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FINAL REPORT

Project / Program Title		Improving Diagnostics in Enteroviral Meningitis
Name		Dr Natalie Martin
Award Received		2015 NZ Research Development Scholarship
Report Date		1 August 2018
Chief Investigator / Supervisor		Professor Andrew Pollard, Assistant Professor Manish Sadarangani, Dr Dominic Kelly
Administering Institution		University of Oxford
Funding Period	Start Date:	1 January 2015
	Finish Date:	31 December 2015

PROJECT SUMMARY

Most meningitis (infection of the fluid around the brain) is caused by viruses nowadays in countries like New Zealand. This is partly due to effective vaccine programmes for bacterial causes of meningitis. The most common viruses causing meningitis are enteroviruses. Most children with viral meningitis are admitted to hospital and receive intravenous antibiotics because of concern that there may be a bacterial cause, however antibiotics are not needed to treat viral meningitis. Therefore, making a diagnosis of viral meningitis rapidly is important to avoid unnecessary treatment. The diagnosis can be made by obtaining a lumbar puncture for microbiological tests, but often this is not done before antibiotics are started and results may take days to become available. In this study, I collected samples from children with suspected enteroviral meningitis including stool samples, throat swabs and blood. Microbiological testing of these samples by enteroviral polymerase chain reaction (PCR) found that all children with enteroviral meningitis also had a positive stool test for enterovirus, and some children who had meningitis with no cause identified also had a positive stool test for enterovirus. There are very few studies that have investigated this, and the findings may help doctors with clinical decision making in children who have suspected viral meningitis but don't have lumbar puncture results available. I also investigated the symptoms and results of blood tests in children with meningitis of different causes. These findings have been presented at conferences, in my DPhil thesis at the University of Oxford, and are being prepared for publication in a scientific journal.

PROJECT AIMS / OBJECTIVES

The aims of this study were:

1. To perform enteroviral (EV)-PCR on stool, serum and respiratory samples, and investigate the proportion of EV-PCR+ samples, in children with EV meningitis or aseptic meningitis of unknown cause.

2. To investigate the time (in days or hours) following onset of symptoms that positive or negative EV-PCR results are obtained for CSF and non-CSF samples in childhood EV meningitis or aseptic meningitis.

3. To compare age, clinical and laboratory features in childhood EV meningitis with CSF pleocytosis to without CSF pleocytosis.

4. To compare clinical and laboratory features in children with EV meningitis, to children with aseptic meningitis of unknown cause who have a EV-PCR+ stool, respiratory or serum sample.

These aims were achieved through a laboratory study which was performed as a sub-study in a UK-wide prospective multicentre cohort study including children presenting to hospital with suspected meningitis or encephalitis. Clinical data were also analysed.

SIGNIFICANCE AND OUTCOMES

Outcomes and Significance

In aseptic (non-bacterial) meningitis, no cause is identified from routine laboratory tests performed at hospital sites for many children, although enteroviruses cause the majority of cases with a defined aetiology. EV infections may also cause many cases of aseptic meningitis when CSF EV-PCR has not been performed. It may be reasonable to consider that EV could also be the cause in children who are CSF EV-PCR- but have EV isolated from a non-CSF site, as has also been suggested by other small studies. Previous studies have shown that most children with viral meningitis are admitted to hospital and treated with intravenous antibiotics. If time to making a diagnosis of probable or definite EV meningitis could be reduced, this may allow earlier cessation of intravenous antibiotics and earlier hospital discharge. Notably, the majority of children included in these present data were infants, predominantly young infants aged <3months. Children included in this study were admitted to hospital between March 2015 and May 2016.

In this subset of participants from the UK-ChiMES study who had EV-PCR performed of non-CSF samples by EV R-gene® kit, all children with EV meningitis (with and without CSF pleocytosis) and an available stool sample for analysis were stool EV-PCR+, and the positive predictive value of a positive stool sample for CSF-EV+ was 87% (table 1.0). The stool EV-PCR+ samples were obtained up to several days following reported onset of symptoms, with some positive samples collected more than a week following onset of symptoms, which could be expected because EVs may continue to be shed in stool for weeks after infection (figures 1.1-1.2). These findings suggest that stool EV-PCR is a sensitive test for diagnosing EV infection in children, and that if CSF pleocytosis is also present it could be reasonable to consider probable EV meningitis the diagnosis. Few children with EV meningitis (with or without pleocytosis) were EV-PCR+ from an upper respiratory sample. Overall, 25% (3/12) of children with EV meningitis had a positive throat swab and 66% (2/3) had a positive NPA sample. No children with aseptic meningitis of unknown cause, and only one child who did not have meningitis had an EV-PCR+ upper respiratory sample. These data suggest that obtaining upper respiratory EV-PCR may be a less sensitive test for EV meningitis compared with stool EV-PCR. Although the majority of the serum EV-PCR results were invalid, the serum EV-PCR+ results obtained were from samples that were collected on day 1 or 2 following onset of symptoms, which would be consistent with viraemic stage of EV infection when virus may cross the blood brain barrier by haematogenous spread.

