Neurodevelopmental Outcomes Following Severe Hand Foot and Mouth Disease in Vietnam

Thesis

NEURODEVELOPMENTAL OUTCOMES FOLLOWING SEVERE HAND FOOT AND MOUTH DISEASE IN VIETNAM

by

SARASWATHY SABANATHAN, MBBS

A thesis submitted to The Open University U.K

For the degree of Doctor of Philosophy in the field of Life Sciences

Oxford University Clinical Research Unit

Hospital for Tropical Diseases

Ho Chi Minh City, Viet Nam

December 2015

DATE OF SUBMISSION : 29 DECEMBER 2015
DATE OF AWARD : 15 AUGUST 2016

Abstract

In 2011, more than 160 children died in an unprecedented outbreak of Hand foot and mouth disease (HFMD) in Vietnam, predominantly associated with enterovirus 71 (EV-A71). The occurrence of encephalitis outbreaks in children at vulnerable developmental stages raised concerns of long-term consequences. Limited retrospective outcome studies in the literature lack either a healthy comparison group or a locally validated neurodevelopmental assessment tool. Brain MRI retrospective observations identified stereotypical patterns of brainstem lesions predominantly in severe EV-A71 HFMD cases with conflicting opinions on the prognostic role of MRI.

I adapted the "Bayley Scales of Infant and Toddler development (3rd edition)" for Vietnam and demonstrated that the adaptation was reliable and valid. I conducted a prospective observational cohort study to test the hypothesis that children with severe HFMD, graded per Vietnam Ministry of Health classification, would have lower cognitive, language and motor Z scores than a healthy comparison group. All HFMD cases had virological samples taken and a sample of severe HFMD cases had brain MRI scans.

All Z score 95% confidence intervals were within 2 standard deviations of the comparative healthy cohort mean suggesting outcomes at six months were not significantly lower than the healthy comparative group. I identified novel non-specific brain white matter abnormalities on MRI in all severity grades, lower motor Z scores in grade 2b children with MRI abnormalities and that Coxsackievirus A10 (CV-A10) was significantly associated with MRI abnormalities.

These findings support surveillance of all enteroviruses during HFMD outbreaks and suggest MRI may be predictive of motor impairment in a subset of severe

HFMD cases. No significant impairment was identified at six months follow-up, but more complex developmental skills are yet to emerge. Hence the study continues for an eighteen-month follow up to robustly determine emergence of long-term sequelae.

Preface

All the work presented in this thesis was conducted under the umbrella of a project agreement between the Hospital of Tropical Diseases in Ho Chi Minh City and the Oxford University Clinical Research Unit.

Acknowledgements

I am indebted to my supervisors Rogier van Doorn, Bridget Wills and Louise Thwaites. They have all been a continued source of inspiration and counsel. The projects described in this thesis owe an incredible amount to the enthusiasm and mentorship of Jeremy Farrar – I remain enormously grateful to his advice at the start of my PhD studies. I thank the Wellcome Trust and the Oxford Li Ka Shing Foundation for sponsoring this research.

At OUCRU and the Hospital for Tropical Diseases, I would like to thank in particular Dr. Phan Tu Qui, Dr. Huynh Trieu and Dr. Pham Ngoc Thanh, Dr. NTH Biet for enthusiasm, motivation and commitment to the study participants; Dr. Le Van Tan, Dr. Vu Thi Ty Hang, Nguyen To Anh for their help with virological diagnostics; Dr. Marcel Wolbers, Dr. Ho Thi Nhan for their statistical support; Dr. Mary Chambers and the public engagement team for community links and advice; Sarah Barton and Laura Merson for their logistical support. In addition, I would like to thank Dr. Biet from PMC district 8 who enthusiastically introduced us to community schools and clinics and has fully supported the community project. Thanks also to Dr. Phan Thanh Hai and his team of radiologists at the Medic Centre for performing the MRI scans. The challenge of adapting the Bayley was made easier with help from Betty Hutchson, Gloria Maccow, Professor Zhu and Sarah Weinberg from the publisher, Pearsons. I am incredibly blessed to have such a wonderful, dedicated and enthusiastic team of assessors: Nguyen Thi Kim Anh, To Thi Mai Dao, Phan Ngoc Thanh Tra, Le Nguyen Thanh Nhan, Cao Van Quang, Vo Khoai, Vo Ngoc Cat Tuong, Pham Tam Dinh, Nguyen Thi Xuan Hoa, Nguyen Thi Ngoc Nhi, plus the exceptional nurses Ms. Pham Thi Lieu and Ms. Huynh Thi Thuy Nga. The successful validation is due to their work above and beyond the call of the project. I learned so much from each member. Above all

thanks to the study participants without whom none of this research would have been possible

Special thanks to Dr. Kling Chong who set aside precious time to review and discuss the MRI scans at Great Ormond Street Hospital, London.

I am grateful for good advice and moral support from Anne Brink and Hannah Schmitt. Thanks especially to my husband, children and parents for their love and support throughout these projects.

ADMG

Abbreviations

ANS	Autonomic nervous system
ATT	Average treatment effect on treated
BSID II	Bayley scale of infant and toddler development 2nd edition
Bayley III	Bayley scale of infant and toddler development 3rd edition
CSF	Cerebrospinal fluid
CDAT	Child development assessment tool
CNS	Central nervous system
CV/ CVA	Coxsackievirus
CRF	Case record form
ECCE	Early childhood care and education
EV71/ EV-A71	Enterovirus 71
G-CSF	Granulocyte colony stimulating factor
HCMC	Ho Chi Minh City
HFMD	Hand foot and mouth disease
IFN	Interferon
IL	Interleukins
MRI	Magnetic resonance imaging
NTS	Nucleus tractus solitarius
PE	Pulmonary oedema
PMC	Preventative Medical Centre
PWS	Propensity weighted scoring
rRT-PCR	Real Time Reverse Transcriptase Polymerase Chain Reaction
TNF	Tumour necrosis factor

Table of Contents

List of Figures

List Of Tables

1. Introduction And Literature Review

Morbidity from central nervous system infections covers a wide spectrum of manifestations. Understanding these is important to identify potential points of intervention, as well as to measure the burden of disease on society and prioritise public health resource allocation.

Over the last twenty-five years, hand foot and mouth disease (HFMD) has emerged as a threat to early childhood development in South East Asia. The infection itself, and the supportive care needed during the acute illness, may both give rise to long-term sequelae but the delicate health care systems operating in these regions are typically unable to provide follow-up care or support after the acute phase.

This chapter will explain the relevance of hand foot and mouth disease to Vietnam and the surrounding region, and outline the rationale for the research project.

1.1 Hand foot and mouth disease (HFMD)

The clinical syndrome of a vesicular rash on the palms of the hands and soles of the feet with ulcers in the mouth was first described in New Zealand in 1957 in 8 children,[1 2] but no virological studies were reported at the time. In 1958, 60 people were investigated following a mild febrile illness with a maculopapular or vesicular exanthem associated with pharyngeal lesions;[3] 34 were children less than 5 years of age and all resided within a housing estate in Toronto, Canada. Virological studies confirmed a group A coxsackievirus (current nomenclature: Enterovirus A)

which was already known to cause a self-limiting febrile illness with mouth ulcerations, termed 'herpangina'.[4] In 1959, the term, 'hand foot and mouth disease' (HFMD) was coined in a publication describing 83 cases, of which 24 occurred in Birmingham, UK.[5] 14 of the 24 were children less than 5 years old. Coxsackievirus A16 was isolated from the vesicle fluid of one patient. By 1970, further reports of HFMD from the United States of America, England and New Zealand confirmed the outbreak nature of this mild clinical disease mainly affecting children.[6]

1.1.1 Viruses associated with HFMD

HFMD associated Group A coxsackieviruses (CVA) belong to the species *Enterovirus A*, genus *Enterovirus*, family *Picornaviridae*. HFMD associated viruses all belong to the species *Enterovirus A*. Since the initial isolation of coxsackievirus A16 from vesicular fluid in 1959, other enteroviruses belonging to the species *Enterovirus A* (types coxsackievirus A2-8, 10, 12, 14, 16 and enterovirus 71, 76 and 89-92), have been associated with HFMD.[7]

1.1.2 Enteroviruses

Enteroviruses are the most common human viruses worldwide, presenting either asymptomatically or with a diverse range of clinical manifestations depending on the age and immune status of the host. The taxonomy of enteroviruses continues to evolve as improvement in molecular and biological techniques identifies further serotypes and re-classifies others. Currently, there are twelve species within the genus Enterovirus, with seven that affect humans as their only host. These are Human Enterovirus A-D and Human Rhinovirus A-C. There are more than 100 serotypes of human enteroviruses A-D in total.[8] Enteroviruses are small RNA non-

enveloped viruses, which are susceptible to chlorine and iodine based detergents under certain conditions, but which are not susceptible to ethanol. Enteroviruses are acid-resistant, replicate in the tonsils and can pass through the stomach after ingestion after which they replicate in the gut. Transmission is generally oro-faecal, although enteroviruses can be found in respiratory secretions. It is also possible that onward transmission may occur from asymptomatic individuals to cause disease. Outbreaks of HFMD are most commonly associated with CVA16, with more recent outbreaks associated with CVA6 and CVA10.[9-13] Sporadic cases of HFMD have been associated with other pathogens (CVA2, CVA4, CVA5, CVA9, Echovirus 4).[14-17] Table 1.1 lists common clinical syndromes associated with enteroviruses.

Table 1.1 The clinical syndromes associated with enteroviruses

Enterovirus A	Enterovirus B	Enterovirus C	Enterovirus D
Herpangina	Pleurodynia	Paralytic Polio	Respiratory infection
Acute lymphatic or nodular pharyngitis	Aseptic meningitis	Aseptic Meningitis	
Aseptic meningitis	Severe systemic infection in infants, meningoencephalitis		
Paralysis	Myocarditis		
Hand foot and mouth disease	Pericarditis		
Pneumonitis of infants	Upper respiratory illness		
Common cold	Hepatitis		
Hepatitis	Aseptic meningitis		
Infant diarrhoea	Guillain-Barré		
Acute haemorrhagic conjunctivitis	Herpangina		
Meningoencephalitis			

1.1.3 Enterovirus 71

Enterovirus 71 (EV-A71) was first isolated from stool in 1969 from a 9 month old child with encephalitis.[18] Retrospective analysis from untyped isolates in the Netherlands identified EV-A71 circulating in 1963.[19] In 1975, Bulgaria reported 705 cases of central nervous system (CNS) disease, of which 149 (21%) presented with acute flaccid paralysis (AFP). [20, 21] In total there were 44 deaths. 92 strains of EV-A71 were isolated from 65 cases. 338 (48%) of total cases, 121 (81%) of paralytic cases and 41 (93%) of fatal cases were in children less than 5 years old.[22, 23] Neurological manifestations were prominent with no mention of rash or oral manifestations during this outbreak of EV-A71. In Hungary in 1978, 1550 cases of CNS disease, with a small number associated with the typical features of HFMD, resulted in 47 deaths.[24] 30 deaths were in children less than 6 years old. This was thought to be a mixed outbreak of a tick-borne encephalitis virus and EV-A71, with EV-A71 affecting mainly children.[21] Although interest in EV-A71 arose before its association with HFMD, this connection with a common disease of childhood, previously considered to be benign, cemented its profile as an emerging viral infection of importance.

1.1.4 HFMD and neurological disease: emergence of Enterovirus 71

In New York in 1972, among 10 cases with CNS disease (e.g. aseptic meningitis, encephalitis), some of whom had clinical signs of HFMD, EV-A71 was isolated in all cases.[25] In the summer of 1972-3, there were 39 cases in Melbourne with aseptic meningitis. In 6 children under 5 years with meningitis a rash was also present, described as '*either a fine erythematous maculopapular rash lasting 24-48 h (4 patients), scattered vesicles on the hands and feet (1 patient), or a mixture of vesicles, macules, and papules (1 patient).*[26] Over the same period, 5 cases presented with rash only, 2 of whom were diagnosed with HFMD. The virus

isolated in all cases was EV-A71. Sweden reported a large outbreak of aseptic meningitis and HFMD associated with EV-A71 between April and September 1973.[27] 41 (21%) cases were in children less than 3 years old. In 1973, there was a large outbreak in Japan with more than 3200 cases.[21, 28, 29] Hagiwara et al. reported the highest incidence of CNS disorders were in children less than 1 year with onset of CNS complications 2-4 days following a rash.[28] They also noted myoclonus in 26% of 81 children as a clinical feature indicating CNS disease onset. Further cases of EV-A71 associated HFMD were reported in New York in 1977 and again in Japan in 1978.[21, 29-31]

1.1.5 South East Asia outbreaks

In 1997, in Sarawak, Malaysia, more than 2000 cases of HFMD were reported with 31 deaths (26 less than 6 years old). 39 cases had aseptic meningitis or acute flaccid paralysis (AFP).[32, 33] Around the same time, mainland Malaysia also reported a few thousand HFMD cases with 4 deaths. Post mortem examinations of fatal cases identified EV-A71 as the causative pathogen of both HFMD outbreaks.[33, 34] Further large EV-A71 outbreaks continued to occur in the region. In 1998, Taiwan reported more than 129,000 cases of HFMD with 78 fatalities;[35, 36] 91% of the fatalities were among children less than 5 years, and in 92% of the fatal cases EV-A71 was isolated. In the next decade outbreaks of EV-A71 associated HFMD were reported in Australia, Japan, Singapore, Taiwan, Thailand, Malaysia, China and Vietnam (Table 1.2, Figure 1.1, Figure 1.2).[37-47]

Table 1.2 Published reports of EV-A71/HFMD outbreaks 1972-2013

Year & Reference	Country	Population: clinical features	Cases with HFMD	EV-A71 (state population)	Fatal cases	Ages	Sequelae
1972[48]	Melbourne Australia	49 hospital admissions Aseptic meningitis 6 other children maculopapular rash +/- vesicles hands and feet	✔ small numbers	All	0	Mostly <10 years. Rash most common in children <5 years	None noted
1972[30, 49]	New York	11 cases Aseptic meningitis and encephalitis	✔	All	0		None noted
1973[28 29 50]	Japan	Cases >3200 fine maculopapular rash trunk and limbs Myoclonus in 26% of 81 children 24% tremor, ataxia and myoclonus/CNS pleocytosis	✔	217 HFMD: 66 isolates EV-A71 36 aseptic meningitis: 11 isolates EV-A71	Yes: unknown number	0-2 year old 64% of all cases. Highest incidence CNS disorders in <1 year. CNS complications 2-4 days after rash onset.	Some left with sequelae, not described further. 2 cases AFP recovered within 40 days
1973[27, 30, 50]	Sweden	195 cases Aseptic meningitis	✔	Not stated	0	21%<3 years	Not stated
1975[50 20 51 29, 50 20,52 28,60]	Bulgaria	705 cases Aseptic meningitis (77.3%) Some myocarditis AFP 149 cases (21.1%) Bulbar palsy 9.6% of which 64% died.		92 EV-A71 strains isolated, 37 from post-mortem studies	44	48% total cases, 81% of paralytic cases and 93% fatalities in <5 years old[20, 52](20, 52)[20, 52][20 52][20,5220,52]	Not stated
1975[49]	New York	11 cases	✔ few	Not stated	0	Not stated	Not stated
1977[30 30, 50]	New York	37 cases AFP 2 polio-paralysis like syndrome	✔small numbers	12	0	10 children, 5 <1 year.	Recovery by 1 year

Year & Reference	Country	Population: clinical features	Cases with HFMD	EV-A71 (state population)	Fatal cases	Ages	Sequelae
1978[29 31]	Japan	36301 cases 8% tremor, ataxia and myoclonus/CNS pleocytosis	✔	Not stated	Not stated	Not stated	Not stated
1978[50 53]	Hungary	1550 cases aseptic meningitis encephalitis cerebellar ataxia AFP 724 encephalitis (6.8 per 100,000) This group had cerebellar ataxia and polio-myelitis-like paralysis and fatal cases	✔ small numbers	Not stated	47	30 of 47 fatal cases were <6 years old A mixed epidemic of tick-borne encephalitis, mainly adults and EV-A71, mainly children 826 aseptic meningitis (7.7 per 100,000)	Not stated
1979[50]	Lyon, France	5 cases Acute respiratory distress with CNS involvement		Not stated	Not stated	Children aged 5-9 years	Not stated
1980[54]	Taiwan	Approx. 20 cases Polio-like flaccid paralysis associated with HFMD/herpangina	✔	EV-A71 isolated in 5	0	3 or 4 years old. All referenced as personal communication.	Not stated
1985[31]	Hong Kong	5 cases total: 1 herpangina and AFP 1 erythematous rash on limbs and AFP 3 cases HFMD and AFP	✔	Yes	Not stated	4-86 months, average 23 months	3 children recovered within 40 days. 2 persistent weakness 8-9 months
1986[55]	Sydney, Australia			Suggested by phylogenetic analysis	Not stated	Not stated	Not stated
1986[56]	Victoria, Australia	114 cases isolated EV-A71 51 CNS disease	✔	114 with 5 cases got EV-A71 in hospital	0	51% admissions <1 year, 85% <5 years	Not stated

Year & Reference	Country	Population: clinical features	Cases with HFMD	EV-A71 (state population)	Fatal cases	Ages	Sequelae
1986[54]	Kaoshiung, Taiwan	HFMD/Herpangina. All referenced as personal communication in	✔	Not stated	Not stated	Not stated	Not stated
1987[57]	Philadelphia	5 cases AFP, >=1 extremity, areflexic at involved cord levels. One child with transient brainstem encephalitis		All	0	3 month-8 years, median 22 months	3 recovered within 60 days 2 residual weakness at 10-11 months
1987[58]	Alaska	Cases: 29 Of which 15 rash (this is referred in personal communication) 2 meningitis, 1 carditis		All	Not stated	Of all 93 cases of EV-A71 in US 1985-9, 49%<1 year 73%<5 years	Not stated
1987[59]	US: Alaska (above), New Jersey, Pennsylvania	Cases: 45 27 CNS		All	Not stated	Not stated	Not stated
1995[60]	Japan	158,677 HFMD		Not stated	Not stated	Not stated	Not stated
1997[32 61]	Sarawak	Cases: 2628 889 admissions, 39 aseptic meningitis or AFP	✔	Not stated	31 62% of fatalities had a rash, and 66% had oral ulcers	26 all <6 years	Not stated
1997[22 34 62]	Kuala Lumpur	Few thousands - 14875 HFMD	✔	Not stated	4 13 deaths	All<5 years	Not stated
1997[63]	Otsu, Japan	12 of which 7 HFMD, 2 herpangina 7encephalitis, 5 meningitis	✔	Not stated	0	11 patients<6 years.	One affected neonate had motor disorder
1998[36 35 64 65]	Taiwan	129,106	✔	EV-A71 in 48.7% outpatients, 75% hospitalised, 92% fatalities 37% of 194 cases	78	91%<5 years	405 cases with sequelae not detailed.

