIGINAL ARTICLE

Clinical and diagnostic findings of an echovirus meningitis outbreak in the north west of England

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Postgrad Med J 2006;**82**:60–64. doi: 10.1136/pgmj.2005.036608

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Submitted 27 April 2005
Accepted 13 June 2005

Introduction: An outbreak of echovirus meningitis occurred in the north west of England in 2001. This paper reviewed the clinical features and the role of different diagnostic methods.
Methods: This was a prospective study of adults admitted to a regional infectious disease unit with a probable diagnosis of meningitis, March to August 2001.
Results: Half the 40 cases were male; median age was 28 (range 16–51) years. Fifteen of 38 (39.5%) were smokers, and 20 of 24 (83.3%) had close contact with children. Median (range) duration of symptoms was 1.1 (0.25–7) days. Symptoms included headache (100%), photophobia (87.5%), and nausea (67.5%), and severity ranged from minimal signs to those consistent with a meningoencephalitis. The diagnosis was confirmed virologically in 29 of 40 (72%); echovirus 30 was isolated from six. Cerebrospinal fluid (CSF) enterovirus polymerase chain reaction (PCR) was positive in 26 of 32 (81%), and CSF virus culture in 3 of 16 (19%). Thirty one per cent of CSF samples had a neutrophil predominance, and 3 of 29 (10%) virologically confirmed cases had normal CSF microscopy and biochemistry.
Conclusion: CSF microscopy may be normal or suggest bacterial meningitis in a substantial minority of cases of echovirus meningitis. CSF PCR for enterovirus seems to be more sensitive than virus culture of CSF, although PCR does not yield information on circulating virus type. Early and accurate diagnosis could reduce both use of parenteral antibiotics and length of hospital stay with both morbidity and cost implications. Close contact with children may be a risk factor, particularly if good hygiene measures are not practised.

Enteroviruses are RNA viruses belonging to the picornaviridae family, and include echoviruses, coxsackieviruses A and B, enteroviruses 68 to 71, and poliovirus. Coxsackieviruses and echoviruses are major aetiological agents of viral meningitis, and account for about 80% of cases in children and adults.[1–3] A total of 64 enterovirus serotypes have been identified, and 30 echovirus serotypes. Clinical manifestations of enterovirus infection range from asymptomatic carriage to life threatening disease. Other disease manifestations include mild febrile illness, respiratory disease (pharyngitis, bronchitis, bronchilitis), rash, hand foot and mouth disease, myocarditis, encephalitis, and neonatal sepsis.[4 5]

In the northern hemisphere, enterovirus infections predominate in summer and autumn, although sporadic cases occur throughout the year. Young children are most susceptible, with viral meningitis being about five to eight times more common in children than adults.[6] Enteroviruses are spread predominantly by the faecal-oral route, although infection may also occur through the oral-oral route and by upper respiratory tract infections.[2] No specific prevention or control measures are available for the non-polio enteroviruses, however good hygiene practices such as thorough hand washing after nappy changes, disinfection of contaminated surfaces, and avoidance of shared utensils may help interrupt transmission.[7]

In clinical practice, rapid diagnostic confirmation of viral meningitis is advantageous, and avoids unnecessary treatment with antibiotics, reduces the length of hospitalisation, and ultimately reduces costs. Virus culture, the standard technique for enterovirus detection, is time consuming, and has poor sensitivity, but provides serotype data on the virus causing disease.[8] While the identification of a specific enterovirus may be of limited interest to clinicians, it is of considerable epidemiological and public health importance in identifying outbreaks and understanding circulation patterns. Molecular methods of diagnosis such as polymerase chain reaction (PCR) and sequencing have increasingly become available, and serotype specific primers have been developed for several enteroviruses. A rapid, sensitive RT-nested PCR assay based on VP1 has been used directly on clinical samples in an outbreak of aseptic meningitis, to type the group B enteroviruses causing aseptic meningitis.[9]

Echovirus 30 is one of the most frequently isolated enteroviruses in Europe, and North America.[10–12] It follows an epidemic mode of transmission, causing large outbreaks, and then becomes quiescent for a period of several years.[13 14] This quiescence is probably attributable to the development of population immunity that occurs in a high infection rate epidemic. Echovirus 30 was also the commonest enterovirus identified in a prospective study of 61 children with enteroviral meningitis.[3]

Between March and August 2001, an outbreak of echovirus 30 meningitis occurred in the north west of England (fig 1). This was mirrored by above normal levels of confirmed echovirus elsewhere in England and Wales.[15 16] The clinical features and different diagnostic methods are reviewed here.