For children with aseptic meningitis who were CSF EV- or who did not have CSF EV-PCR performed, 35% (6/17) were stool EV-PCR+ by EV R-gene® kit, but none were serum or throat swab EV-PCR+ (figure 1.3). Two of these children did not have CSF EV-PCR performed at hospital site, and the remaining four were CSF EV- but had no other possible cause defined from

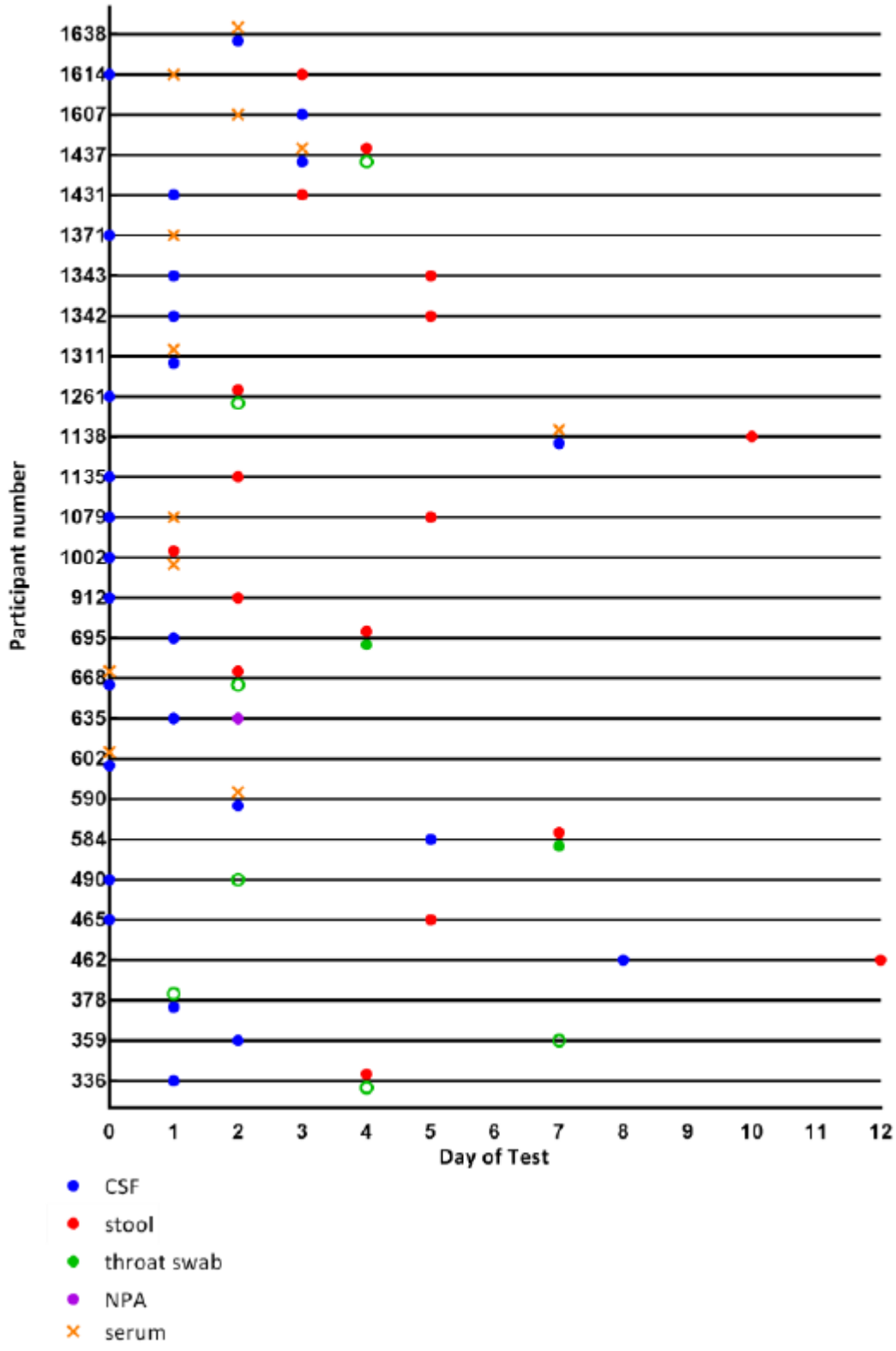
hospital site investigations. One further child with CSF EV- aseptic meningitis was stool and throat swab EV-PCR+ at hospital site laboratory. Although these data are limited by sample size, the infants and children who were CSF EV-PCR- and stool EV-PCR+ presented with generally similar clinical features to children who were CSF EV+ including vomiting, rash, fever and altered consciousness.