Year & Reference	Country	Population: clinical features	Cases with HFMD	EV-A71 (state population)	Fatal cases	Ages	Sequelae
1999[66 67]	Perth, Australia	Cases: 6000 29 severe neurological disease: aseptic meningitis, acute cerebellar and AFP.	✔	14	0	12/14 EV-A71 cases <5 years	2 children sequelae 1 year after initial infection
2000[60 68]	Japan	205365 HFMD	✔	Not well described	1 with EV-A71	Not stated	Not stated
2000-1[55 69]	Sydney	200 HFMD 6 neurogenic pulmonary oedema (PE)	✔	Some samples were EV-A71 positive but not clear which cases	0	All <5 years	Follow–up 6 cases with PE 17 to 86 months: Minor focal weakness to ventilator depended respiratory failure
2000[70]	Republic Korea	HFMD No data on case number	✔	12	0	12 cases < 6 years	Not stated
2000[41 64 65]	Taiwan	HFMD 291 confirmed severe cases	✔	25 EV-A71 152 EV-A71 35% of 983	18 of 181 EV-A71	91% of fatal cases <5 years	Not stated
2000-1[40 62]	Singapore	Sentinel surveillance 3790 HFMD 9000 ref 3 in	✔	76 (73.1%) of 104 cases	7	78.8% of 138 HFMD cases <4 years.	Not stated
2000[71]	Malaysia	Few hundred HFMD	✔	76 (68.5%) EV-A71 of 111 cases	Not known	2 fatal cases <5 years	One five-year-old child six weeks post discharge residual bulbar dysfunction.
2001[41 64 65 72]	Taiwan	HFMD 398 severe 628 reported severe EV disease	✔	26 EV-A71 182 EV-A71 28% of 1569 cases	58 deaths of 393 severe	Not stated	Not stated
2001[73]	Singapore	5187 HFMD	✔	45.6% of 178 cases	3	75% of cases <4 years	Not stated
2002[73]	Singapore	16228 HFMD	✔	3.8% of 210 cases	0	75% of cases <4 years	Not stated

Year & Reference	Country	Population: clinical features	Cases with HFMD	EV-A71 (state population)	Fatal cases	Ages	Sequelae
2002[74 64]	Taiwan	315 HFMD 162 severe cases	✔	16% of 1164 cases	30	93% cases < 4years	Not stated
2003[59]	Denver, Colorado	8 cases: Meningitis, AFP, pulmonary oedema	✔	All	1	All <5 years	1 child with PE (age 5 years) ventilator depended >3 years post illness, one child (aged 6 months) residual monoparesis.
2003[60]	Japan	HFMD 172659	✔	Not stated	Not stated	Not stated	Not stated
2003[64]	Taiwan	HFMD 139 severe	✔	594 of 900 between 1998-2005	8	93% cases < 4years	Not stated
2003[46]	Vietnam	HFMD 12 patients with encephalitis	✔	173 isolates from 764 children	3	Peak age-specific EV-A71 incidence age 1-2 years	Not stated
2004[64]	Taiwan	HFMD 148 CNS involvement	✔	594 of 900 between 1998-2005	5 of 30 EV-A71	93% cases < 4years	Not stated
2005[59]	Denver, Colorado	8 cases Encephalitis, meningitis, AFP	✔	8 EV-A71 in 2003 and 8 in 2005	0	One 9 year old. 7 <5 years.	The 2 year old and 7 month old with AFP had persistent monoparesis
2005[73]	Singapore	15256	✔	52.7% of 76 cases EV-A71	0	Highest incidence 0-4 years	Not stated
2005[64]	Taiwan	275 CNS involvement	✔	20% of 2003 cases	16 6 of 47 EV-A71	Children <1 year highest case-fatality rate	Not stated
2005[46]	Vietnam	764 enrolled in prospective study CH1 51 CNS disease,	✔	173/411 specimens EV-A71	3	Primarily children age <5 years	Not stated
2006[73]	Singapore	15282	✔	45.5% of 145 cases EV-A71	0	Not stated	Not stated

Year & Reference	Country	Population: clinical features	Cases with HFMD	EV-A71 (state population)	Fatal cases	Ages	Sequelae
2006[75]	Brunei	1681 HFMD	✔	42 EV-A71 from approximately 100 cases sampled	3	Not stated	Not stated
2006[62]	Thailand	HFMD 3961	✔	5	13	Not stated	Not stated
2006-7[76]	South Vietnam	305 CNS	✔	36	3	Not stated	Not stated
2007[62]	Thailand	16826 HFMD	✔	5.6% of 2744 specimens EV-A71	2 (Deaths probably due to EV-A71 but no lesions)	Not stated	Not stated
2007[76]	South Vietnam	HFMD 5719	✔	Not stated	23	Not stated	Not stated
2008-9[76]	South Vietnam	HFMD 21590	✔	Not stated	48	Not stated	Not stated
2007[77]	Linyi City, Shandong Province, China	HFMD 1149 Eleven of the HFMD cases (0.96%)were severe, presenting with neurologic complications, such as brainstem encephalitis and aseptic meningitis.	✔	55 EV-A71 (52.4%) out of 105 hospitalised cases	3	84.4% <5 years old	Not stated
2007[72]	China	83344	✔	Not detailed but EV-A71 cases	17	Not stated	Not stated
2007[73]	Singapore	20003 HFMD	✔	8.5% of 94 EV-A71	0	Children <5 years	Not stated
2008[76]	Singapore	15030 HFMD	✔	EV-A71 in 33.2% of samples tested	0	Not stated	Not stated

Year & Reference	Country	Population: clinical features	Cases with HFMD	EV-A71 (state population)	Fatal cases	Ages	Sequelae
2008[78]	Anhui province, North China	488,955 HFMD	✔	EV-A71 confirmed as major pathogen	126	Not stated	Not stated
2008[45]	Fuyang, China	HFMD March-May 6049 cases	✔	specimens from 161 cases. Six (46%) of 13 fatal cases, 36 (36%) of 99 severe cases, 17 (44%) of 39 mild cases were EV-A71	22 All of the 22 fatal cases were < 3 years old, and the youngest was 3 months.	78% of the cases < 3 years old.	Not stated
2008[79 80 81]	Prospective surveillance: All china includes above	HFMD 489540	✔	83.33% of deaths EV-A71	127	Children younger than 5 years old were the majority of the victims in the outbreaks, which accounted for 91.3% (446,263 cases) of the reported cases in 2008	Not stated
2008[82]	Mongolia	3210 HFMD	✔	245 samples, 102 (41.6%) EV-A71	Not stated	Not stated	Not stated
2008[62 83]	Taiwan	19530 cases 714 Hospital admissions HFMD/herpangina[83]	✔	109/714 (15.3%) EV-A71	One fatality 3 year old	199/280 (71%) cases than 3 years old.	90 (92.8%) recovery. Six patients (6.5%) had sequelae, including limb weakness in two patients, epilepsy in three patients, and two-limb disability (one upper limb and one lower limb) due to the complication of post-extracorporeal membrane oxygenation, with resultant amputation in one patient

Year & Reference	Country	Population: clinical features	Cases with HFMD	EV-A71 (state population)	Fatal cases	Ages	Sequelae
2008[84]	Hong Kong	98 cases herpangina 11.2% meningitis, 6.1% encephalitis	✔	98 EV-A71cases	1 (11 months old)	Highest incidence reported in children aged 0–4 years (27.9/100 000). 63.6% of complications in children <5 years	Not stated
2009[72 79 80 81]	China	HFMD 614901 ref 180 in 1,155,575 Severe cases 13835	✔	88.89% of deaths EV-A71	200-353 deaths	Children <3 years accounted for 93.2% (1,086,793 cases) in 2009	Not stated
2009[85]	South Korea	Prospective surveillance: 2,427 CNS symptoms, 519 HFMD/herpangina	✔	92 (82%) of 112 HFMD with CNS complications was EV-A71 and in 2 without HFMD/herpangina.	2	Mean age 46 months, SD 29, range 1 month to 12 years	4 cases with MRI brainstem changes developed ataxia
2010[80]	China	1774669	✔	94.12% of deaths EV-A71	905	Mainly children < 5 years	Not stated
2011[86]	Vietnam	116,000	✔	EV-A71	200	Mainly children < 5 years	Not stated
2012[80]	China	1619706	✔	94.12% of deaths EV-A71	509	Mainly children < 5 years	Not stated
2012[87]	Cambodia	78	✔	EV-A71	54	Mainly children < 5 years	Not stated
2013[80]	China	1832141	✔	100% of Deaths EV-A71	256	Mainly children < 5 years	Not stated

Year & Reference	Country	Population: clinical features	Cases with HFMD	EV-A71 (state population)	Fatal cases	Ages	Sequelae
2013[88]	North Sydney	37 with meningoencephalitis	✔	24 (65%) EV-A71	0	20/24 (83%) confirmed cases <35 months.	33/36 (92%) of cases full neurological recovery at 6 weeks. 2 children primarily focal weakness

AFP: Acute Flaccid Paralysis, PE; pulmonary oedema, HFMD; Hand foot and mouth disease, EV-A71; enterovirus A 71, CNS; central nervous system.

Figure 1.1 Map of HFMD/EV-A71 Outbreaks 1970-1995

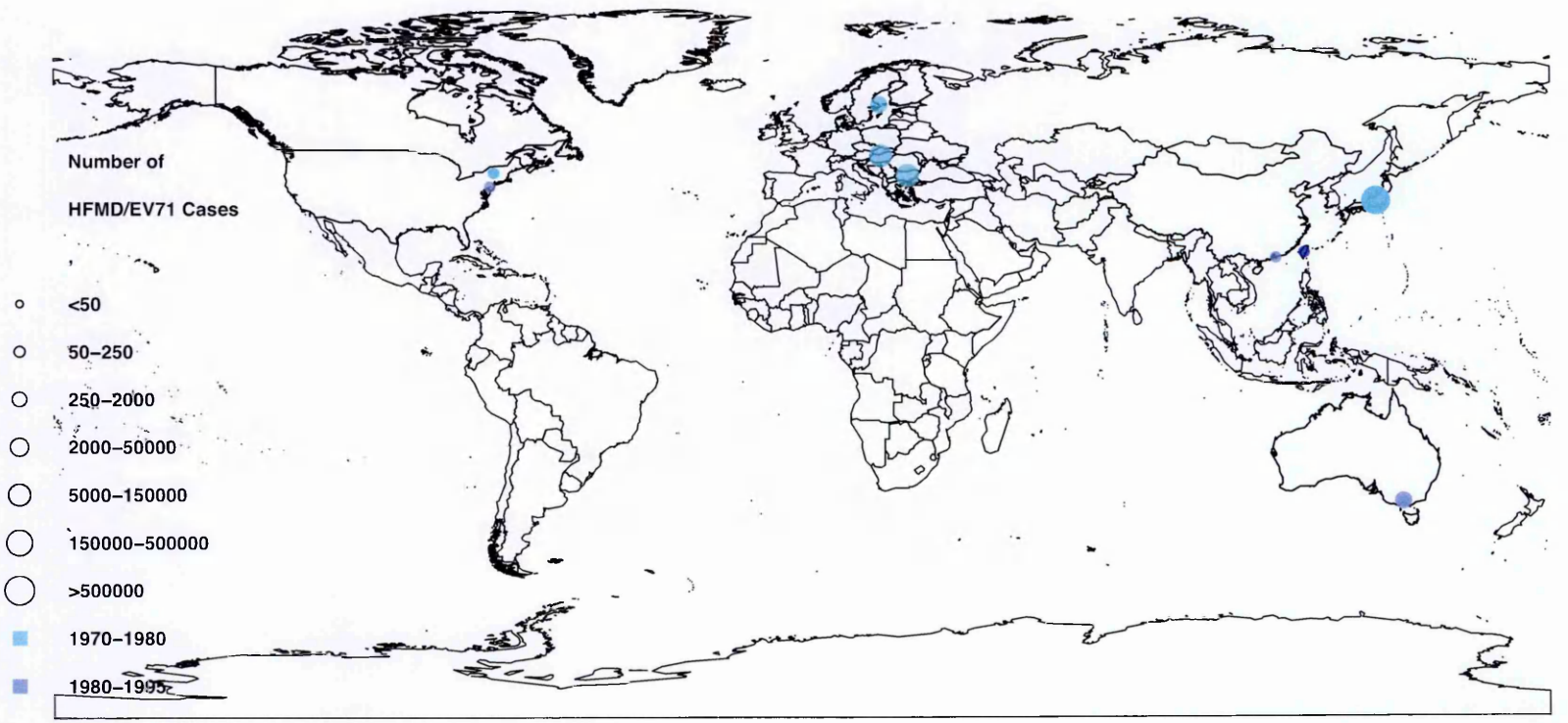
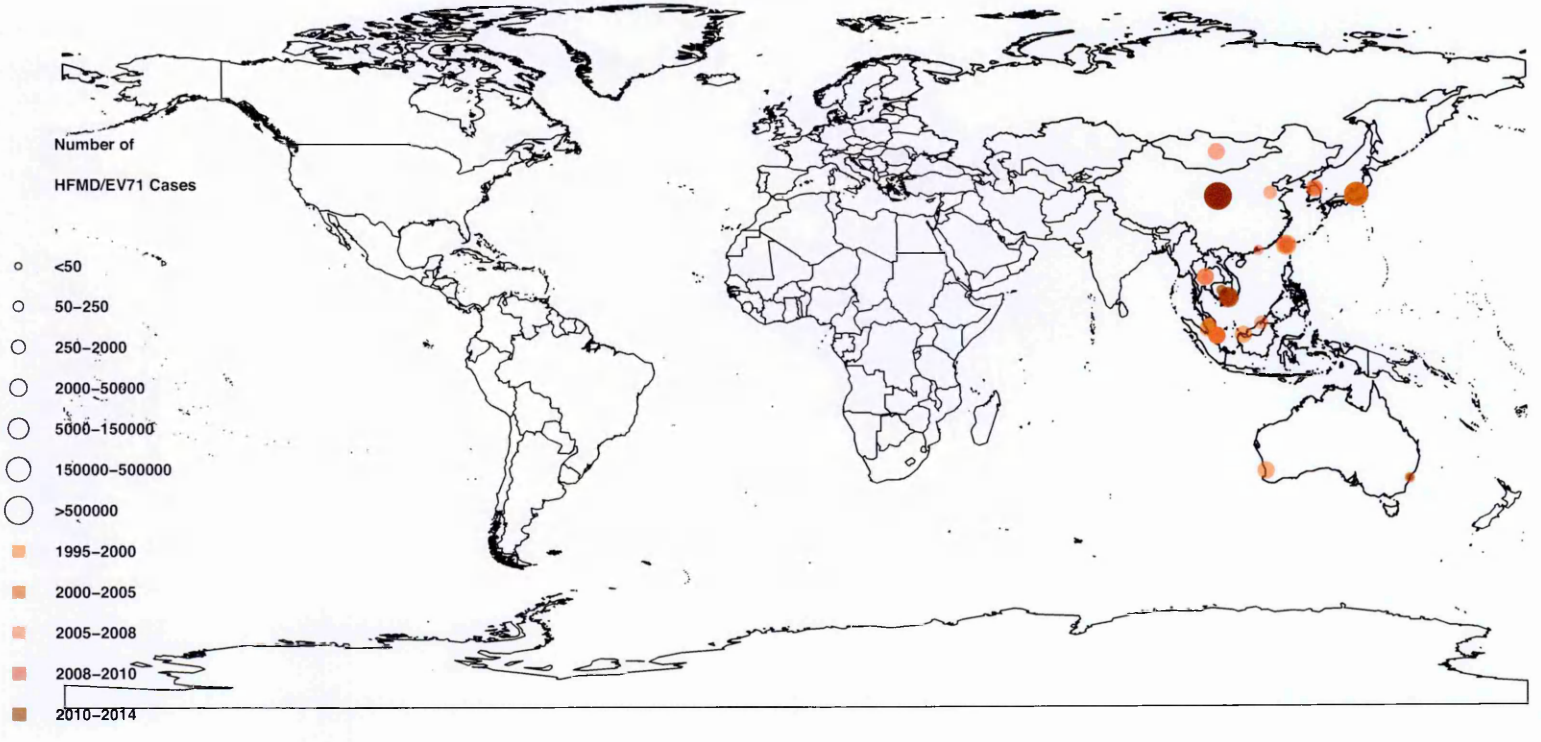


Figure 1.2 Map of HFMD/EV-A71 Outbreaks 1995-2013



1.1.6 Incidence and disease burden

Estimations of the incidence of HFMD vary by region and year. An epidemiological study in Singapore reported that the HFMD annual incidence rate per 100,000 in the general population increased from 125.5 in 2001 to 435.9 in 2007.[73] In Taiwan seroepidemiological surveys estimated the incidence of EV-A71 infection among children between 1994 and 1999 to range between 13-22%, depending on the age of the child.[54] Estimations from the 2008 Hong Kong outbreak suggest an EV-A71 incidence of 1.4/100,000 in the general population, but the rate was higher, at 27.9/100,000, in children under 5 years.[84] Of note, the EV-A71 seroprevalence rate prior to the Taiwan 1988 outbreak in the age range 6 months to 3 years was inversely related to this age group's specific mortality rate during the epidemic, and their severity of disease.[89] A prospective study in Taiwan measured EV-A71 antibody levels in serum of 749 healthy neonates at 0, 6, 12, 24 and 36 months during the 2008-2009 HFMD epidemic.[90] There were 28 EV-A71 infections, and of these 8 were asymptomatic, including 2 infants less than 6 months of age. The authors calculated a cumulative incidence of 15% for EV-A71 by 36 months, despite losses to follow-up, which resulted in samples from only 51% of the cohort being obtained at 36 months. The risk of EV-A71 infection increased after 6 months of age, in line with the expected trajectory for decay of maternal antibodies.