METHODS

All adult patients admitted to University Hospital Aintree with a diagnosis of viral meningitis were recruited. Patients were identified from the laboratory records of all cerebrospinal (CSF) samples processed for possible meningitis between 30 March 2001 and 16 August 2001, throat swabs or faeces samples for culture for enteroviruses, and of serum samples sent for pathogens implicated in viral meningitis. These searches were supplemented by a review of the prospectively

Abbreviations: PCR, polymerase chain reaction; CSF, cerebrospinal fluid

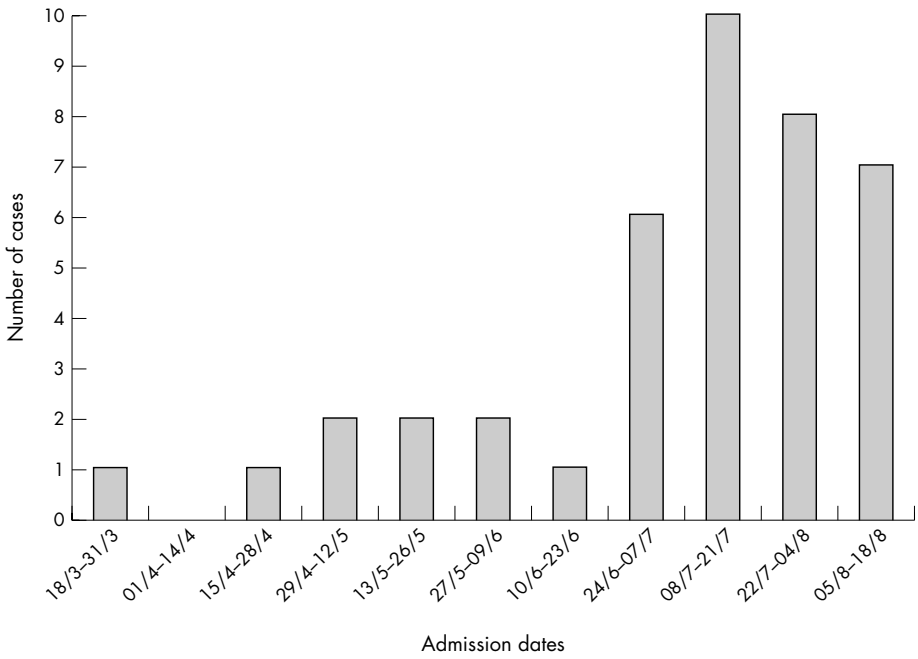

Figure 1 Fortnightly distribution of cases of viral meningitis presenting to a regional infectious disease unit.

maintained ward based diagnostic index on the infectious disease unit and review of unit discharge letters covering the same period. All patients were admitted to the infectious disease unit either directly or from the accident and emergency unit. Clinical and laboratory parameters were recorded on a specifically designed form. CSF cell total and differential counts and biochemistry were done in all patients. Virus was cultured using two cell lines, secondary rhesus monkey kidney (RMK) and MRC-5 (human embryo lung) cells. After inoculation the cells were examined daily for 10 days for evidence of cytopathic effects. Enteroviruses were initially identified by the characteristic cytopathic effect produced, and individual serotypes identified by fluorescent antibody testing using polyvalent and individual fluorescein labelled antibodies. Detection of enterovirus by nucleic acid amplification used a two step reverse transcriptase Taqman real time PCR.[17] The assay is specific for enterovirus and paraechovirus, and does not amplify other viruses. Throat swabs were sent for viral culture, as were faecal samples in a minority of patients (although universally requested).