Children with CSF EV infection who presented with CSF pleocytosis had higher blood WBC, but lower CRP, and were slightly older than those without CSF pleocytosis. Almost all children with aseptic meningitis (including EV meningitis) received IV antibiotics (95%, 58/61), and 32% (19/60) received IV or IM antibiotics prior to LP. Children with CSF pleocytosis had a slightly longer LOS compared to without pleocytosis (4.2 days versus 3.4 days, p=0.001) in the full UK-ChiMES dataset.

Table 7.4. Results of Enterovirus R-gene® RT-PCR from different sites for children with aseptic meningitis or suspected meningitis

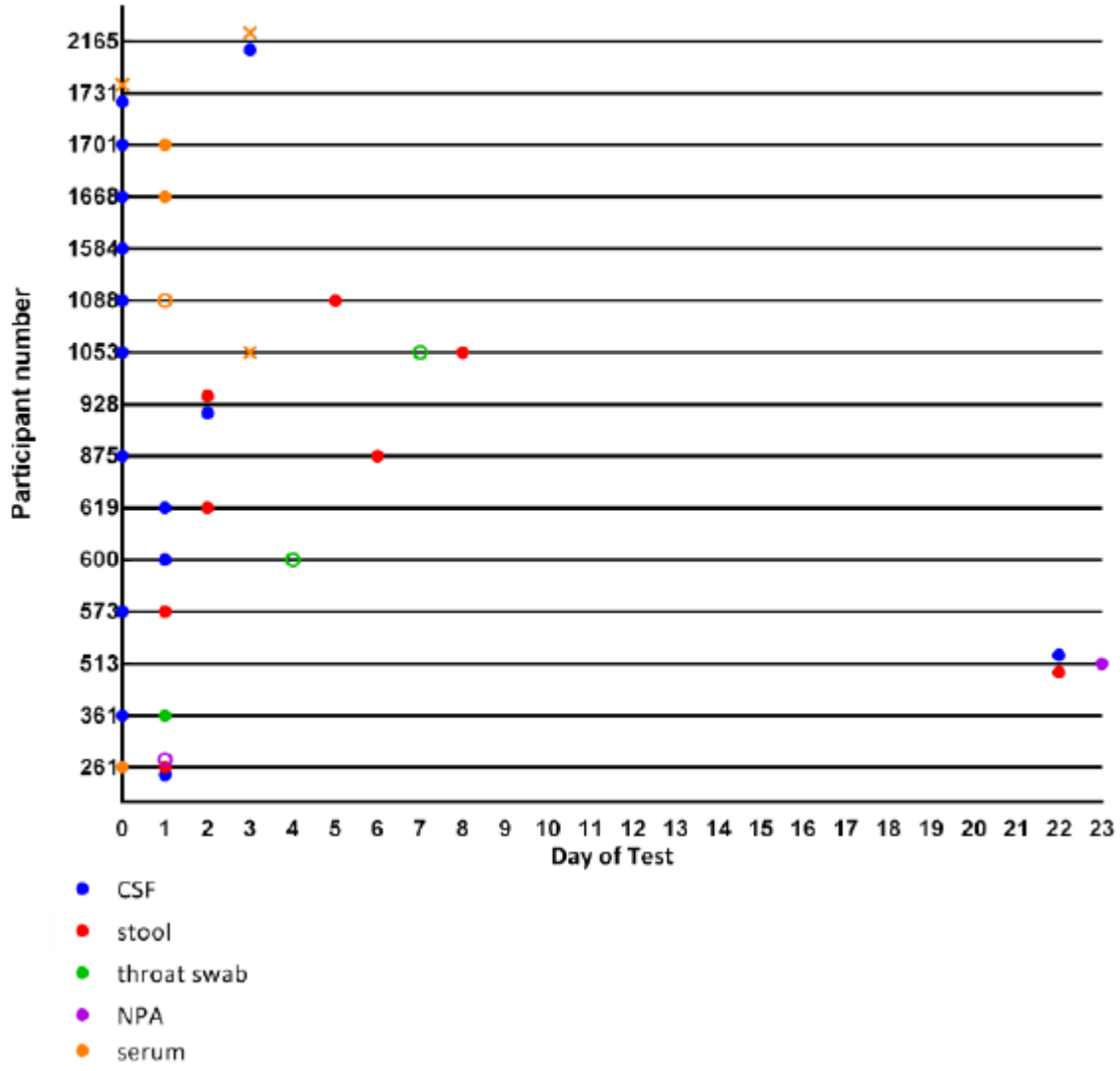
	Percentage positive enteroviral PCR results			
	Stool % (n/N)	Throat Swab % (n/N)	NPA % (n/N)	Serum % (n/N)
Group 1: CSF EV+, with pleocytosis (n= 27)	100% (17/17)	22% (2/9)	100% (1/1)	(0/0)
Group 2: CSF EV+, no pleocytosis (n= 15)	100% (9/9)	33% (1/3)	50% (1 /2)	75% (3/ 4)
Group 3: Aseptic meningitis, unknown cause, CSF EV- (n=19)	29% (4/14)	0% (0/6)	(0/0)	(0/5)
Group 4: Unspecified viral illness, normal LP (n=14)	0% (0/10)	13% (1/8)	(0/0)	0% (0/7)