The first cases of EV-A71 were reported in Vietnam in 2003, from 12 patients with encephalitis complicating HFMD.[46] Further HFMD outbreaks occurred from 2005 onward with increasing numbers, in 2008 HFMD became notifiable and for the year 2011, 110,897 cases and 166 deaths were reported.[91]

A meta-analysis of 14 studies from Vietnam, Singapore, Hong Kong, South Korea

and China over the period 1998-2012 representing a total population of 112,546 children calculated a pooled case-fatality of 1.7% (95% confidence interval 1.2-2.4).[92] The largest population based study on HFMD was carried out in China. Chinese surveillance data from 2008-2012 of over 7 million children with HFMD reported an annual incidence rate of 1.2 per 1000 person-years between 2010 and 2012. The highest HFMD incidence was in children aged 12-23 months (38.2 cases per 1000 person-years). Children aged less than 6 months had the highest risk for severe and fatal disease, with the risk declining with increasing age. 1.1% of the children had neurological or cardiopulmonary complications, of which 3% died.[93]

Case fatality rates in Taiwan range from 96.96/100,000 in children age 6-11 months to 3.34/100,000 in children aged 3 years.[54] Hong Kong reported a case fatality rate of 1.0%.[84] The case-fatality rate of EV-A71 HFMD cases from a prospective referral hospital based study in Ho Chi Minh City in 2011 was 0.2%, lower than the 2005 case-fatality rate of 1.7%.[46, 86] This may be due to increased awareness of the disease and its complications, earlier hospital admission, and consistent use of treatment guidelines. The Chinese population based study reported a case-fatality rate of 0.03% (n=2457), with 93% of the laboratory confirmed deaths (n=1737) associated with EV-A71.[93] This study likely gives the best estimates of incidence and case-fatality in endemic/epidemic settings, based on clinical diagnosis of probable HFMD cases.

1.1.7 Structure of *Picornaviridae* and EV-A71 receptors

EV-A71 and the other HFMD associated types of *Enterovirus A* belong to the family of *Picornaviridae*. They are non-enveloped, small (30nm in diameter) virions with a positive single stranded RNA genome. The genome is

approximately 7500 nucleotides long. The enterovirus (EV) virions have an icosahedral capsid made of 60 identical subunits. Each subunit consists of the 4 structural proteins, VP1-VP3, plus a small internal peptide VP4.[94] VP4 is hidden within the virion, facing inwards to the RNA, and is not detected by the host defence system.[95, 96] Five subunits create a pentamer, and 12 pentamers make the virion. VP1 is located around the five-fold axis, whilst VP2 and VP3 are around the three-fold axis. EV subtypes are commonly identified using the complete coding sequence of VP1.

Viral replication occurs in the host cell cytoplasm. EV needs to attach to a cell receptor in order to initiate a configuration change in the virion, which uncoats the virion and then expels the RNA into the host cell.

Suckling mice, 3-4 week old cotton rats, vervet monkeys and rhesus macaques are susceptible to EV-A71, and are used as animal models to investigate the pathophysiology of the disease.[97] So far two receptors for EV-A71 have been identified: scavenger receptor class B member 2 (SCARB2), a ubiquitous membrane protein and P-selectin glycoprotein ligand-1 (PSGL-1). Both have been found to increase sensitivity to EV-A71 infection in mouse models.[98, 99] Antibodies to both receptors reduce mouse infectivity. PSGL-1 is primarily expressed on leucocytes so does not explain the infection of neurons and other host cells. SCARB2 also appears to play an important role for CVA7, CVA14, and CVA16, which are closely related to EV-A71. However CVA2, CVA3, CVA4, CVA5, CVA6, CVA8, CVA10, and CVA12 appear not to be dependent on SCARB2 for cell infection in vitro.[100] It is not clear how binding to these receptors results in conformational change in the virion and host cell entry. One study looked at SCARB2 and PSGL-1 expression in tissues of fatal EV-A71 cases from China.[101]

They identified that SCARB2 expression occurred in tissue cells with evidence of viral infection, such as the CNS and tonsillar crypts, but that it was also expressed in tissue not infected by EV-A71. This suggests SCARB2 alone does not determine EV-A71 infection. PSGL-1 was expressed in various cells which did not show evidence of EV-A71 infection suggesting its role in infection may be limited.[99]

1.1.8 EV-A71 lineages

EV-A71 is hypothesised to have evolved from coxsackievirus A16 around 1940, subsequently diverging into 3 lineages, A, B and C.[102] Lineages B and C are further divided into subgenotypes, B1–B5 and C1–C5 according to nucleotide sequence divergence. Although genetically different, all lineages and sub-lineages represent one serotype of EV-A71. Molecular epidemiological studies have identified that all lineages are circulating in Asia, with a few in Europe. Lineage B predominated in the Malaysia and Singapore outbreaks, while lineage C predominated in the Australian, Vietnamese and Chinese outbreaks.[103] Analysing genetic diversity for the VP1 region of EV-A71 suggested a correlation between the HFMD outbreaks in the 1990s and transient increases in genetic diversity of certain subgenotypes.[102] However, genetic diversity persists between outbreaks and the cyclical 2-3 yearly outbreak patterns observed in countries such as Japan, suggests that accumulation of a susceptible population also contributes to triggering outbreaks.[102] In vitro studies suggest there is cross-neutralisation of antibodies between subgenotypes of EV-A71, but there are differences in antigenicity between subgenotypes.[104] In vitro, subgenotype C appears more difficult to neutralise and hence may be more transmissible which has implications for vaccine development.[104] Sera taken weeks post vaccination with the C4 subgenotype cross neutralise B and other C subgenotypes.[105] It is not

clear if this persists with time but persistence may be related to multiple infections with related subgenotypes. Supporting this theory is the broad range of neutralizing activity found in sera from healthy individuals in Japan, where EV-A71 is endemic.[104]

1.1.9 Seasonality and seroepidemiology

Based on US surveillance data from 1970-2006 a seasonal pattern has been identified, with EV infections being more common from June to October.[106] The dominance of different serotypes changes over time with both epidemic and endemic circulation apparent. Endemic serotypes demonstrate stable low-level circulation whereas epidemic serotypes show large fluctuations, at times becoming the most prevalent serotypes in circulation. Since 1983, EV-A71 cases reported in the US were mainly from children less than 5 years. Seventy per cent of reported infections were within the *'enterovirus season'* (summer and autumn) with incidence suggesting endemic circulation.

Following the 1997 Sarawak HFMD outbreak, Malaysian surveillance data identified a 3-year cyclical pattern of EV-A71 HFMD outbreaks.[107] Surveillance data in Japan also identified a 3 yearly pattern with increased EV-A71 HFMD cases in 2000 and 2003, while CVA16 HFMD was more prevalent in 2001-2.[39] A longitudinal study in Taiwan found a decrease in circulation in EV-A71 in the 4 years prior to the large 1998 outbreak. It was suggested that during this time of low circulation, there was an accumulation of susceptible, immunologically naive hosts that exceeded the threshold density needed to trigger an outbreak.[108] This may be one of the factors that contribute to this cyclical pattern.[109]

Studies from Germany, Singapore, Taiwan and China consistently find lower seroprevalence of EV-A71 in children less than 5 years old compared to adults.[89, 110-113] Maternal antibodies detected in infants at birth were undetectable by 6 months of age in cohorts from Taiwan and Vietnam.[114, 115] This suggests that children between 6 months and 5 years are at greatest risk. A Singapore seroprevalence study, using residual hospital sera (excluding patients with HFMD symptoms), identified that there was lower prevalence of EV-A71 antibody in the age group 1-6 years but higher neutralizing titres in this age group compared to older children.[116] This suggests most children acquire the infection asymptomatically during early childhood.

1.1.10 Diagnostics

There is no cheap sensitive rapid diagnostic test available for EV-A71, so formal laboratory diagnosis is usually reserved for severe hospitalised cases. The traditional method is isolation in cell culture followed by serotype identification using neutralization with monoclonal antibodies. Direct methods of identification include the indirect immunofluorescence assay (IFA) and Real Time Reverse Transcriptase Polymerase Chain Reaction (rRT-PCR). Primary specimens that can be sampled for culture include throat swab, vesicle fluid, stool samples or rectal swabs, cerebrospinal fluid (CSF) and blood. The yield for both CSF and blood is very low, but suggests disseminated disease when positive.[68, 117, 118] EV-A71 may be isolated in a variety of human and primate cell lines including African green monkey kidney (Vero) cells, human lung fibroblast (MRC5) cells and human rhabdomyosarcoma (RD) cells. However, cell culture is laborious, slow (weeks) and costly.

rRT-PCR can be applied directly to clinical samples such as throat and rectal swabs, vesicle fluid, stool samples and cerebrospinal fluid (CSF). Caution must be used when interpreting the findings for samples from non-sterile sites, such as throat and rectal swabs/stool, to distinguish asymptomatic shedding from disease. EV-A71 can persist in respiratory secretions for up to 4 weeks and in stool up to 5 weeks, although shedding for several months is also reported.[119] Between 2000 and 2003, Ooi et al. attempted virus culture on 2,916 samples from 628 children with HFMD.[120] Throat swabs were the most useful specimen, although the authors advise to sample vesicles when possible. Otherwise they advise throat swab combined with rectal swab. Vesicle fluid is most desirable as it is a sterile site. CSF from three of 102 HFMD cases with aseptic meningitis grew enterovirus on viral culture (of which two were EV-A71). A similar trend was found from a prospective study of 66 children with encephalomyelitis in Taiwan.[121] These 66 children had a clinical diagnosis of herpangina/HFMD with neurological signs (one or more of; ataxia, myoclonic jerks, cranial nerve dysfunction, MRI brain stem changes); and positive pleocytosis, defined as more than 5 white cells per mm^3 with no bacteria detected in CSF. Eleven (16.7%) cases had EV-A71 detected in the CSF by RT-PCR, versus none detected by viral culture. This CSF detection rate was lower than for throat and rectal swabs, in which RT-PCR detected EV-A71 in 40.9% and 42.4%, respectively.

Aseptic meningitis is defined as CSF pleocytosis without bacteria detected. A variety of aetiologies may result in these findings including viral, fungal, and parasitic infections, autoimmune pathologies and malignancy. With the advent of RT-PCR, the most commonly detected causes of aseptic meningitis are viral infections, particularly enteroviruses during annual spring outbreaks of viral meningitis or HFMD, when they can be detected in CSF, and in swabs from throat and/or rectum.[122, 123]

rRT-PCR diagnosis depends on knowledge of the most up-to-date sequences of circulating subgenotypes of EV-A71, requiring molecular surveillance.

1.1.11 Pathophysiology: viral entry and spread to central nervous system

Enteroviruses typically replicate in the tonsils and the Peyer's patches in the gut. Spread to the regional lymphoid tissue results in a mild primary viraemia. Most infections are controlled at this point although asymptomatic carriage may occur, with continued shedding in respiratory secretions and stool. It is not clear how CNS infection arise. This may be from spread through the reticulo-endothelial system and then through a disrupted blood-brain barrier. Another route that has also been implicated from autopsies of EV-A71 cases and in animal models is retrograde axonal transport.[124] Seven autopsies from Malaysia found the degree of inflammation decreased from the anterior horn cells to the motor cortex, along the distribution of the motor pathways.[125] It was hypothesised that secondary viraemia infecting skeletal muscle may infect the neuromuscular junction. From here there may be spread through the peripheral motor nerves to the anterior horn cells, followed by further spread up the corticospinal tracts to the motor cortex. The sensory and autonomic neuronal pathways appeared spared. However evidence of viral replication in skeletal muscle or peripheral nerves has not been found from autopsy material.[101, 125] Typical autopsy features indicate inflammation of the central grey matter of the spinal cord and medulla oblongata, specifically involving the dorsal nucleus of the vagus, the tractus solitarius, the reticular formation of the medulla and pons, the inferior olivary nuclei, various cranial nerve nuclei, the basis pontis, the substantia nigra and red nuclei of the midbrain, the hypothalamus, and the subthalamic and dentate nuclei, with sparse viral inclusions found at these sites.[101, 126-128] The function and connections of

these regions are illustrated in Figures 1.3-1.5 and Table 1.3. The inflammatory changes observed were mainly perivascular cuffing by mononuclear cells with occasional neutrophils, and areas with clusters of neuronal necrosis and neuronophagia, microglial nodules and microabscesses. One autopsy reported involvement of focal areas of the white matter in the cerebellum.[22] Enteroviral antigens and nucleic acids have been reported in neurons and neuronal processes in the spinal cord and brainstem, with electron microscopy visualizing picornavirus like particles and viral inclusions in neurons from spinal cord tissue.[128] Additionally, autopsy findings in 5 neonates with fatal EV-A71 infection included cerebral oedema and brain herniation. (Abstract only in English)[129] These findings are not consistent with Lui et al. noting panencephalitis and mild non-specific changes in the spinal cord of an 8 month old fatal case.[130] Zang et al ran a prospective study of EV-A71 encephalitis cases admitted to the Fuyang Hospital PICU in 2008. Thirty-six children required mechanical ventilation. Seven patients died with one patient noted to have brainstem oedema.[131] Brainstem oedema may not be routinely identified on MRI scans or post mortem resulting in variability of its reporting in the literature.

Autopsy evidence in a case with pulmonary haemorrhage showed no significant alveolar damage or inflammation and no viral inclusions. The heart did show evidence of discrete myocardial mononuclear infiltrates in the right ventricle, but with no viral inclusions or myocyte damage.[128] In contrast, in ten autopsies of infants aged 13-36 months EV-A71 antigen was found in the heart, and in the bronchial lining epithelial cells as well as in alveolar macrophages.[132] Additionally some antigens were detected in the kidney and spleen. The majority of antigens were detected in the central nervous system. The absence of Nissl bodies was evidence of irreversible neuronal necrosis. There were a large number of

inflammatory cells in the small and large intestine, which also had a significant presence of EV-A71 antigen. Autopsies from Taiwan and China identified reactive hyperplasia in the Peyer's patches.[101, 127] Viral antigens have been found localised to the squamous epithelial cells of the tonsillar crypts, suggesting that this may be an important site for viral shedding and transmission and a possible entry route to the CNS.[101] Autopsy results from an 8 month old child who died in China showed the highest tissue viral loads in the brainstem and mesenteric lymph nodes, with the lowest viral loads in the ileum and stomach.[130] These findings support the hypothesis that the mesenteric lymph nodes are the main replication site and the brain the main target for viral invasion.

Figure 1.3 Outline of Brainstem

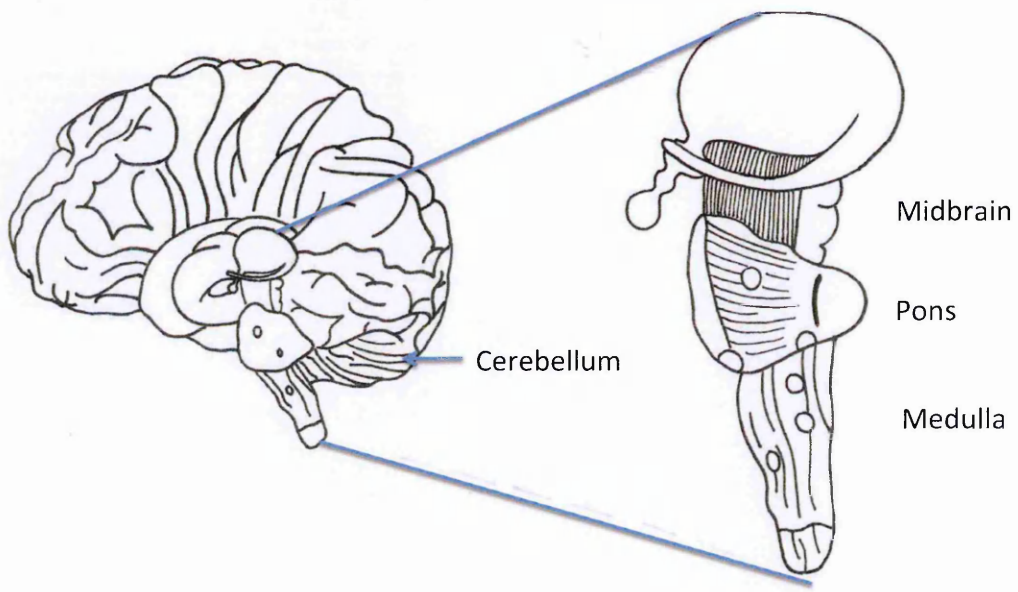


Figure 1.4 Locations of regions affected by brainstem encephalitis in severe HFMD (purple text). Cranial nerves are numbered.

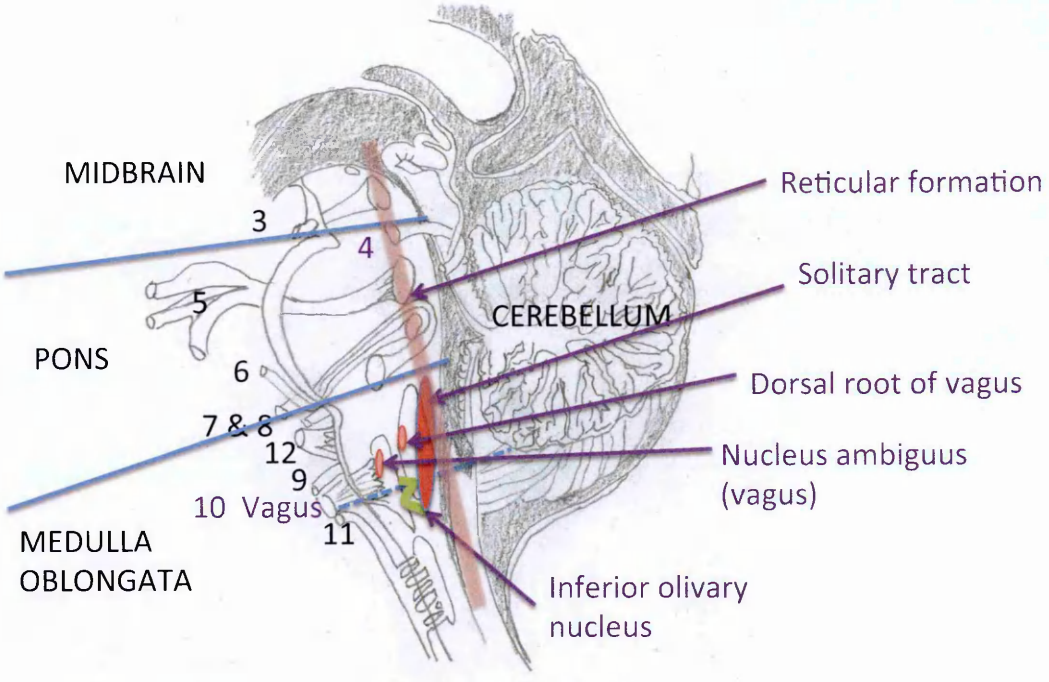


Figure 1.5 Image of dentate pathway and red nucleus affected by severe HFMD (pathway involved in movement planning)