The following definitions were used;

- Probable case: symptoms of meningitis (fever, headache, neck stiffness, vomiting, photophobia).
- Confirmed case: symptoms of meningitis (fever, headache, neck stiffness, vomiting, photophobia) and either positive enterovirus PCR, positive CSF viral culture or CSF pleocytosis (>5 WCC/ml) and positive serology.

Statistical analysis was performed using SPSS version 11 for Windows.

RESULTS

Forty adults with viral meningitis were identified during the period March to August 2001 inclusive. Twenty (50%) were male, and the age range was 16–51 years, median age 28 years. Fifteen of 38 (39.5%) were smokers, and 20 of 24 (83.3%) had close contact with young children (parent, grandparent, school teacher, paediatric nurse). Symptom duration ranged from six hours to seven days, median 1.1 days. The most frequent symptoms were headache (100%), photophobia (87.5%), nausea (67.5%), vomiting (65%), fever (65%), and neck stiffness (62.5%). Specific gastrointestinal symptoms (abdominal pain and diarrhoea) only occurred in 2 of 40 (5%) of patients. Myalgia was specifically inquired about, and reported in 7 of 40 (17.5%).

Twenty three of 40 (58%) had fever on admission, 30 of 38 (79%) had clinical photophobia, and 27 (68%) had

Table 1 CSF characteristics in patients with echovirus meningitis

Range Median (95% CI)	All patients (n = 40)	Culture positive (n = 3)	Culture negative (n = 13)	PCR positive (n = 26)	PCR negative (n = 6)
White cell count ($/mm^3$)	1–405	14–211	1–365	2–378	1–194
	38 (20 to 140)	26 (14 to 211)	55 (26 to 187)	36 (18–112)	29 (1–167)
% Lymphocytes	2–100	5–85	5–100	2–100	5–100
	85 (33 to 95)	40 (5 to 85)	85 (27 to 95)	88 (38 to 95)	85 (5 to 100)
% Neutrophils	0–98	15–95	0–95	15–95	0–95
	15 (5 to 68)	60 (15 to 95)	15 (5 to 73)	60 (15 to 95)	15 (0 to 95)
Protein (g/l)	0.25–1.6	0.4–1.4	0.3–1.4	0.3–1.4	0.3–0.9
	0.7 (0.5 to 0.8)	1.2 (0.4 to 1.4)	0.7 (0.5 to 0.9)	0.6 (0.5 to 0.8)	0.7 (0.6 to 0.9)
% Abnormal protein	31/39 (79.5%)	2/3 (66.7 %)	11/13 (84.6%)	20/26(76.9 %)	5/6 (83.3%)
Glucose (mmol/l)	2.5–7.3	2.6–3.2	2.6–4.7	2.6–7.3	3.0–4.2
	3.4(3.1 to 3.6)	3.0 (2.6 to 3.2)	3.6 (3.3 to 4.0)	3.3 (3.1 to 3.6)	3.6 (3.2 to 4.0)

Table 2 CSF culture compared with CSF PCR in patients with echovirus meningitis

	CSF PCR			
CSF virology	Positive	Negative	Not done	Total
Positive	3	0	0	3
Negative	8	4	1	13
Not done	15	2	7	24
Total	26	6	8	40

meningism on examination. Kernig's sign was positive in 3 of 22 (14%), and fundoscopy was abnormal in 2 of 30 (7%). Nine of 40 patients had a CT scan of the head performed, all of which were normal.

All patients had a CSF cell count performed, but a differential count was only recorded in 32 of 40 (80%) cases. Table 1 shows a breakdown of CSF characteristics in culture positive and negative cases and PCR positive and negative cases. There was a lymphocyte predominance in 22 (69%) and a neutrophil predominance in 10 (31%). A total of six patients had a CSF white cell count of $\leqslant 5$, 3 of whom had a positive CSF enterovirus PCR. CSF glucose was normal in all patients. Three of 40 (7.5%) had normal CSF cell count and biochemistry, but positive virology (positive CSF enterovirus PCR). There was a trend suggesting lower CSF glucose in culture positive compared with culture negative cases ($p = 0.05$), and in PCR positive compared with PCR negative cases ($p = 0.31$), but these differences were not significant.