Figure 1.1. Day of sample following onset of symptoms and result of PCR test from different sites for each participant, in children with confirmed EV meningitis with CSF pleocytosis (Group 1)



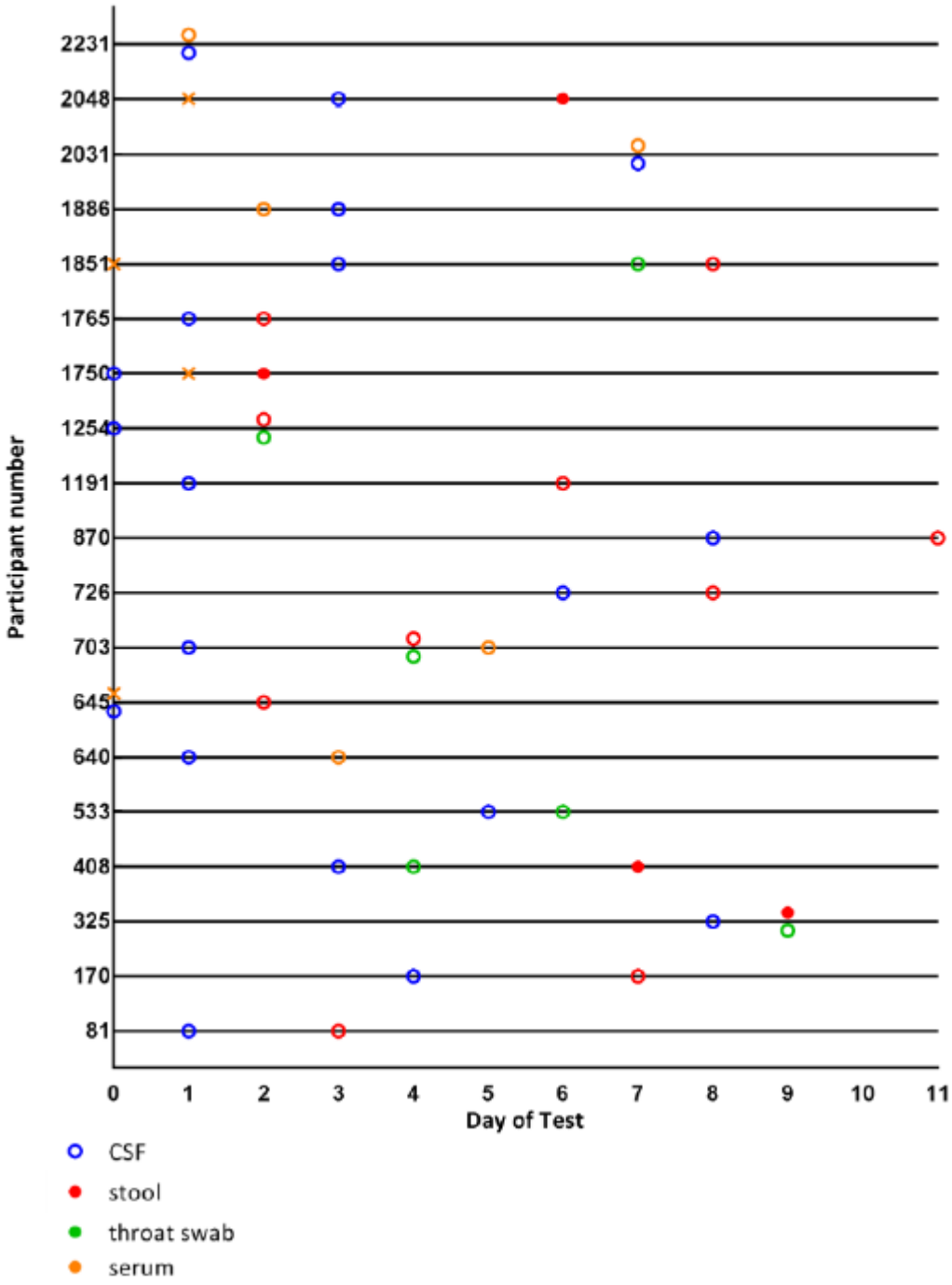
Notes: Filled circle indicates positive result, empty circle indicates negative result, x indicates invalid result. Results of Enterovirus R-gene® kit for stool and respiratory samples test, results of testing at hospital site laboratories for CSF samples.