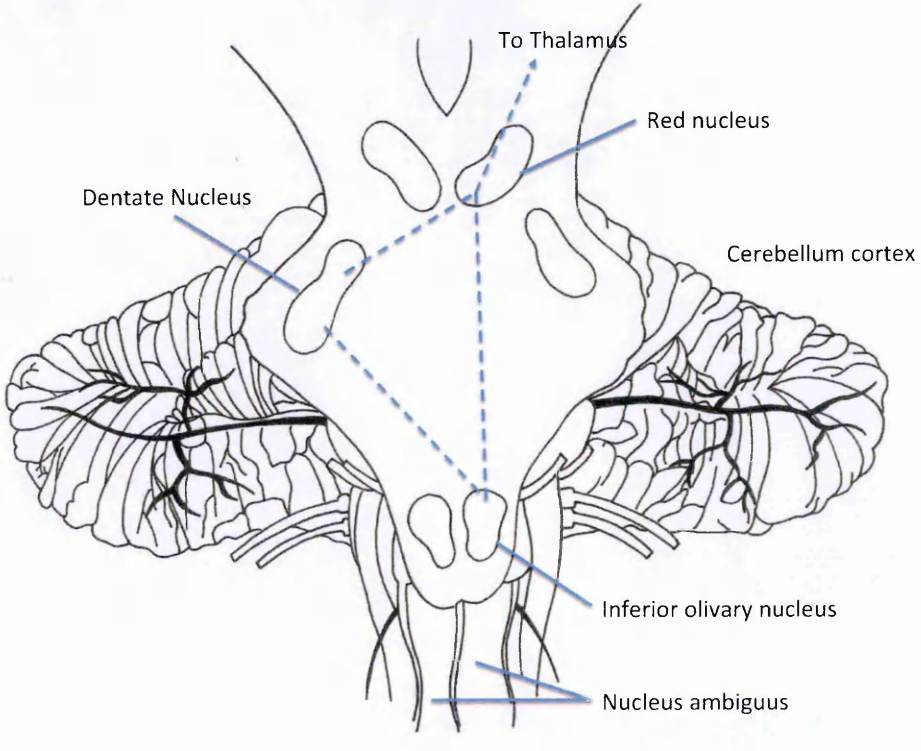


Table 1.3 Brainstem regions, connections, function and reports on pathology[133-136]

	Connections	Function	Clinical Effects
Hypothalamus	Afferent: sensory information from brainstem via nucleus solitary, also from thalamic nuclei and several regions in the cortex Efferent: To cortex, pituitary gland, thalamic nuclei and brainstem through reticular formation, dorsal motor nucleus, vasomotor and respiratory centres and preganglionic parasympathetic nuclei	Control of autonomic function, temperature regulation, appetite, thirst, endocrine functions, and involved in limbic system controlling emotion.	Hormonal deficiencies resulting in growth failure, thyroid dysfunction. Eating disorders, memory loss, sleep disturbances and autonomic dysfunction.[137]
Subthalamic nuclei (STN)	Afferent: Cerebral cortex, thalamus Efferent: nuclei of basal ganglia	Pathway that influences control of motor, oculomotor, cognitive and emotional functions	STN stimulation in adults with Parkinson's disease can cause transient confusion, apathy, and reduced verbal fluency; impaired attention, working memory and response inhibition; and depression, anxiety.[138, 139]
Midbrain			
Red Nucleus	Afferent: Cerebellum of opposite side and same side cortex Efferent: same side inferior olive, reticular formation and spinal cord of opposite side	Minor role in control of upper limb movement and coordination	Tremor and ocular and palatal myoclonus[140]
Substantia nigra	Afferent: striatum (subcortical structure receiving input from cortex)	Eye-movement, motor-planning, learning	Parkinson's disease results in tremor and akinesia.[141]

	Connections	Function	Clinical Effects
	Efferent: several projections to movement pathways (nigrostriatal pathway) and thalamus		
Pons			
Reticular formation Complex groups of nuclei throughout brainstem	Afferent: many sites dependent on individual nuclei but includes; hypothalamus, pre-motor cortex, spinal cord and adjacent brainstem nuclei Efferent: many sites dependent on individual nuclei but includes; cerebellum, ventral and dorsal horns of spinal gray matter, cranial nerve nuclei and autonomic nuclei in brainstem	Involved in regulating the autonomic nervous system, awake, sleep and arousal states, perception of pain, control of movement.	Pathologies affect level of consciousness and can lead to coma. High cervical lesions result in respiratory and cardiac failure.[142]
Dentate nuclei (cluster of neurons deep in cerebellum's white matter)	Afferent: pre-motor and supplementary motor cortex Efferent: pathway through cerebellum and red nucleus to contralateral thalamus	Visuo-spatial function and sensory input (weight) for motor control, planning, initiation and control of voluntary movement	Metronidazole induced lesions in the dentate nucleus resulted in ataxia and dysarthria in case reports.[143]
Basis pons	Afferent: includes motor cortex Efferent: contralateral cerebellum	Correction of motor error: learning motor skills	Rapid correction of hyponatraemia can cause central pontine myelinosis resulting in spastic bulbar paralysis, quadriplegia, stupor or coma, or the locked-in syndrome.

Medulla	Connections	Function	Clinical Effects
Inferior olivary nucleus	Afferent: spinal cord, dentate nucleus, and motor cortex, and the central tegmental tract originating from multiple nuclei at midbrain levels Efferent: cerebellum	Not completely clear but believed to be involved in motor learning.	Lesions in the triangle of Guillain-Mollaret (dentate efferents ascending through the superior cerebellar peduncle and crossing to the red nucleus, to reach the inferior olivary nucleus and back to original dentate nucleus) can cause palatal myoclonus.[144]
Dorsal nucleus of vagus (secretomotor)	Afferent: hypothalamus, olfactory system, autonomic centres in the reticular formation, especially the nucleus solitarius Efferent: parasympathetic innervation to gastrointestinal tract, lungs, and other thoracic and abdominal regions	Increased gastric peristalsis and secretion of gastric and intestinal juices. Control larynx and heart rate.	Lesions may result in respiratory sinus arrhythmias, cardiac arrhythmias and reduce gastric secretions.[145]
Nucleus ambiguus of Vagus (motor)	Afferent: pharynx and tonsils Efferent: muscles involved with swallowing	Parasympathetic cardioinhibitory effect on the heart and decrease pulmonary bronchial airflow. Swallowing includes innervation from nucleus of solitary tract. Innervates pharynx and larynx.	Atrophy and paralysis of most of palatine muscles, nasal speech, dysphagia, dysphonia.[146]
nucleus tractus solitarius (sensory)	Afferent: taste information from back of the throat, visceral information such as blood pressure changes, stretch receptors in the gut. Efferent: reticular formation, hypothalamus, amygdala and other regions of brainstem	Mediates gag reflex, involved in circuits modulating autonomic function	Lesions can affect taste and increase the heart rate, cardiac arrhythmias.[146]

1.1.12 Autonomic dysfunction and cardiopulmonary compromise

The brainstem contains important physiological systems that regulate temperature, heart rate, and respiratory rate and pattern. It contains the important nuclei that form part of the autonomic nervous system (ANS). The ANS regulates visceral organs such as the heart, stomach and intestines. It consists of the sympathetic nervous system that stimulates the 'fight flight response' of increasing heart rate and blood pressure and is generally activated during stressful situations, and the parasympathetic system, which regulates the organs when not under stress. The parasympathetic system, reduces blood pressure and heart rate, and stimulates digestion. The sympathetic nervous system mainly releases noradrenaline at the effector organs and stimulates the adrenal medulla to produce adrenaline. Acetylcholine is the neurotransmitter for the parasympathetic system. The ANS mediates communication between the peripheral nervous systems and the brain. Most organs, including the heart receive parasympathetic innervations through the vagus nerve. The vagus nerve nuclei are located in the brainstem. Inflammation of the vagal nuclei, as found in EV-A71 autopsies, may result in unbalanced sympathetic stimulation of the heart and a resulting tachycardia. The respiratory centre is located in the reticular formation in the medulla. (Figure 1.6). Inflammation of the nucleus and tractus solitarius and nucleus ambiguus will result in irregular breathing. Temperature regulation occurs through circuitry involving the thalamus, with autonomic control located in the hypothalamus. Peripheral information is relayed through the spinothalamic tracts to the thalamus and hypothalamus, and the hypothalamus has effector pathways that mediate vasomotor tone, sweating, shivering and piloerection. Autopsy results from 4 deaths following EV-A71 associated pulmonary haemorrhage identified extensive destruction of the ventral medulla, which is a central depressor area. Kao et al. hypothesised that excessive sympathetic activation

results in systemic vasoconstriction resulting in shift of blood to the pulmonary circulation. To evaluate this, they ran a 2 year prospective study recruiting 48 HFMD patients (age 6-18 years) whom they evaluated during periods of respiratory distress. Peripheral arterial pressure (AP) and heart rate (HR) were continuously recorded with use of a polygraph and a tape recorder. These were used retrospectively to determine HR variability. Four cases died and the authors state "Consent for autopsy of patients who died of acute PE was obtained from the patients themselves (in 4 cases) or from their relatives." [147] All cases who died were noted to have acute respiratory distress syndrome (ARDS) and developed severe haemorrhagic oedema (post-mortem finding). Prior to onset of ARDS (not defined in the paper) the AP and HR were reported to be within normal limits but changed following the onset of ARDS. The study identified that arterial pressure and heart rate variability suggested sympathetic activation one hour after onset of ARDS and 30 minutes prior to death.

1.1.13 Immune pathways

The inflammatory response is regulated by cytokines, which influence immune cell generation and specialization. In the CNS they are predominately produced by glial cells and the immune T helper cells and macrophages.[148] Outside the CNS, macrophages, monocytes, lymphocytes and vascular endothelial cells produce cytokines. Cytokines are signalling molecules, which a large variety of cells produce to communicate with other cells in response to infection or inflammation. They can have local and systemic effects. Cytokines consist of many different molecules, such as monokines, interleukins (IL), colony-stimulating factors, interferons (INFs), tumour necrosis factor (TNF), and can act on non-immune cells. Chemokines, such as IL-8 attract immune cells to sites of inflammation or infection. They also communicate between the neural-endocrine-immune

system.[149] Cytokines can be pro- or anti-inflammatory, neuroprotective or destructive, depending on their state and concentration.[150] In the CNS, blood borne or CNS immune cells, brain endothelial cells, astrocytes, microglia, and neurons can stimulate cytokine production. Pro-inflammatory cytokines include TNF-α, IFN-γ, IL-1, IL-6, and IL-18, while anti-inflammatory cytokines include IL-1 receptor antagonist, IL-4, IL-10, IL-11, and IL-13, and transforming growth factor (TGF)-β, with IL-6 falling in both categories.[151]

The hypothalamus is sensitive to peripheral and central cytokines such as IL-1β and TNF-α. In disease states, the brain upregulates IL-1β and TNF-α expression which can exacerbate the inflammatory response, resulting in a cytokine storm.[152] IL-1β is produced by blood monocytes as a result of infection or injury. It leads to fever, hypotension and stimulates other pro-inflammatory cytokines such as IL-6.[153] A small study in 33 children found significantly raised IL-1β, TNF-α and IL-6 levels in CSF and blood of severe EV-A71 HFMD cases.[154] Direct inflammation of the hypothalamus/thalamic areas plus up regulation of CNS released cytokines may contribute to the persistent hyperthermia seen in some cases with severe EV-A71 HFMD.

1.1.14 Autonomic nervous system and immune modulation

The autonomic nervous system (ANS) plays a fundamental role in mediating interactions between the nervous and the immune system. Constituents of the immune system enlist the ANS to coordinate sympathetic and parasympathetic discharges, which modulate immune organs and cell activities. As reviewed by Kenney et al, intravenous or intrathecal IL-1β administration changes sympathetic nerve inputs to the kidney, spleen, and adrenal glands.[155] IL-1β also adapts the sympathetic system to acute physical stress and in vagotomised rats IL-1β

release was enhanced, suggesting a vagal parasympathetic role in modulating the sympathetic response to raised IL-1β.[156] Similarly, systemic TNF-α and INF-α enhance sympathetic activity in animal models.[155]

Granulocyte colony stimulating factor (G-CSF) is a hematopoietic growth factor, involved in myeloid cell differentiation.[157] It is used therapeutically to mobilise haematopoietic stem cells from the bone marrow to the blood. One study suggests that G-CSF alters noradrenaline and adrenaline levels in humans.[158] Baseline levels of G-CSF are usually undetectable but rapidly increase with an infection and reduce with recovery. Endothelial and epithelial cells can produce G-CSF when stimulated by TNF-α, INF-β and IL-1. Raised G-CSF stimulates neutrophil mobilisation out of the bone marrow and may even modulate the function of the neutrophil at the site of infection and inhibit its apoptosis.[157] In mice models, G-CSF mobilisation is interrupted by pharmacological sympathetic blockade.[159]

Prostaglandin E2 (PGE2) production in blood-brain barrier microvascular and endothelial cells is induced by cytokines. PGE2 activates the sympathetic nervous system and the hypothalamic–pituitary–adrenal axis (HPA axis) resulting in fever.[160]

The vagus is believed to play an important role in communication to the central nervous system and IL-1β receptors are found on abdominal branches of the vagus paraganglia.[161] However, the lack of a parasympathetic effect of IL-1β receptor stimulation in rats in vitro raises questions over the significance of the pathway.[162] Other non-neural pathways include a) cytokines accompanying the transport across the blood-brain barrier through TNF/IL-1 receptors, b) lipopolysaccharide (LPS, a bacterial endotoxin) and IL-1 systemically inducing

PGE2 (in brain microvascular cells) and cytokines at the blood-brain barrier, and c) direct entry of immune mediators into the brain parenchyma in regions where the blood brain barrier tight junctions are deficient around the third and fourth ventricles.[163] Different immune mediators can activate differential or multiple pathways to communicate with the central nervous system, depending on the dose and route of entry of the immune stimulant.

The literature suggests that increased cardiac vagal tone is associated with decreased levels of IL-6 in healthy adults and in adults with cardiovascular disease, supporting the parasympathetic down regulating role in immune pathways.[164] The parasympathetic system also modulates the peripheral inflammatory response to suppress pro-inflammatory cytokines and activate anti-inflammatory cytokines.[155] This process may occur with low levels of cytokines in a specific location and before there are cytokines present in the systemic circulation. The vagus can also activate the innate humoural immune system by relaying information to the medullary reticular formation, locus coeruleus and hypothalamus and indirectly stimulate the anterior pituitary gland to secrete adrenocorticotrophin (ACTH), stimulating glucocorticoid production.[165] Animal models of vagus nerve stimulation suggest there are beneficial anti-inflammatory responses in haemorrhagic and toxic shock syndrome and in myocardial ischaemia/reperfusion.[166] Inflammation and pathological states of the vagal nuclei in the brainstem may inhibit protective anti-inflammatory systemic responses of the vagus in severe HFMD.

1.1.15 Immune mediators and autonomic nervous system dysfunction in HFMD

Systemic hypertension is believed to be an early indicator of potential progression to cardiopulmonary compromise. It may be the effect of unopposed sympathetic

stimulation and catecholamine release on the systemic and pulmonary circulation. This leads to increased peripheral vasoconstriction and raised pulmonary pressure resulting in pulmonary oedema or haemorrhage. This is termed 'neurogenic' pulmonary oedema.[167] There are reports of left-ventricular dysfunction associated with raised catecholamine levels.[168] The same researchers identified changes consistent with catecholamine cardiotoxicity in seven cardiac autopsies. In a retrospective comparison of 5 fatal cases of EV-A71 meningoencephalitis with 16 survivors, significantly reduced cardiac function and raised troponin I levels was found in the fatal cases.[169] The authors suggested that there may have been non-ischaemic myocardial damage although the timing of echocardiography and troponin I measurements was uncertain and cannot be conclusively assumed to be taken prior to shock. Prospective invasive measurement of pulmonary arterial and central venous pressures in five children with EV-A71 brainstem encephalitis complicated by pulmonary oedema found minimal changes in pulmonary arterial and central venous pressures.[170] The authors hypothesised that pulmonary oedema resulted from changes in pulmonary vascular permeability following a systemic inflammatory response induced by brainstem damage.

It is hypothesised that the cytokine profiles reflect severity of disease in HFMD/EV-A71 brainstem encephalitis. In a small study significantly raised IL-1β, TNF-α and IL-6 levels were found in the CSF and blood of 8 children with cardiopulmonary compromised EV-A71 associated HFMD compared with 21 healthy controls.[154] There were no significant differences in levels between controls (21 cases) and those with encephalitis (8 cases) and uncomplicated EV-A71 (17 cases) infection. However, in another study when the levels of CSF IL-1β and IL-6 were measured in the first 48 hours of admission in 24 EV-A71 cases

with diverse clinical manifestations, no differences were seen in levels between those with CNS manifestations, despite progression in some cases to cardiopulmonary failure within the same timeframe.[171] The authors speculate that CNS inflammatory derivatives may cross the disrupted blood-brain barrier and set off a systemic inflammatory response. However, the serum cytokine levels in this cohort were not as high as those in CSF and did not seem to correlate with the CSF levels. Wang et al. found high levels of IL-6 and IFN-γ in CSF samples from 9 children with pulmonary oedema (PE), when compared to 48 less severe EV-A71 associated disease manifestations.[172] Both IL-1β and IFN-γ are neuro-inflammatory and may result in disruption of the blood brain barrier. IL-1β was only found in the CSF of the 9 PE cases. The authors hypothesised its role was to permit cytokines to be released from the CNS into the systemic circulation[172, 173] IL-1β may additionally increase CNS catecholamine production through its effect on the locus coeruleus, the most important source of noradrenaline in the brain.[174] To explore whether CNS mediation could activate a systemic response resulting in increased pulmonary vascularity, 73 EV-A71 cases were age matched to 15 healthy controls and specific cytokines were measured in plasma.[175] Levels of plasma IL-10 was significantly higher in the group with pulmonary oedema than those with brainstem encephalitis. IL-10 is an anti-inflammatory cytokine, reducing lung permeability and is closely related to catecholamine release. The authors suggest IL-10 has an immune-regulatory role in the CNS and is activated by unopposed sympathetic stimulation following direct EV-A71 brainstem invasion. Although IL-10 may protect the pulmonary vasculature from leakage, it can increase thrombocytopenia and this process may adversely affect the pulmonary vasculature and contribute to pulmonary oedema. The study also found raised plasma IL-13 in all EV-A71 cases. The authors' hypothesis that the increased

airway hyper-responsiveness and over production of mucus as a result of raised IL-13, may contribute to PE formation.