Table 2 shows a comparison of CSF culture with PCR. All CSF culture positive cases were also PCR positive. Overall, the diagnosis was confirmed virologically in 29 patients (72%). Echovirus 30 was isolated from six patients (three throat swab, three CSF). CSF enterovirus PCR was positive in 26 of 32 (81%) compared with CSF viral culture 3 of 16 (19%), ($p<0.0001$, Fisher's exact test) or 3 of 31 (9.7%) throat swab cultures ($p = 0.65$).

As CSF and PCR positivity may be related to duration of symptoms, we examined the relation between duration of symptoms and diagnostic confirmation and found no relation.

Table 3 compares the performance characteristics of CSF PCR against the gold standard, CSF virus culture, in several published studies. Our study showed low specificity and positive predictive values, which is probably because of the small number of culture positive cases. Our results are similar to some of the other studies.

DISCUSSION

This prospective observational study took place in response to a developing viral meningitis outbreak. The symptoms and signs were similar to other those seen elsewhere.[16 23] However, in a study from Munich, preceding signs of gastrointestinal infection were reported in 14 of 19 (79%) patients with acute aseptic meningitis secondary to enterovirus infection.[24] Our study failed to support such an association, as only 2 of 40 patients (5%) patients had specific gastrointestinal symptoms. We consider that the nausea and vomiting that occurred in most of the patients were symptoms of meningeal irritation and meningitis, and should not be regarded as separate gastrointestinal symptoms secondary to enterovirus infection. In a larger study from Marseilles, only 11% of patients had diarrhoea as a presenting symptom.[23] Our study found that nearly a fifth of patients (17.5%) reported myalgia, which was more commonly reported than from another recent study of an echovirus 30 outbreak (3.8%).[23] Myalgia as a presenting symptom of sepsis, if not specifically inquired about, can often be missed.[25]

As there is no control group, it is impossible to comment on the possible significance of smoking as a risk factor. In a study carried out on Greek military recruits, meningococcal carriage was significantly associated with smoking,[26] but there are no studies confirming such an association in viral meningitis. Eighty three per cent of our patients had close contact with small children as parents, grandparents, schoolteachers, or paediatric nurses. As the disease is usually spread by the faecal-oral or oral-oral routes, one could speculate that failure to observe thorough hand washing after nappy changes, assisting young children with toileting, and kissing small children might facilitate transmission to adult carers. Previous community based studies have shown high attack rates for enteroviral illness in household members of day care centre children (13%) and day care centre employees (5%). Household members of ill day care centre children were 15 times more likely to have met the case definition for enteroviral illness than those of non-ill day care centre children.[27] Another study reporting an echovirus 30 outbreak confirmed that contact with an ill household member (odds ratio, OR, 6.3), day care attendance (OR 2.6), and playground use either two or three times a week (OR 3.7) or daily (OR 4.3) were risk factors for transmission, and therefore, illness.[28]

The echovirus 30 serotype has been responsible for numerous outbreaks in the Rhone-Alps region of France between 1976 and 2000, and was partly responsible for the outbreak of viral meningitis seen in that region in 2000 (together with echovirus 6 and echovirus 13). The echovirus 30 serotype mainly affected patients between the ages of 15 and 49 years.[29] This is similar to our cohort, most of whom were between 16 and 40 years old. Possible reasons for the predilection of the virus for this age group are decreasing levels of neutralising antibody in adolescents and adults, or genomic variation in echovirus 30.[29 30]

This study confirms that CSF parameters are not reliable markers for excluding viral meningitis, and may be normal in nearly 10% of confirmed cases. This is in keeping with previous studies, which have shown that in routine medical and laboratory practice, quantitative and qualitative CSF