Figure 1.2. Day of sample following onset of symptoms and result of PCR test from different sites for each participant, in children with confirmed EV meningitis without CSF pleocytosis (Group 2)



Notes: Filled circle indicates positive result, empty circle indicates negative result, x indicates invalid result. Results of Enterovirus R-gene® kit for stool and respiratory samples test, results of testing at hospital site laboratories for CSF samples.

Figure 1.3. Day of sample following onset of symptoms and result of PCR test from different sites for each participant, in children with aseptic meningitis of no known cause and CSF enteroviral PCR negative (Group 3)



Notes: Filled circle indicates positive result, empty circle indicates negative result, x indicates invalid result. Results of Enterovirus R-gene® kit for stool and respiratory samples test, results of testing at hospital site laboratories for CSF samples.

Summary:

The majority of meningitis nowadays is viral, with most cases where a pathogen is identified caused by enteroviruses. There are few previous studies which have assessed enteroviral positivity from non-CSF sites, particularly in children. In this study, in childhood with enteroviral

meningitis, all stool samples obtained were EV-PCR positive, and EV was isolated from stool samples many days following onset of symptoms. Although EV is not always isolated from CSF, the finding of an EV-PCR positive sample from another site could support the diagnosis of probable EV meningitis, and influence clinical decision making. This could lead to a reduction in unnecessary antibiotic use.

There are also few studies which have investigated differences in infants and children who presented with EV meningitis with and without a raised CSF WBC count. This study provided unique data comparing these different disease presentations. Ongoing studies assessing outcomes in these children will provide further knowledge about the relatively recently described phenomenon of infants who are CSF EV-PCR positive but have no raised CSF WBC count.

I have applied for grants to continue research into viral and enteroviral central nervous system infections in New Zealand children. I gained excellent research skills at the University of Oxford, UK which I am keen to apply to improving health outcomes for New Zealand children.

PUBLICATIONS / PRESENTATIONS

Peer Reviewed Conference Proceedings (poster attached):

Martin NG, Sadarangani M, Willis L, Beckley R, Coxon A, Kadambari S, Kelly DF, Heath PT, Nadel S, Solomon T, Pollard AJ. Detection of enterovirus by real-time PCR of stool, serum, and respiratory samples in children with suspected or confirmed viral meningitis – findings from UK-CHiMES. ESPID Conference (European Society for Paediatric Infectious Diseases), 2016.

Oral Presentations

The results of this study have also been included as part of oral presentations I gave at the Paediatric Society of New Zealand Annual Scientific Meeting, November 2016 and at the George Abbott Symposium, Christchurch, August 2016

DPhil thesis chapter, University of Oxford

Martin NG, Sadarangani M, Kelly DF, Pollard AJ. Detection of enterovirus by real-time PCR of stool, serum and respiratory samples in children with suspected or confirmed viral meningitis. Childhood meningitis: current and previous UK epidemiology, clinical and laboratory characteristics and outcomes. DPhil thesis, Chapter 7. Oxford Record Archive; University of Oxford, 2017.

Manuscript in preparation, planned submission 2018

Martin NG, Sadarangani M, Willis L, Beckley R, Coxon A, Kadambari S, Kelly DF, Heath PT, Nadel S, Solomon T, Pollard AJ. Detection of enterovirus by real-time PCR of stool, serum, and respiratory samples in children with suspected or confirmed viral meningitis.

The findings from this study are also being prepared for publication in a scientific journal.