The study evaluated IFN-γ levels in 73 EV-A71 cases and 15 controls since this cytokine is known to mediate microvascular leakage. IFN-γ levels were elevated in 34 patients with brainstem encephalitis and 14 cases with pulmonary oedema but not elevated in the 25 cases with autonomic dysfunction.[176] Kinetic evaluation from a child with PE found IFN-γ levels rise a day after IL-10 and may play an important role in PE genesis. Sympathetic release of catecholamines including adrenaline and noradrenaline stimulate pre- and post-synaptic receptors on immune cells.[177] These catecholamines inhibit the production of TNF-α, IL-1β and IL-6 whilst up-regulating anti-inflammatory IL-10.[178] Catecholamines influence the differentiation of CD4 T cells and their proliferation, although the exact nature of the relationship is not understood.[178]

Wang et al. identified depleted CD4+ T cells, CD8+ T cells, in 14 patients with pulmonary oedema and suggest that as a result of sympathetic activation on the systemic circulation, immune responses are impaired resulting in delayed viral clearance of EV-A71.[175] Zhang et al. evaluated the cytokine and chemokine profile in 153 children with differing severity of HFMD, of which 99 were EV-A71 positive and 31 CVA-16 positive.[179] Levels were compared to 19 healthy controls. They found certain cytokines and chemokines were more common in EV-A71 disease than CA16, and others had higher concentrations in the CSF compared to plasma in cases with CNS complications. Griffiths et al measured serum and CSF chemokine and cytokine profiles in 88 EV-A71 infected children, of differing severity, of whom 4 died.[180] They identified that a serum G-CSF to IL-5 ratio >100 on admission was predictive of death in their study cohort. Raised IL-1β and TNF-

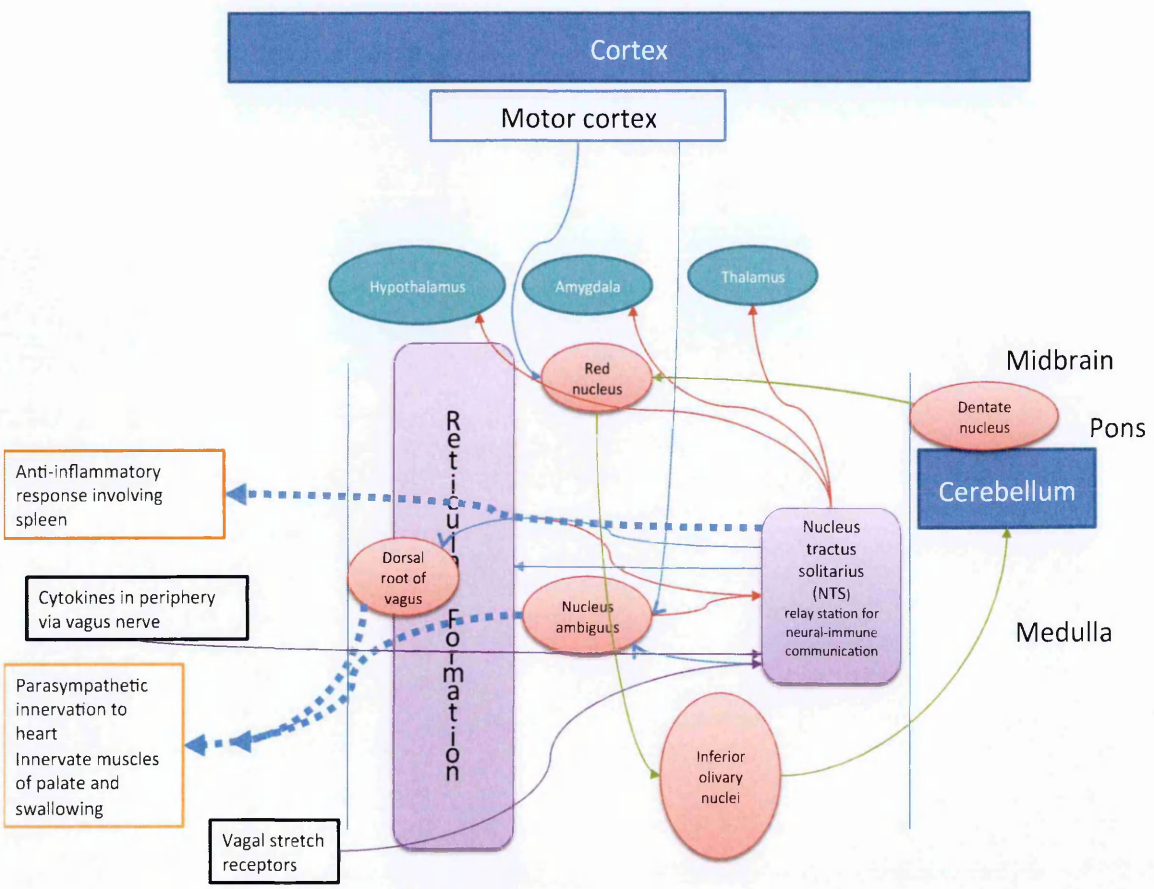
α levels were associated with the need for inotropic intervention, consistent with their known negative inotropic effects on cardiac contractility. The patterns of serum and CSF mediators detected in the study suggest CNS activation of the peripheral immune system. This has also been observed following stroke, possibly due to sympathetic activation of the spleen and lymph nodes.[181]

In summary, the HFMD immune mediator profile in the CSF and serum are different, suggesting that the CNS response may modulate the systemic response through the autonomic nervous system (Figure 1.6). However the complexity of the pro- and anti-inflammatory mediator levels found in each compartment indicate that further prospective evaluation is needed to establish whether any markers show a prognostic association with short or long term outcomes.

Figure 1.6 Schematic diagram of brainstem connections

Damage to vagus nerve centres in the brainstem inhibits the anti-inflammatory response following cytokine detection by the peripheral nervous system (efferent vagus response), and inhibits the parasympathetic response controlling heart rate, respiration and blood pressure.

The NTS (nucleus tractus solitarius) has projections to several structures in the limbic system including the hippocampus, thalamus and midbrain, and modulates emotion and attention.



1.2 Clinical features and stage based management

HFMD is usually a benign, self-limiting febrile disease of childhood. Typically children present with a maculopapular rash or vesicular lesions on the palms, soles and buttocks, and/or ulcers in the mouth. Rarely severe complications can arise with CNS features. There is an increased risk of CNS complications such as aseptic meningitis, acute flaccid paralysis and brainstem encephalitis associated with EV-A71 as highlighted in the South East Asian HFMD outbreaks. There are 3 stages of severe disease; a) CNS involvement, followed by b) autonomic dysfunction which may lead to c) cardiopulmonary collapse and shock. Children may develop a) or b) without progressing to c). Disease progression tends to be rapid, usually occurring within 3 days of disease onset.[182]

Clinically, CNS signs such as myoclonus or cranial nerve palsies may herald disease progression, but there may also be more subtle signs of brainstem encephalitis such as autonomic nervous system (ANS) dysfunction. However, ANS dysfunction can be difficult to identify, and requires continuous monitoring to detect the changes in blood pressure and heart rate before progression to cardiopulmonary compromise. Early outbreaks highlighted the lack of CNS signs prior to rapid cardiopulmonary collapse.[182] However one consistent sign that was identified was a raised white cell count in CSF, indicating CNS involvement but requiring lumbar puncture.

Current aims of the stage-based management are to identify early signs of CNS involvement, instigate early invasive monitoring for pre-emptive supportive care.

Following the large HFMD outbreak in Taiwan and based on the management system there, a local stage-based management system was developed in Vietnam.[183] It grouped the clinical features of HFMD as listed in Table 1.4. The WHO published a similar management strategy.[47]

Table 1.4 Vietnam stage based management system[86, 184]

Grade 1	Grade 2a	Grade 2b[a]	Grade 3*	Grade 4*
Rash with or without ulcers in the mouth	History from carers of myoclonus > 2times in 30minutes	Ataxia*	HR>170beats/min (afebrile and at rest)	Apnoea
Ulcers in the mouth	Irritability	Nystagmus*	Peripherally cold	SpO2<92%
Fever		Weak limbs (<4/5)*	Systolic Blood pressure • <1year >110mmHg • 1-2 years >115mmHg • >2 years >120mmHg	Shock: Undetectable BP
		Cranial nerve paralysis*	Raised respiratory rate	Low systolic BP: <12 mths: <70mmHg >12 mths: <80mmHg
		Myoclonus witnessed by clinician > 2times in 30minutes	GCS<10	Pulse pressure (systolic – diastolic)<25mmHg
		Lethargy	Irregular breathing pattern	
		HR>150 beats/min (afebrile and at rest)	Abdominal respiration	
			Temp>39, with antipyretics	

[a]Indication for admission to paediatric intensive care (PICU) *Indications for intravenous immunoglobulin (IVIG). HR: Heart Rate, Temp: Temperature, GCS: Glasgow Coma Scale

1.2.1 Current therapeutics

Intravenous immunoglobulin (IVIG) consists of the pooled immunoglobulin G (IgG) fraction from the plasma of a thousand or more blood donors. It is sterile and contains more than 95% unmodified IgG, with minimal amounts of immunoglobulin A (IgA) or immunoglobulin M (IgM). Pooled IVIG contains neutralizing antibodies against a wide spectrum of common infectious agents including enteroviruses. Components of IVIG are also believed to suppress various inflammatory mediators, including cytokines and chemokines.[185] There is anecdotal evidence that intravenous immunoglobulin (IVIG) may be beneficial in severe HFMD. IVIG may influence the cytokine network, and neutralise EV-A71.[186] Neutralising EV-A71 antibodies levels rise quickly in HFMD and do not correlated with clinical severity.[187] Cellular immune responses are believed to be more critical in determining disease progression. Wang et al. administered IVIG to 22 children with EV-A71 infection and compared specific cytokine levels within 12-24 hours to 13 age matched healthy children.[188] 12 children had ANS dysfunction and 10 had pulmonary oedema. There was no significant change in EV-A71 antibody titres post administration of IVIG, but there was a reduction in the plasma concentration of IL-6, IL-8, IL-10, IL-13, and IFN-γ in both groups with EV-A71. The authors suggest that IL-8 affects vascular permeability and reduction of this cytokine may inhibit progression to pulmonary oedema.

An observational study using historical controls suggests some benefit of IVIG in HFMD, but multiple management changes occurred between the two study periods resulting in earlier referral and supportive intervention in severe cases.[183] Another Malaysian observational study found more survivors of severe HFMD had received IVIG compared to fatal cases. However interpretation is confounded by the time-dependent bias, where the most severe cases may not have had time to

receive IVIG.[189] A randomised controlled trial is needed to determine the efficacy of IVIG in severe HFMD.

Antiviral therapies against EV-A71 are limited. Ribavirin has activity against DNA and RNA viruses including respiratory syncytial virus and herpes viruses. In mice models, it inhibits EV-A71 replication in infected cells but was less effective in 1 day old mice compared to 12 day old mice.[190,191] Pleconaril is another agent which inhibits picornavirus uncoating and blocks attachment to host cell receptors. In mice models it appears more promising against EV-A71[191] with small studies showing benefits in paediatric enteroviral meningitis.[192] A multi-site placebo controlled double blind study in 221 children, aged 4–14 years old with a clinical diagnosis of viral meningitis (181 had confirmed enteroviral infection), randomised the children into three groups.[193] One group received placebo and the other two groups received different doses of pleconaril for 7 days. There was statistically significant improvement in a clinical score in the pleconaril-treated groups, but this improvement was small.[194] In those receiving high dose pleconaril, the proportion of subjects reporting headache was reduced initially but the effect was not sustained.[193] A study of 21 infants with enteroviral meningitis was insufficiently powered to confidently show benefit with pleconaril.[194, 195] However a prophylactic study in picornavirus respiratory infection suggested that pleconaril may interfere with metabolic pathways, which could result in reducing the efficacy of contraceptives and HIV medication.[194] As a result the FDA did not license pleconaril as the modest benefits were insufficient to outweigh the risks for the intended use in mild respiratory infections.[194]

Milrinone, a phosphodiesterase inhibitor class 3 (PDE3) elevates intracellular cyclic adenosine monophosphate (cAMP), resulting in and increase of the heart's contractility and a reduction of pulmonary vascular resistance and peripheral

vascular resistance.[196] It is used for congestive cardiac failure and low-output cardiac conditions post cardiac surgery.[196] However there is a risk of arrhythmias with prolonged use so it should be used with caution.[197] In the last decade milrinone has been increasingly used in neonates with persistent pulmonary hypotension[198] post cardiac surgery for congenital heart disease,[199] and as adjuvant therapy for vasocontricted septic shock.[200, 201]

Elevated cAMP can inhibit inflammation-related chemotaxis, lysosomal enzyme and histamine release, mitogenesis, and lymphocyte mediated toxicity.[202] Cardiopulmonary bypass (CPB) surgery results in acute inflammation which predisposes to postoperative complications such as respiratory dysfunction, and potentially to multi-organ failure.[203, 204] In a randomised controlled trial on 24 patients undergoing CPB surgery, 12 received intravenous milrinone from induction of anaesthesia until 24 hours post surgery, 12 did not.[205] Clinical outcomes were the same in both groups, with no deaths and no difference in length of ITU stay or duration of intubation. However the levels of IL-1β and IL-6 were significantly lower in the milrinone group, supporting its immune modulatory role. Increasing pulmonary cAMP may protect or strengthen the microvascular barrier and thus increase clearance of alveolar fluid, reducing the reperfusion injury post CPB surgery.[206]

A small historical controlled trial suggested milrinone might help survival following cardiopulmonary collapse due to pulmonary oedema.[207] An open label randomised controlled trial of milrinone was carried out in Vietnam;[208] 22 children were administered milrinone intravenously within 2–6 hours of the onset of cardiopulmonary collapse (brainstem encephalitis with cardiopulmonary collapse, hypotension, and/or pulmonary oedema) and compared to 19 children with

standard treatment. Mortality at one week was significantly lower in the milrinone treated group.[208] Despite no evidence that milrinone has any beneficial effect when given prior to cardiopulmonary collapse, it has become standard management in managing hypertension associated with autonomic dysfunction with severe HFMD in Vietnam. The rationale is that milrinone may suppress the cellular immune processes associated with disease progression in addition to its vasodilator effects.[186]

1.2.2 Drugs and vaccines research

There are promising results from phase III trials of a Chinese inactivated EV-A71 vaccine (sub-lineage C4), with 90-97% efficacy against HFMD associated with EV-A71.[209-211] However, there are still many questions regarding whether the vaccine is suitable for the whole region, whether it will protect from severe disease, and particularly if the C4 sub-lineage will also provide protection against other EV-A71 sub-lineages or related viruses associated with HFMD. Taiwan and Singapore are also currently conducting vaccine research.[212, 213] Further research is needed to determine the target population, the age at which they should be vaccinated, and the overall cost effectiveness of vaccination.[214]

1.3 Risk factors for severe HFMD

A prospective observational study carried out in Malaysia between 2000 and 2006 identified three independent risk factors for CSF pleocytosis (used by the authors to define neurological involvement) among 725 children admitted with HFMD.[215] These were total duration of fever ≥ 3 days, peak temperature ≥ 38.5°C and history of lethargy. A retrospective questionnaire and serological study from the 1998 HFMD outbreak in Taiwan identified the following factors to be associated

with severe disease: attendance at a kindergarten/child care centre; contact with HFMD/herpangina cases in 1998; a greater number of children in a family; and residence in a rural area.[89] During an EV-A71 HFMD outbreak in China in 2008, 176 children were compared to 201 asymptomatic healthy children who were frequency matched by status of residence.[216] Retrospective questionnaires were administered to all parents to identify specific exposures and behaviours. The children with HFMD were more likely to have visited an outpatient clinic for another problem less than one week prior to onset of symptoms, played with neighbourhood children, and had poor hand washing hygiene practices, as did their parents. The community exposure in the HFMD cohort was significant even in those infants that did not attend kindergarten or pre-school. Hence the authors advocate control measures to include strict hand-washing and hygiene practices as well as restriction of community gatherings during an outbreak.

A case-control study in China, found exclusive breastfeeding in the first 6 months of life was possibly protective up to 28 months of age against severe HFMD, compared to mixed feeding (breast and supplements).[217] In a small retrospective study from the 1998 Taiwan outbreak, three groups of EV-A71 culture confirmed cases were compared.[182] 11 children with pulmonary oedema were compared to 38 children with CNS involvement without pulmonary oedema and 105 uncomplicated cases. The study identified hyperglycaemia, leucocytosis, and limb weakness as risk factors for pulmonary oedema following CNS involvement. Another small study found fatal outcomes in 8 children following cardiopulmonary shock were significantly associated with elevated troponin I ($p=0.001$) and CSF leucocytosis ($p=0.002$) compared to 19 survivors.[218] Following the 2000 HFMD outbreak in Singapore, 7 fatal cases were compared to 131 controls.[219] A raised total white cell count, vomiting and absence of mouth ulcers were predictive of a

fatal course. Similarly, Yang et al. ran a retrospective frequency matched case control study in 2010 and 2011 with 89 severe HFMD cases and 267 mild cases.[220] The study found oral ulcers were associated with mild disease whilst persistent fever >39 degrees for more than 3 days, "*leg trembling*" (authors clarify as "*clinical manifestation of legs shaking uncontrollably which may be due to weakness after illness*") and detection of EV-A71 infection were significantly associated with severe disease and neurological complications. In 665 EV-A71 virus culture confirmed cases, myoclonic jerks appeared to have poor positive predictive value for EV-A71 infection (0.48), severe disease (0.2) and neurological complications (0.09) but good negative predictive outcome for severe disease (0.95) and neurological sequelae (0.99).[221] A systematic review of 19 studies including those published in Chinese concluded that the following factors significantly increased the risk of severe HFMD: duration of fever for more than 3 days, odds ratio (OR) 10.09 (95% confidence interval (CI) 6.22–16.35); body temperature more than 37.5 °C (OR 4.91, 95% CI 1.26–19.18); lethargy (OR 7.75, 95% CI 3.78–15.89); hyperglycaemia (OR 2.77, 95% CI 2.06–3.71); vomiting (OR 8.83, 95% CI 1.05–74.57); increased neutrophil count (weighted mean difference (WMD) 0.61, 95% CI 0.52–0.70); EV-A71 infection (OR 5.13, 95% CI 3.11– 8.46); and young age (WMD -0.44, 95% CI 0.69 to –0.19).[222]

A prospective cohort study from Taiwan investigated the relationship between viraemia (RT-PCR positive for EV-A71 in a blood sample) and clinical severity in EV-A71 cases.[223] 224 children were enrolled and 26% were confirmed to have viraemia, of which 68% were detected within 3 days of illness. There was no association between the presence of viraemia and complications, although those who had viraemia after 3 days had significantly more complications. No viraemia persisted after 7 days, even if clinical symptoms persisted, suggesting that

progression after 7 days is unlikely. However the study did not have a large enough cohort with complications and did not take kinetic samples to determine correlation of viraemia with severe disease, with the single sampling method reporting a snapshot rather than trends. Due to the neurotropic nature of EV-A71, prolonged viraemia may permit CSF viral entry but is only one aspect of the pathogenesis.