Table 3 Comparison of performance characteristics of RT-PCR with CSF virus culture (gold standard) from previous studies

Reference	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Verstrepen *et al*[18]	100	91	69	100
Corless *et al*[17]	100	19	28	100
Pozo *et al*[19]	100	11	28	100
Guney *et al*[20]	89	66	74	84
Gorgievski-Hrisoho *et al*[21]	100	19	28	100
Buxbaum *et al*[22]	68	54	52	70
Carrol *et al* (this study)	100	33	27	100

examination results are of little value in ruling out viral meningitis.[3 23] Verstrepen *et al*[18] found that of 41 patients with positive enterovirus PCR, 8 (19.5%) had a WCC $<10/mm^3$. A CSF neutrophil predominance was seen in 31% of patients in our study, which is similar to the CSF neutrophil predominance of 42%,[3] 41%,[31] and 55%[23] reported by others.

Only 3 of 31 (9.7%) throat swab samples were positive by virus culture, and only three patients submitted samples for stool virus culture (all of which were negative). Attempts to isolate enterovirus from CSF are frequently less successful because of the lower viral titre in clinical specimens, and also because some serotypes grow slowly,[19] this was less sensitive in our series compared with previous reports.[3 32] This is a limitation of the usefulness of cell culture techniques.[20] In an echovirus 30 associated outbreak of aseptic meningitis in Taiwan, virus was more frequently isolated from throat swab (85%), and to a lesser extent stool (76%) or CSF (70%).[33] In another study from Germany, more isolates of enterovirus were isolated from stool samples than from CSF samples over three consecutive years.[22]

CSF PCR for enterovirus is more sensitive than CSF viral culture, and increased diagnostic success about threefold in our hands. This contrasts with experience during an epidemic of echovirus 30 meningitis in Marseilles in 2000, where CSF PCR had similar sensitivity to cultures of faeces or of throat swabs.[23] We had access to a two step reverse transcriptase real time PCR method that has been optimised to produce rapid results, with excellent sensitivity in CSF and throat swab samples.[17] RT-PCR is more rapid and sensitive than virus culture, which is traditionally used as the ''gold standard''. This makes it difficult to assess the performance of RT-PCR against a true gold standard,[21] and RT-PCR should now be used as the gold standard.

Several authors have argued that performing CSF PCR for enteroviruses on patients with probable viral meningitis may affect clinical decision making, reduce hospitalisation times, and reduce diagnostic or therapeutic interventions including prolonged administration of unnecessary antibiotics or dexamethasone.[3 8 16 34 35] It has been estimated that rapid availability of PCR results on CSF specimens from adults could result in cost savings for England and Wales of over £250 000 ($450 000) per year,[16] and the savings would be further increased if processing of specimens from children was taken into account.[35] We would support this view, particularly if it can be introduced as a rapid routine test, because our results confirm that PCR significantly improves the ability to specifically diagnose enteroviral meningitis, irrespective of CSF parameters.

Unfortunately, specific antiviral agents such as pleconaril that showed initial promise,[36–40] are not licensed for routine clinical use, and there is therefore no effective treatment available, even if confirmatory diagnostic tests results were available rapidly.

CONCLUSION

This prospective study of an echovirus outbreak in the north west of England confirms that headache, photophobia, nausea, vomiting, and temperature are the most common symptoms seen in adults. CSF parameters alone cannot be reliably used to confirm or exclude viral meningitis. CSF PCR for enterovirus is more sensitive than viral culture, although PCR does not yield information on circulating type. The implications of early and accurate confirmation of viral meningitis using PCR are to reduce parenteral antibiotic use and length of in-patient stay. Both these factors have implications for patient morbidity and the cost of patient management. Finally, close contact with children could be a significant risk factor, especially if good hygiene measures are not practised.

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Funding: none.

Conflicts of interest: none declared.

This research was presented in part at the Federation of Infection Societies eighth conference, Manchester, 28–30 November 2001 (poster).

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