1.4 Magnetic resonance imaging (MRI) findings in HFMD

The central nervous system consists of grey and white matter. The grey matter contains mainly neuronal cell bodies, glial cells and synapses, with very few myelinated axons. In contrast, the white matter consists mainly of myelinated axons, communicating between sections of grey matter. Grey matter participates in muscle control, hearing, vision, language, memory, cognition, self-control and emotions. The cerebellar and cerebral cortices consist of grey matter, whilst the interior of the hemispheres and cerebellum are mainly white matter. However there are grey matter structures within the hemispheres such as the basal ganglia. In the spinal cord, the interior consists of grey matter surrounded by white matter. Different disease states, such as multiple sclerosis,[224] Japanese encephalitis (JE)[225] and herpes simplex encephalitis (HSE)[226] can be distinguished by their localization or pattern of distribution in brain tissues. Magnetic Resonance imaging (MR) is the modality of choice for imaging grey-white matter differentiation and lesions in the brainstem. MR works on the principle that when a magnetic field excites protons from different tissues, the protons relax (return to their previous resting state) at different rates when the excitation stops. Protons from water and fat containing tissues relax differently and hence MR is useful in differentiating soft tissues. Increased proton density results in darker areas. There

are several types of MR imaging sequences where pulses of magnetic forces are applied and relaxation of protons are measured; T1 and T2. T1 images are bright when there is haemorrhage or calcification and are useful for identifying the border between brain and CSF. Water and fluid are bright on T2 scans and so T2 is preferred to assess oedema. Diffusion weighted imaging (DWI) measures the diffusion of water into surrounding tissue, which may be restricted in stroke or altered in demyelination. Fluid attenuated inversion recovery (FLAIR) is a technique where T2 weighted images are used and the CSF signal is nulled or cancelled out. It is useful for differentiating grey matter lesions.

The brain tissue changes of encephalitis typically go through an acute phase in the first few days of illness, followed by a healing process of gliosis (scarring) and atrophy which becomes increasingly evident from the end of the first week or early 2nd week through to the 4th week. The chronic phase of disease is when the final stages are relatively static and this is usually reached at 2 – 3 months and beyond. The MR sequence, T1, T2, DWI or Flair, that gives the most information, depends on the specific pathology being investigated and the time of the scan in relation to the illness onset. For example, DWI has been shown to be useful in HSE, but has less utility in adults with scans taken more than 7 days after onset of JE.[227, 228]

Availability of MRI is increasing in low and middle-income countries (LMIC), sparking interest in its possible prognostic utility. In practice, expertise in transportation of critically ill patients in such countries is lacking and very sick ventilated children are often unable to undergo scans even within the same hospital. Also, MRI requires children to be very still (for approximately 10-20 minutes for brain imaging), usually requiring some form of sedation. These factors

currently limit widespread application for patients with less severe disease, although imaging is more likely to be used in severe cases and may contribute prognostic information.

In EV-A71 HFMD outbreaks, MR scans have been carried out for clinical indications and then retrospectively reviewed. Published papers are detailed in Table 1.5. Typically lesions were found in the pontine tegmentum, medulla oblongata, midbrain and dentate nucleus within the first week of illness. Later scans, performed after 7-12 days, identified atrophy and gliosis of the brainstem. However, a variety of retrospective grading systems were used to classify disease severity and in some cases the timing of the scans was uncertain. Most lesions were identified on T2 series but there is a suggestion that DWI may be more sensitive in the acute phase in detecting lesions.[229] Early findings (day 1-5) may not persist and it is difficult to collate data from scans taken at different time points. This may explain why there does not appear to be a clear correlation between MRI changes, clinical presentation and outcome. In addition, scans have rarely been performed in patients with mild disease meaning that similar changes to those observed in severe disease may be present but go undetected. Since EV-A71 can present without rash and in many different clinical guises, it is possible that MRI scans could be very useful in aiding diagnosis. Currently there is no information on the correlation between MRI changes and outcome, especially long-term outcome. Despite its cost, access to MRI in LMIC is improving. However, research is needed to define how much the findings from this imaging modality can contribute to improving diagnosis and guiding management, potentially resulting in better outcomes.

Table 1.5 MRI changes in severe HFMD/EV-A71

Year	Country	Cases	Findings	Age at disease onset	Time to scan	Comment
1998[230]	Taiwan	28 culture confirmed EV-A71 with CNS disease	3 of 4 acute flaccid paralysis (AFP)- anterior horn and ventral root changes but full recovery. Abnormal scans in: 6(46%) of 13 myoclonus /tremor/ataxia 9(100%) of myoclonus & cranial nerve 2(100%) of cardiopulmonary (CP) Locations: 72% pontine tegmentum 55% medulla oblongata 44% midbrain 22% dentate nucleus Late scans showed brainstem atrophy and cavitation.	3 months to 8.2 years. Mean age 2.5years	26 scans done within 5 days of neurological symptoms, 2 scans in CP cases done 2 months after clinically stable.	No obvious difference in scans between different clinical stages. Small sample size.
5 cases from 2000-1 1 case from 1995[69]	Sydney, Australia	6 Culture proven EV-A71 +cardiopulmonary complications	Acute lesions in pons, brainstem, medulla, cerebellum. Late scans showed encephalomalacia, myelomalacia, or T2-hyperintense signal abnormalities concordant with clinical signs	0.5-4.2 years, mean 1.75 years	Acute scans done within 2 weeks of neurological symptoms. Not clear when late scans were done.	Small group with no detail on timing of late scans
1999[67]	Perth, Australia	14 EV-A71 positive CNS disease	2 cases transverse myelitis acute MRI (<3 days of onset): diffuse abnormalities dorsal columns, lower medulla to spinal cord. MRI at 3m and 8m: residual gliosis or atrophy from medulla to T5. MRI 12 days post 2 cerebellar ataxia: diffuse cerebellar swelling. One patient scanned at 3 months showed resolution of swelling and post inflammatory gliosis.	Age range 2m-12 years	Acute within 3 days and follow-up 3-8 months	Authors comment immunopathological disease rather than direct viral infiltration

Year	Country	Cases	Findings	Age at disease onset	Time to scan	Comment
1998[231]	Taiwan	14 EV-A71 +CNS disease	Normal scan in 1 meningomyelitis. 9 (69%) brainstem encephalitis had oedematous change over pons ± medulla	3m-12year, 87% <5 years	Not stated	Retrospective unclear grading. No details of timing of scans
1998[232]	Taiwan	20 HFMD and CNS disease. 18 were EV-A71 culture positive.	Abnormal in: 10(67%) Grade III (,fever, vomiting, lethargy, tachycardia, cranial nerve palsies, myoclonic jerks, paresis, dyspnoea, ataxia). 5(100%) in Grade IV (hypothermia, pulmonary oedema, respiratory failure) Locations: Posterior aspect of medulla oblongata Posterior aspect of pons Midbrain Dentate nuclei of cerebellum, thalamus, putamen, cervical cord	2m-7 years. Mean 25 months	Grade III -5 patients rescanned 2 weeks to 2 months later and lesions resolved. All neurologically normal by 1 month. Grade IV, 3 who had acute scans recovered. 2 late scans at 3 months showed persistent abnormal signal in medulla oblongata and pons in one, and old brain-tissue destruction in the other.	No clear timing of acute scans. Acute stage T2 scans revealed hyper intense areas not seen on T1 scans, believed to be reversible as patients recovered.
1998[233]	Taiwan	7 EV-A71 culture positive with AFP	5 scans within 2 days of disease onset and 2 scans at 2 and 7 months after diseases onset. 6 showed unilateral or bilateral hyper intense lesions in the anterior horn regions of the cord on T2-weighted images in six patients. 1 patient normal scan.		2 days-7 months	Residual weakness in 3(43%) at 1 year follow-up.
2010-11[234]	Korea	11EV-A71 culture positive with CNS disease	5 abnormal scans. 2 cases HFMD: both extremity weakness, CSF pleocytosis. 2 cases fever, lethargy, seizures, CSF pleocytosis. 1 Herpangina, both lower extremity weakness Typical findings in medulla and pons but Flair detected hippocampal involvement. All had repeat scans later and in one child, there was hippocampal atrophy. All other lesions resolved and clinical condition resolved	21m-4 years	Not stated	Authors speculate the findings correlate with retrograde spread of EV-A71 via motor pathways.

Year	Country	Cases	Findings	Age at disease onset	Time to scan	Comment
2010[235]	Shenzhen, China	42 HFMD EV-A71 with brainstem encephalitis Retrospective	MRI enhancement disease day 6 to 10 days from its onset and peaked at 7 to 8 days with the enhanced rate of 82%, higher than the average enhancement rate of 60% for the whole early stage. Six to 10 days after disease onset was the inflammation period of brainstem lesions; therefore, MRI enhancements were intensified. Later, either improvement of the inflammation or progression to encephalomalacia	5months -11 years	4-28 days	Good methodology Statistical comparisons done.
1998[236]	Taiwan	1HFMD, 1 herpangina both EV-A71.	Both monoplegia. Signals in anterior horn of spinal cord	5 months and 13 months	Not stated	
2008-2010[237]	China	21	All brainstem encephalitis. Rear of medulla oblongata, rear of pons varolii, rear of midbrain, thalamus, white matter of the cerebral hemisphere Dentate nucleus Ventral horn of spinal cord Subarachnoid cavity broadening at the bilateral parietal lobe Ventricle Authors classified according to Type I or II according to MRI. Type I cases exhibited unilateral or bilateral symmetrical patch-like hyperintense T1 and T2 signals from the posterior brainstem that were restricted mainly to gray matter nuclei. Type II brainstem encephalitis cases based on lower contrast, speckled patterns of hyperintense T1 and T2 signals that involved a wider area of the posterior brainstem than most type I cases. Authors suggest type I children may have poorer outcomes.	6-37 months	Not stated. Follow-up scans	

Year	Country	Cases	Findings	Age at disease onset	Time to scan	Comment
2008-2010[238]	China	35	24 brainstem encephalitis -12 grade I (myoclonic jerks+/- tremor+/- ataxia), 4 grade II (myoclonus + cranial nerve), 8 grade III (cardiopulmonary) Brainstem encephalitis: posterior medulla		Not stated.	
2008-2010[239]	China	35	7 AFP: long T2 signal intensity in the posterior part of spinal cord. Symmetrical, well-defined hyperintensity lesions in the spinal cord were seen in T2-weighted transverse images. 21 cases of pontine encephalitis: long T1 and long T2 signal intensity was seen in the posterior portions of the medulla oblongata, midbrain, pons, one lesion in thalamus. 7 aseptic meningitis: nonspecific MRI changes including widening of subdural spaces and ventricular system.	Median 19 months range (5-41 months)	Not stated	Of the 21 cases of pontine encephalitis, 3 patients died and 6 had mild neurological problems.
2009-2012[240]	Korea	21 total: 18 HFMD, 3 with no rash. I died so 20 MRI scans	13/17 brainstem encephalitis: lesions dorsal brainstem, bilateral cerebellar dentate nuclei 3/17 brainstem encephalitis: no abnormality 1/2 meningitis: small lesion left dorsal pons Encephalitis: no abnormality 1 AFP: contrast-enhancement of bilateral ventral nerve roots	12 months to 12 years.	Not stated	Note that MRI and clinical features do not always correlate: normal cerebellar on MRI had ataxia
2010-2011[229]	China	26: one left with neurological impairment and 1 died.	15: abnormal signal intensities in brainstem (posterior portions of medulla oblongata, posterior portion of midbrain, posterior portion of pons). 4 abnormal signal intensities in cortex and subcortical white matter, diffuse meningeal enhancement. Some additional lesions in corpus callosum, dentate nuclei, head of caudate nucleus and thalamus.	4-60 months	2-6 days after onset of illness (acute phase)	Diffusion Weighted images were more sensitive than T2 or Flair. Not much difference between T2 and Flair. DWI suitable acutely.

Year	Country	Cases	Findings	Age at disease onset	Time to scan	Comment
2001-2006[241]	Taiwan	42 HFMD (29, 69% EV-A71 +ve), 4 herpangina (2, 50% EV-A71 pos) with neurological involvement	26 had MRI: 15 abnormal with 13/15 (86.7%) EV-A71 pos. 2 EV neg had MRI changes and neurological sequelae. Lesions in the midbrain (3/15, 20.0%), pons (10/15, 66.7%), medulla (11/15, 73.3%), cerebellum (5/15, 33.3%), and spinal cord (6, 40.0%). No abnormal findings in the cerebral cortex.	Range for whole study, not just MRI scans: 5 months to 11 years median 22 months	Not stated	
2010-2012[242]	Korea	21 EV-A71 +ve in PCR cases: 18/21 (86%) had HFMD. No herpangina. brainstem encephalitis (n = 16), aseptic meningitis (n = 2), encephalitis (n = 2), and acute flaccid paralysis and brainstem encephalitis(n = 1) 20 cases MRI	1 child with brainstem encephalitis died within 15 hours. All others survived with complete recovery 12/15 (80%) brainstem encephalitis lesions in dorsal midbrain, dorsal medulla, pons, dentate nuclei. ½ (50%) meningitis had lesion in left dorsal pons. No lesion in 2 encephalitis cases. AFP and BE 1: lesions Dorsal midbrain, pons, dentate nuclei and both ventral nerve roots of Lumbar spinal cord 7/13 cases with abnormal MRI had scans 7days-1 month after first MRI with improvement in lesions.	Mean 3.4 years (range 12 months to 12 years)	Not stated	Authors state discrepancy between clinical features and MRI scans.

Year	Country	Cases	Findings	Age at disease onset	Time to scan	Comment
2008-2011[243]	China	12 EV-A71+ve cases	6 brainstem cases: changes in medulla and pons, 2 aseptic meningitis cases had abnormal widening of fronto-parietal subarachnoid space and ventricles, 4 AFP: enhancement of anterior roots	Range 6-37 months	first acute, then 4 months, then 2 years	Correlation between clinical outcomes I and persistence of MRI changes at 2 years, although lesions improved.

1.5 Outcomes following HFMD

There are limited data on long-term clinical outcomes of survivors. Since children less than 5 years are at particular risk, there are concerns about the long-term effects of infection during a potentially critical period of brain development. However, there is poor understanding of when the critical periods of brain development are, and the role of brain plasticity in recovery.[244, 245]

1.5.1 Biological plausibility: normal brain development

There is a preset programme of brain development from gestation onwards, with neurons migrating to specific sites to form neural networks.[246] Different regions and layers of the brain develop at different rates and the whole process is influenced by gene expression and environmental conditions such as maternal nutrition.[247] The prenatal period sees the development of the neural tube into recognizable cerebral and CNS structures. Interruptions to normal development at this point result in gross abnormalities of brain structure, or dysplasia of cortical or subcortical structures.[247] There is a careful balance between proliferation of neurons, dendritic development, synaptogenesis (a prerequisite for cerebral connectivity), differentiation (i.e. commitment to specialised systems with specific function) and apoptosis (programmed cell death).[247] After birth, there is on-going maturation in a set sequence into early adulthood.[248] Between 8 months to 2 years there is rapid dendritic growth and synaptogenesis, concomitant with pruning of the CNS as a result of environmental influences.[247 249] It is thought that the rapid production of dendrites and synapses allows for more adaptability of the brain, with the potential for functional reorganization if an insult occurs at this time.[250] Myelination ensures efficient transport of electrical signals. Peaks of myelination occur at 2, 7-9 and 11-12 years.[244]

The concept of 'critical' or 'sensitive' periods of brain development arose as there are specific times when inputs to the brain stimulate reorganization resulting in either a particularly good or bad outcome.[251] An example is the visual system. Failure of normal visual input due to squint during early life leads to reorganization of the visual pathways resulting in amblyopia. The same lesion in later childhood once development of the vision has occurred does not result in a permanent visual deficit. However, the timing and nature of the different critical periods relevant to different areas of the brain and its function remain poorly understood.[252]

Injury to the brain may result in neuronal death, damage to axonal tracts and/or altered neurotransmitter balances.[253] Later the impact of scarring can delay recovery. Following injury to the brain the recovery processes are hypothesised from adult studies to consist of two main processes; restitution and reactivation of pathways, and substitution/functional reorganization from damaged to healthy areas.[254] Most examples of restitution and substitution were identified in animal models, and it remains uncertain whether these processes are necessarily beneficial or preserve function, even if they do occur with more efficiency in early childhood.

Two patterns of recovery are well described following insults such as stroke, meningitis or trauma: either an apparent absence of deficit at the initial assessment but problems with emerging functions, or a delay in initial development followed by catch-up growth later.[244] These two patterns are dependent on the age at which the child had the insult and how long afterwards they were assessed. Short-term evaluation may not be able to discriminate emerging skills, or evaluate the effect of age at insult has on outcome.[244] A review

of outcomes following viral encephalitis in children identified most recovery occurred within 6-12 months of the insult, and the authors advised follow-up for at least 1 year.[255] With time however, there may be catch-up of skills that were delayed, although there may be secondary consequences on higher demanding skills in related functions. Thus for example a cohort of children who had bacterial meningitis were followed up at both 7 and 12 years following the insult;[256] initial findings included impaired reading ability at 7 years which had resolved by the 12 year follow-up, but had lead to later verbal learning impairment. In addition more functions need to be carried out by the non-damaged areas of the brain as time goes on. Attention, emotional control and executive function rely on a complex system of neural interaction and pathways, so functional reorganization may be difficult. As a result these functions may suffer disproportionately more following brain injury, than areas where functional reorganization is efficient.[257] Recovery post insult depends not only on the age of the child, the timing of the event and whether the pathological process is progressive or not, but also on external factors that can influence recovery such provision of a rich and stimulating environment.[253] This is particularly important in the younger child, and the impact is not usually seen until after the acute phase of the insult has resolved. Children who are exposed to an enriching, stimulating environment have been shown to increase their learning capacity, and hence this provides a potential opportunity for therapeutic intervention.[258] However the optimal timing for intervention is not known, and neither the simplistic theories of plasticity or vulnerability can explain the variety of outcomes seen following brain insults.[244]

1.5.2 Role of the brainstem on child development

The brainstem is evolutionarily the oldest part of the brain. It consists of the medulla, pons and midbrain,[259] and is comprised of a complex system of nuclei

and cranial nerves which are part of the sensory and motor systems.[260] The brainstem plays a vital role in coordinating the behavioural and physiological responses that occur during foetal development, including breathing movements, heart rate, and gross movements of the body and head. Later the brainstem takes on a major role in determining the sleep-wake cycle, and influencing heart-rate variability (i.e. physiological variation in beat to beat heart rate changes modulated by the autonomic nervous system).[259, 261] These hard-wired functions do not require higher input from cortical reasoning. There is evidence of some plasticity in neuronal network development within the brainstem through evidence of habituation to sound, and preference to maternal voice up to 6 weeks before birth.[262] However, it is generally agreed that the brainstem mediates reflex responses, as opposed to engaging true cognitive processing to result in purposeful behaviours and higher cognitive function.[259] Postnatally, the brainstem mediates the infant's reaction to hunger, resulting in crying. Smiling at 6 weeks is also a reflexive response and can be produced by brainstem stimulation. Later, as the child and the brain develop, the hypothalamus begins to exert some regulatory control over the midbrain. Normal development sees the appearance and then loss of some brainstem mediated reflexes such as the stepping reflex, and the grasp and Moro reflexes, as the forebrain begins to exert higher control.[263]

Porges et al. developed a theoretical model of the autonomic nervous system as hierarchically mediated, with social communication (vocalization, expression and listening) designated as the most superior function, followed by the defensive mechanisms - firstly mobilization through fight-flight responses, and lastly immobilization through vaso-vagal syncope and "behavioural shutdown".[123] According to this model, following exposure to a challenge the evolutionarily

newest circuit, i.e. social communication, is employed first. If that fails, then the mobilisation circuit comes into play next, followed lastly by the oldest immobilisation circuit. The newest circuit is operative by 6 months of age, correlating with increased myelination of the vagus nerve.[264] Disruptions occurring at this time due to illness or environmental stresses (such as neglect), may result later in difficulties with self-control and reciprocal social communication, as reviewed by Calkins et al.[265]

1.5.3 Outcome following brain stem pathology

The major CNS manifestation in HFMD is brainstem encephalitis (BE). Irrespective of cause, BE is rare in early childhood and, thus, literature on outcomes in this age group is limited. There is some data from adults. In 7 adults with brainstem lesions due to ischaemia or haemorrhage, cognitive deficits and attention problems were detected at follow-up.[266] Neuropsychological tests suggested that the fronto-cortical areas, which are involved in attention and executive function, were affected, indicating that the brainstem has intrinsic neural circuitry connecting it with the hemispheres. Hence it appears that the influence of the brainstem on the cortex and higher functioning is significant in the fully formed brain. In children however, in whom cortical development is still on-going, it remains unclear what effect the brainstem may have on cognition or attention and emotional regulation of responses.

1.5.4 Immune system and cognitive development

The immune system plays an important role in tuning brain activity during normal functioning, with complex immunological and brain interactions responding to environmental demands.[267] Neural plasticity is the process of fine-tuning neural

circuits by formation and pruning of axons and dendrites with synapses throughout life. This process steers learning and memory formation following experience. The baseline neuroimmune circuits, involving IL-1β, IL-6 and TNF-α also interact with the endocrine and autonomic nervous systems.[268] Animal studies in proinflammatory states suggest IL-1β, IL-6 and TNF-α may have detrimental effects on learning and memory although this has not been a consistent finding with in vivo studies in disease states such as multiple sclerosis, underlining the poor understanding of the mechanisms involved.[267]

In summary, predicting developmental outcomes after brainstem encephalitis in children is difficult, in part due to the wide variety of possible effects on the developing brain that may occur across the range of ages that are commonly affected. Detailed assessment, covering as many aspects of development as possible, is necessary to ensure problems are identified and appropriate management strategies / rehabilitation are put in place.

1.5.5 Literature summary of outcome assessments following EV-A71 associated HFMD

The age range of children affected, as well as the increased severity of disease seen in younger children, has prompted several research groups to try to assess developmental and neurological outcomes after severe HFMD. However, in general these studies have been conducted retrospectively rather than prospectively.

For the purposes of formal assessment, child development is categorised into a number of domains, cognitive, language, motor, adaptive, and socioemotional, as

described in Table 1.6. One limitation of measuring child development in low and middle-income countries (LMIC) is the lack of locally standardised tests with local normative comparative data. In particular socioemotional and adaptive skills are commonly assessed by parental report using questionnaires, and these domains can be challenging to adapt, as cultural expectations differ widely in different contexts. Unfortunately, attempts to develop more relevant questions based on engagement with local focus groups can be problematic, and in at least one report from an LMIC have resulted in poor psychometric quality.[269] More research is needed to develop appropriate tools in the social and adaptive areas, which can be used to monitor child development in LMICs.

A summary of outcome studies in HFMD is listed in Table 1.7. The largest study, involving 142 children with confirmed EV-A71 infection, found that a younger age at disease onset may have consequences for verbal comprehension and that worse outcomes were associated with more severe disease.[270] However, the researchers also found that parental education influenced the IQ scores, making it difficult to draw clear conclusions about how large the impact of age of onset was on verbal comprehension. As with all retrospective studies, there is also the inherent risk of sampling bias since the patients that were not traceable may have been the most severe cases or from lower socioeconomic groups.

Extracorporeal life support was incorporated into the Taiwanese management guidelines for severe HFMD disease in 2010.[271] This was amidst controversy as it was not clear what the long-term outcomes were following this high-risk intervention. The 10 patients who were followed up had MRI scans, of which in 5 there was evidence of complications of extracorporeal life support. Survival improved but this study failed to demonstrate improved long-term outcomes.

Mechanical ventilation and ECMO also carry risks to neurodevelopment.[272] It is estimated that MRI changes due to ECMO occur in 30-50% of cases and severe neurological complications occurs in 14-20% of cases.[273, 274]

Overall, the literature suggests that language, cognitive and motor difficulties occur in the most severe cases associated with isolation of EV-A71, and perhaps that behavioural and later cognitive difficulties may occur in less severe cases without cardiopulmonary failure. However, it is clear that both detailed assessments and long follow-up are needed, to ensure better understanding of the difficulties that may arise as complex higher functions become established.

Table 1.6: Child development domains quotes from "Child Developmental Assessments in Low and Middle Income Countries: How can we use them more appropriately?" [275]

Developmental domains and subdomains		Description of domain
Cognitive[276, 277]		Strategies and processes children develop to interpret and respond to their environment and experiences including:- memory (the ability to encode, retain, and recall information over time) attention (the ability to choose what to focus on for a sustained period) which influences memory language skills, (as the brain develops children acquire and refine language skills). Newborns explore the world by mouthing objects, later exploring the world by imitating actions, manipulating objects and planning two-step strategies to get what he/she wants. From 2 years, children increase their use of language and start make-believe play. In children age 3-5 years there is rapid development of information processing (the speed and fluency of response following stimuli), cognitive flexibility (the ability to make and change strategies as required, and to simultaneously process multiple stimuli) and goal setting (the ability to plan strategies in a coherent and efficient order).
Language[278]	Receptive	Understanding of spoken word and sentence structure
	Expressive	Spoken vocabulary
Motor[279]	Fine Motor	Ability to manipulate small objects
	Gross Motor	Ability to walk, run and coordinate complex physical activities
Social and Emotional[279, 280]		Ability to identify and understand one's own feelings and to accurately read and comprehend emotional states in others. Ability to regulate one's own behaviour, to develop empathy for others, and to establish and maintain relationships
Adaptive behaviour[281]		Collection of conceptual, social, and practical skills that have been learned by people in order to function in their everyday lives

Table 1.7 Outcomes HFMD by domain

Year and Country	Cases	Age at disease onset	Follow-up time and testing tools	Cognition	Language	Motor/neurological deficit	Behaviour
1998[126] Taiwan	41 culture confirmed EV-A71 with CNS disease	3 months to 8.2 years. Mean age 2.5years	Not specified. Clinical examination.	NA	NA	7/41 (17%) 2 cases with AFP had residual limb weakness Rhomboencephalitis: 1 myoclonus, 2 cranial nerve deficits, 2 ventilator dependent.	NA
5 cases from 2000-1 1 case from 1995[69] Sydney, Australia	6 Culture proven EV-A71 with cardiopulmonary complications	0.5-4.2 years, mean 1.75 years	17-86 months. Clinical examination.	NA	NA	5/6 (83%) bulbar dysfunction, central and peripheral respiratory failure, and flaccid quadriparesis	NA
1999[67] Perth, Australia	14 EV-A71 positive CNS disease	Age range 2m-12 years	Clinical examination. 1 year follow-up if still residual problems.	NA	NA	4/14 (29%) (opsoclonus syndrome responded to steroids, quadriplegic and ventilator dependent, Guillain-Barré syndrome)	NA
1998-2004[282] Taiwan	63 culture proven EV-A71 with CNS disease	0.3-7.1 years, mean 2.4 ± 1.4 years	1.4-4.9 years mean 2.8 ±1.0 years. Cognitive Wechsler pre-school (WPPSI-R), Motor Movement ABC, Visual motor integration Beery-Buktenica Developmental test	3/63(5%)	NA	6/63(10%) (cerebellar dysfunction, cranial nerve palsies)	2/63(3%) also included in motor
1998-2003[270] Taiwan	142 proven EV-A71 with CNS disease	0.1-13.5 years	1-7.4 years, median 2.9 years Denver developmental screening test (DDST II) if <6 years. Wechsler Intelligence Scale for Children, third edition (WISC-III)>6 years	14/90 (15%) 3/90 (33%) special education	12/90 (13%) verbal IQ	32/142(23%) Focal limb weakness, dysphagia, central hypoventilation, cranial nerve palsy, seizures.	6/81(7%) ADHD

Year and Country	Cases	Age at disease onset	Follow-up time and testing tools	Cognition	Language	Motor/neurological deficit	Behaviour
1998-2003[232] Taiwan	86 virus culture confirmed EV-A71 and CNS disease 172 age, sex and parental education matched controls	0.10 to 12.68 years, mean 2.52±2.12	0.42 to 7.45 years, mean 4.58±1.56 years Chinese Version of the Conners' Parent Rating Scale-Revised: Short Form and Conners' Teacher Rating Scale-Revised: Short Form Strengths and Difficulties Questionnaire Wechsler Intelligence Scale for Children, third edition (WISC-III)	NA	NA	NA	20% EV-A71 group vs. 3% matched control have elevated ADHD-related symptoms
2000-2008[271] Taiwan	10 patients EV-A71 culture confirmed and left heart failure who survived >3months after extracorporeal life support.	Age range 4-45 months	5 months-11 years 2 months, median 7 years and 2 months. Child< 5years not clear what assessment done. 'developmental milestones.' Children> 5years Wechsler Intelligence Scale for Children, third edition (WISC-III)	4 unable to assess. 2/10 (20%) mild or borderline cognitive dysfunction	NA	4/10 (40%) 1 hemiplegia, 3 motor clumsiness	NA
1998-2012[283] Taiwan	27 AFP EV-A71 positive -18HFMD, 8 Herpangina, 1 just fever 26 had MRI	6 months - 9 years 1 month	The duration of neurologic follow-up of acute flaccid paralysis ranged from 1 month to 5 years 10 months, with a median of 6 months. Own classification of motor function outcome Class I (complete recovery)-V(unable to walk/elevate hand)	NA	NA	19/27(70%) 13 could elevate hand to the shoulder level or lower or walked with foot drop, 3 unable to walk.	NA
1997-2002[284] Taiwan	202 EV-A71: 72 encephalitis all required ventilation. Of these 14 had tracheostomy and 10 gastrostomy		Minimum 3 years follow up Movement ABC, Visuo-motor integration capacity, WPPSI-R and WISC-III	29/81 (36%) IQ <95% CI	44/72 (61%) children assessed and all had some speech	23/72(32%) 23 had swallowing difficulties. (9 cases had repeated aspiration pneumonia. 10 cases required gastrostomy tubes for feeding, of which 6 died and 4 had them removed after	No exact numbers but stated Autism spectrum disorder

Year and Country	Cases	Age at disease onset	Follow-up time and testing tools	Cognition	Language	Motor/neurological deficit	Behaviour
					delay	average 4 years. 44/72 (61%) did not reach normal motor milestones by 3 years	
2010-2012[285] China	Prospective study. 220 EV-A71: 40.5% (89) had neurological involvement (abnormal CSF/CT/MRI/Acute flaccid paralysis/ataxia/tremor/motor weakness) 23 (10.5%) needed paediatric intensive care unit care and 7 (3%) died.	median age was 27 months (range: 4 months to 8 years)	2 months follow-up clinical examination	NA	NA	At discharge 5/202 (2%)(4 with ataxia and 1 with arm paralysis). 2-week follow-up, all but the child with paralysis had fully recovered. By 2 months, all children had fully recovered.	NA
2001-2006[241] Taiwan	42 HFMD (29, 69% EV-A71 pos), 4 herpangina (2, 50% EV-A71 +ve) with neurological involvement 26 had MRI: 15 abnormal with 13/15 (86.7%) EV-A71 +ve 2 EV –ve had MRI changes and neurological sequelae.	5 months to 11 years median 22 months	Duration of follow-up not specified. Bayley Scales of Infant Development, the Wechsler Intelligence Scale for Children, and the Leiter International Performance Scale.	3/11 (27%)(unclear if the severe children could be assessed or not).	1/11 (9%) hearing impairment	9/11 (82%) vocal cord paralysis, cranial nerve palsy, respirator failure, swallowing difficulties	NA
2008-2011[243] China	12 cases: 6 Brainstem encephalitis, 2 aseptic meningitis, 4 AFP	6-37 months	4 months and 2 years. Clinical examination.	NA	NA	At 2 years follow-up, in total 3/12 (25%) cases residual neurological deficits: 1brainstem had right sided facial weakness, 1 brainstem had mild limb weakness. 1 AFP mild upper limb weakness.	

NA: Not assessed

1.6 Limitations of outcomes literature for HFMD/EV-A71

A variety of tools have been used in different studies, ranging from screening tests to formal assessments with a control comparison group. However, screening tools have poor diagnostic utility and are not appropriate in high risk groups.[286] Also, as mentioned above retrospective studies have an inherent selection bias, and in the only prospective study performed to date the follow-up consisted of a single clinical examination at 2 months. It is clear that this is insufficient to allow identification of problems occurring during the period of rapid development in early childhood. The studies were also inconsistent in their findings between outcomes at different grades of illness severity, probably due to limited sample sizes. In addition, most countries do not have standardised neurodevelopmental assessment tools validated either for clinical or research use. However, despite these difficulties the studies so far have suggested that, aside from the major sequelae that are usually apparent at discharge, problems may develop in verbal comprehension in children who were affected by HFMD when aged less than 2 years. Detailed follow-up, repeated at several time points during the crucial period for rapid brain development in early childhood should help to elucidate whether these areas of concern are real, and if present, whether the problems persist or not.

Severe HFMD has emerged as a serious CNS infection in early childhood across Asia. Understanding long-term outcomes is of great importance for the design and implementation of vaccination programs, as well as for management and intervention during the acute phase of illness or during convalescence. Current research has been limited by a number of factors, including the retrospective nature of the classification of disease severity, which has not always been

consistent or comparable between studies. Secondly, neurodevelopmental assessments have not always included the major domains commonly assessed, and the duration of follow-up has been short. Information about the quality of testing and about the assessment tools used is lacking, and comparative control groups (generally considered essential to provide a culturally relevant gold standard) were not routinely included. Finally, neuroimaging, if performed, was carried out during different phases of the illness and/or recovery period, making it difficult to assess or quantify the evolution and resolution of lesions according to the evolution of the clinical disease, and thus limiting use of neuroimaging findings as potential predictors of severe disease or sequelae.

EV-A71 is commonly implicated as the main pathogen associated with severe HFMD and development of sequelae, but outbreaks with neurological complications have continued to occur with other co-circulating enteroviruses.[255] In the literature the focus has been on EV-A71 positive cases in HFMD outbreaks, and only Tsai et al. noted that two EV-A71 RT-PCR, serology and culture negative cases had neurological sequelae and MRI changes.[241] A prospective study is needed to evaluate the potential of other pathogens to cause severe HFMD and to increase the body of knowledge on the entire spectrum of 'severe HFMD'.

1.7 Research in Vietnam: Background

The total population of Vietnam in 2012 was 88.78 million, with gross national income (GNI) per capita of 1,550 USD, putting it in the bracket of lower middle income countries.[287] It is estimated that 43% of the population are under 25 years. The median age is 28 years and 31% of the population reside in urban areas.[288] Government policy in reducing fertility has been successful but the desirability for

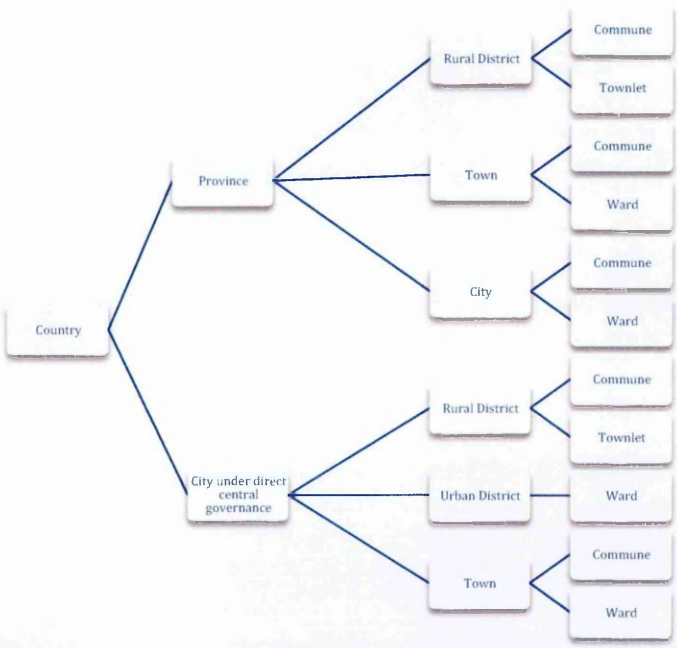
a son in the Vietnamese culture has seen a sex shift with more boys born than girls (1.12:1).[288] Approximately 20% of the population live below the national poverty line.[289]

The National Assembly holds the constitutional and legislative rights of the Socialist Republic of Vietnam. It consists of 498 members, and has the responsibility to elect the President of the Republic, the Chairman of the National Assembly and the Prime Minister as well as approve all appointments of Ministers. The Head of State is elected by the National Assembly from among its deputies to represent the Socialist Republic of Vietnam.

1.7.1 Local government

Vietnam is distributed into administrative units called provinces and cities under direct central rule. The province is further divided into provincial cities, towns and rural districts. The cities are divided into urban districts, rural districts and towns.

Figure 1.7 Vietnamese administrative divisions. [290]



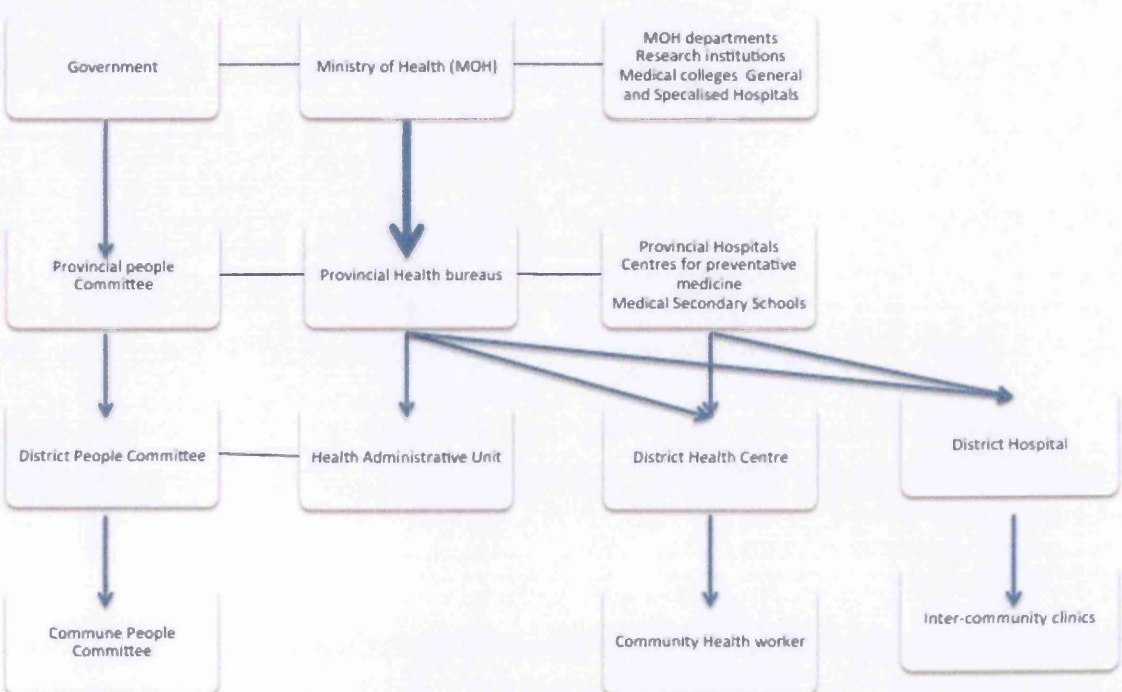
The country is made of 58 provinces and 5 municipalities (including the capital Ha Noi and the largest city: Ho Chi Minh City), which are further divided into districts. Each district is again split into wards, and further split into communes. Ho Chi Minh City is made of 19 inner districts and 5 suburban ones.

1.7.2 Health Provision

The health care administration in Viet Nam is organised in a three-level system centrally controlled by the Ministry of Health (MoH). The MoH has the responsibility to execute health policy and programmes in the country.

At primary level are the district health centres, commune health stations and village health workers. The organisations are summarised in Figure 1.8.[291] In 2013, there were 1.19 doctors per 1,000 population.[292]

Figure 1.8 Healthcare provision in Vietnam



The review by Dao details the changes in healthcare provision in the last three decades and the on-going challenges the system faces.[293] In summary, prior to 1986, primary healthcare was free, but there were limited services and staff to provide appropriate healthcare for the nation. In 1986, economic reform allowed commercialisation of the health sector, with the introduction of private primary care clinics and user fees. From 1992, there was government health insurance for government employees and war heroes and voluntary participation by the public. However, general knowledge about the system was poor, resulting in limited extension out of government sector staff. By 2002, only 17% of the population had government health insurance and user fees were now a major financial burden on the poor and rural Vietnamese households. Out of pocket expenditure is common for many developing countries, where formal taxation is difficult as the populations are farmers or self-employed. However, significant health costs can cause catastrophic expenditure, pushing households into debt that claims all assets and high interest loans, which prevent the household escaping poverty. Recognising rising inequalities in healthcare provision, the government used tax revenue to pay for health insurance for the poor, rural communities called Healthcare fund for the Poor (HCFP). In 2005, the government started Free Healthcare for Children under 6 years (FHCCU6). This insurance covers inpatient and outpatient care in public facilities, lab and generic medicines approved by the Vietnamese Ministry of Health. It was estimated that in 2010 approximately 81% of children in the country were insured under FHCCU6.[294] Nguyen et al. reported that the FHCCU6 was helping relieving the pressure off tertiary centres, as to claim the insurance required formal referral from secondary care.[295] However, due to constant overcrowding, the hospitals commenced services, which were quicker but non-FHCCU6 and usually paid out-of-pocket (OOP). These include services such as a private room. If a private room service was used, none of the hospital episode

cost could be claimed through FHCCU6. Hence OOP payments remain high, estimated to be >50% of all the healthcare financing in Vietnam.[296]

Despite the inequalities, Vietnam has made significant progress in reaching the Millennium Development Goals in child and maternal health. There has been a decline in under-5 mortality from 58 per 1000 live births in 1990 to 24 per 1000 in 2009.[297]

1.7.3 Early childhood education

Under the administrative regions described previously, each commune has at least one pre-school centre, one primary school and one lower secondary school. In towns or districts there is at least one upper secondary school and higher education or technical colleges. Provinces have further specialised colleges such as educational centres and the major universities are located in the large cities including Hanoi, Ho Chi Minh City, and Da Nang.[298]

Early childhood care and education (ECCE) starts at subsidised crèches that take children from 3 months to 3 years and kindergartens, which start at 3 years up to 6 years of age. ECCE units are under the authority of the District People's Committee but there are also privately run centres. The main focus of the ECCE is to ensure school readiness.[298]

1.7.4 Childrens' rights and role in Vietnam

Vietnam was the second country in the world to ratify the United Nations Conventions on the Rights of the Child. The rights are detailed in Vietnam's 2004 Law on Child Protection, Care and Education.[299]

1. The right to have birth registered and acquire nationality (Article 11)
2. The right to be cared for and brought up (Article 12)
3. The right to live with parents (Article 13)
4. The right to be respected and have their life, body, dignity and honour protected (Article 14)
5. The right to health care (Article 15)
6. The right to study (Article 16)
7. The right to join recreational, entertainment, cultural, art, physical, sport and tourist activities (Article 17)
8. The right to develop aptitudes (Article 18)
9. The right to have assets (Article 19)
10. The right to access information, express opinions and participate in social activities (Article 20)

Additionally there are 5 social pillars described by President Ho Chi Minh for children to follow Vietnamese cultural values. (Final draft 25/2004/QH11 on Child Protection, Care and Education, June 15 2004).[300]

1. To love, respect and be dutiful to grandparents and parents, to respect teachers, to be polite to adults, to love minors and unite with their friends, and to help the elderly, disabled people and people with difficulties, according to their capabilities.
2. To study diligently, to observe hygiene, to do physical exercise, to observe public order and traffic safety, to protect public properties, respect the properties of other people and to protect the environment.
3. To love labour and to help their families through jobs suitable to their health.

4. To be modest, honest and ethical, to respect the laws, to observe school rules, to live a civilised lifestyle and build cultured families, and to respect and preserve the national cultural identities.
5. To love their homeland, their country and fellow country folk; to have sense of building and defending the Fatherland of the Socialist Republic of Vietnam, as well as of international solidarity.

Article 22 also stipulates what a child must not do:

1. Drop out of school or leave their families to lead a vagrant life.
2. Infringe upon the life, body, dignity, honour or assets of others or disturb the public order.
3. Gamble, use alcohol, cigarettes or other stimulants harmful to their health. Exchange, share or use violence-provoking or culturally depraved products or play with toys or games harmful to their healthy development.

Childs rights and responsibilities show that Vietnam's cultural deference to its history, nationality and family influence the relationship between parents and children. Expectations on children's behaviour and their role in family and society are enshrined in law. This has implications when adapting child developmental tools for Vietnam, where social expectations and behaviours from children differ from the culture the tool was originally developed.

1.7.5 Disability in Vietnam

Population census data collected in 2009, report almost 6.1 million people over the age of 5 years, or 7.8 per cent of the population, live with one or more disability in seeing, hearing, walking or cognition with 385,000 individuals classified as having severe disabilities, which was the total number of individuals

receiving regular support from the Ministry of Labour, Invalid and Social Affairs (MOLISA).[301] In addition, the 2009 Census data show that 3.8 per cent of the population aged 5 years or older or as many as nearly 3 million persons have multi-domain disability. Disability is disproportionately distributed with higher prevalence in rural areas. Disabled individuals have fewer opportunities to attend school, and shorter durations of study impacting on employment opportunities, which contribute to individuals remaining in poverty. Research within Vietnam found the development of infrastructure and services for rehabilitation improve families' capacity to care for members with disability.[302]

There has been expansion in legal provisions for people with disabilities (PWD) in Vietnam. The 1992 and 2001 constitution guarantees the rights of all citizens including government support for PWD.[301] In 2007, Viet Nam signed the United Nations (UN) Convention on the Rights of Persons with Disabilities (CRPD) - a major international convention that has rapidly gained recognition around the world (UN, 2006).

Shin et al. evaluated the predictors of parenting stress by studying 120 children aged 3-6 years, enrolled in schools and kindergartens and identified by the teachers as having cognitive delay (of which 22% also had additional difficulties, such as epilepsy and cerebral palsy).[303] Fourteen percent of the parents did not agree with the teachers' report of cognitive delay. 119 children without developmental delays were used as a comparison. Using standardised questionnaires, carried out by trained staff, the mothers of children with cognitive difficulties reported higher levels of stress, tended to be older, poorer, less educated and have less social support. After controlling for demographic and psychosocial predictors of stress, having a child with cognitive delay was the most

robust predictor. Further research is needed to identify which aspects in the child's difficulties that contribute most to parental stress, and may be a focus of intervention.

1.7.6 Services for children with disabilities

There is recognition of the discrimination children with disabilities face with regards to access to health and education. Vietnam is moving towards inclusive education with a combined project with UNICEF to train teachers on inclusive education.[304]

In 2009, 66.5% of children with disabilities (CWD) aged 6-10 attended primary schools, compared to the national rate of 97.0%. Many CWD repeat grades until they are too old for general education, as the system does not adapt for their needs. Approximately 33.0% of all CWD who have been to school leave prior to completion of education.[305]

A mixture of government and non-governmental organisations (NGOs) provide health and rehabilitation services for CWD. The government plans to develop a systematic and comprehensive rehabilitation network for the country but is limited by the lack of trained professionals, infrastructure and funding.[306] Many NGO projects are focused on a specific need and fail to integrate well within government systems. Information about programs is not well disseminated and may be short-term and region–specific. Government therapy services are provided in large hospitals, which are oversubscribed. These services are not covered by the government health insurance system.

1.8 Outcome measures

Outcome measures are used to evaluate areas affected by a disease process or intervention. In HFMD we required an objective measure of neurological function and child development (cognitive, motor and language domains) that could monitor changes in children aged 0-4 years. Outcome measures can be objective or subjective. Velentgas et al. discuss the conceptual models of health outcomes, incorporating objective clinical measures, quality of life measures and biological and societal influences as well as models incorporating economic costing.[307] The HFMD study age group makes self-evaluation or reporting a challenge and parental reporting on child development may be biased and miss subtle developmental disorders.[286] Hence, objective measures of child development using standardised child developmental assessment tests (CDATs) and neurological assessment were used. Neurological examinations have an important role in predicting outcome, particularly as later predictive validity of a CDAT can be moderate at best.[308, 309] In encephalitis, acute focal neurological signs are associated with worse outcomes at discharge,[310] but normal neurological examinations at discharge do not exclude long-term problems.[255]

1.8.1 Child developmental assessment tools (CDATs) in low middle income countries (LMICs)

There are two types of CDAT that either assess or screen children's abilities. Screening tools are administered quickly, using a limited sample of items representing an area of ability (domain) and rely on pre-determined cut-off points. Screening tools are designed to identify children who may have impairment and require a comprehensive assessment. However, screening tools have poor utility in assessing subtle delays that may have a significant impact on subsequent child

development.[286]

In order to characterise a child's strengths and weaknesses more accurately, or to monitor changes over time or due to an intervention, specialised standardised CDATs are required.[286, 311] These require skilled assessors with specific training. All CDATs have a threshold that a child must achieve for the assessor to be confident that there are no current developmental concerns. A norm-referenced test is usually administered in a standardised manner and the individual child's scores in each domain are compared to scores from a large representative sample of children of the same age and sex (normative data). Scaled scores allow comparison between scales evaluating the same domain and monitoring of individuals at different ages.[312] However, there are concerns that the rapidly changing nature of society means that normative data become out-dated very quickly.[313] To counter this, inclusion of a control group is now considered crucial for research studies, even when the normative population is contemporaneous and comes from the same linguistic and cultural background as the study population.[314]

CDATs in LMICs follow one of the following formats: a) a standard western CDAT with no adaptations; b) a western CDAT translated (linguistic equivalence) and/or adapted for the local cultural environment (cultural equivalence); c) an amalgamation of a number of translated and/or adapted items from several different western CDAT; or d) a locally developed, culturally specific CDAT consisting of original items designed to be relevant to the population of interest.[315-317] The format of CDAT adaptation depends on criteria set by the particular study and whether the study will compare within same cultural groups or cross-culturally.

It is important that the original psychometric properties of the western CDAT are robust. Developmental domains are a theoretical concept, typically referred to as 'constructs' in the psychometric literature. These cannot be directly measured but are inferred through the child's performance on a number of observed variables (test items).[318] Reliability is the variability of scores obtained by an individual if repeatedly given the same test. There are widely cited levels of acceptable reliability for testing[319], however the result should be interpreted in context. For example, re-test reliability can be influenced by developmental maturation over the time interval, or training by the caregiver following mistakes seen in the first assessment.[320] Validity is the accuracy of the score representing the construct of interest.[318] There should be sufficient evidence of the CDATs reliability and validity as detailed in Table 1.8.

There are no locally normed CDATs in Vietnam. Clinicians and psychiatrists in Vietnam use an internationally recognised screening tool called Denver-II. This measures social contact, fine motor skill, language, and gross motor skill. It was originally created and validated in Denver, Colorado, USA in 1967 and re-standardised to the current version in 1990.[321] Glascoe et al. evaluated Denver-II in 104 children aged 3-72 months attending day-care centres in the USA.[321] They found Denver II had a high sensitivity rate (83%), but over half of the normally developing children had "abnormal, questionable or untestable" scores. The conclusion was to avoid using Denver II till further modifications and research was done to improve the psychometric properties of the test, and is currently excluded from several US states list of acceptable screening tools.[322]

In view of a lack of locally available tools, and the unacceptable high time and cost in creating and validating a detailed assessment tool from scratch, the study adapted a western CDAT for Vietnam.

A list of mandatory and ideal criteria was created in order to decide which western CDAT would be adapted for the study (Table 1.9). Following review of the literature on adaptation of CDATs for LMIC[275], the "Bayley Scales of Infant and Toddler development (3rd edition)" (Bayley III) fulfilled the majority of the criteria, despite the high cost of the license fee, equipment and training.

1.8.2 Bayley scales of Toddler and Infant Development, 3rd Edition (Bayley III)

The "Bayley Scales of Infant and Toddler development (3rd edition)" was created by Nancy Bayley, who directed the Berkeley Growth study (birth years 1928-1929, n=61)[323, 324] which followed up a birth cohort for 36 years.[325] The aim of the study was to describe psychological, motor and physical development from birth with growing maturity. She devised the 'California First-year and Pre-school' assessments to evaluate early years development of the cohort.[326] The experiences from the birth cohort lead to the first edition of Bayley Scales in 1969. The scale was then standardised on 1262 American children aged 2-30 months (referenced by Niccols et al.)[327] This standardization was not from a wide range of socioeconomic backgrounds. The significance of this work was recognised in 1966, with Bayley the first woman to receive the APA Distinguished Scientific Contribution Award.[328]

1.8.2.1 Bayley Scales worldwide

The influence of Bayley Scales has been global. Items from Bayley Scales (1969) were used to develop Baroda norms, which became widely used in India.[329] Studies in Kenya and the Netherlands also adapted the Bayley for local language and culture as an outcome measure.[330, 331] A paper published in 1986 highlighted inflated scores above the mean in 436 low socioeconomic 12 month olds in the US.[332] This raised concerns that the normalization sample from Bayley (1969) was out of date. Subsequently, revisions were made, extra items added and a new Bayley Scales of Infant Development (BSID-II) was published in 1993.[325] Fernald et al. reviewed early child development in LMIC and found more than 60 studies in low-income countries had used Bayley (1969) or BSID-II, mostly without detailed adaptation for local use.[318] In the UK, the BSID-II was chosen as the assessment tool for follow-up of neonatal cohorts in the original population based study of survival and later health status in extremely premature infants, Extremely Premature Infants Cure (EPICURE) cohort.[333] The EPICURE studies continue to influence the debate on the limits of viability in perinatal medicine.[334]

1.8.2.2 Bayley III development

In 2004, the Individuals with Disabilities Education Improvement Act (IDEA) were passed in the United States. This law ensures early intervention for children and stipulates assessment should occur in five domains: cognitive, language, motor, social-emotional and adaptive behaviour.[335] Prior to this, the Bayley scales (1969 and BSID-II) measured a mental development and physical developmental index only. The scale was modified to measure the five domains as stipulated by the IDEA (2004). The Bayley III standardization was on year 2000 U.S. census-stratified sample of 1700 children aged 1 month to 42 months. Children who did

not speak or understand English were excluded from the sample.[336] The technical manual reports detailed data regarding the reliability and validity of Bayley III, with additional evaluation of special groups to increase its clinical utility. The special groups assessed in validation process were; children with Down's syndrome, pervasive developmental disorder, cerebral palsy, specific language impairment, at risk for developmental delay, birth asphyxia, prenatal alcohol exposure, small for gestational age and premature or low birth weight. This extensive information on Bayley III has continued to support its use and it remains one of the most widely used assessment tools for early years development.

1.8.3 Neurological assessment: Amiel-Tison

In severe HFMD, many children under the age of 2 years are affected and are at risk of motor developmental problems, hence a standardised examination that can monitor children under the age of 6 years is appropriate. One of the most long-standing and established tools is the Amiel-Tison.[337, 338] Recent research identified that in extreme pre-term infants (<28 weeks gestation), those identified by Amiel-Tison evaluation at 9 months corrected age as having a transient neurological abnormality (an abnormality which resolved by 12 months corrected age) had lower cognitive and academic skills when aged 5 years compared to extreme pre-term infants who had normal neurological examinations at 9 months.[339] This highlights the need for complementary neurological and neurodevelopmental assessments, particularly in younger infants at risk of motor developmental delay.

Table 1.8 Basic psychometric properties used to evaluate child development assessment tools (CDAT)[275]

Reliability	**Internal consistency**	Evaluates the similarity of test items assessed in one domain. One measure is split-half reliability, which compares the scores on two halves of a test in a single domain.	High internal consistency suggests that some items are too similar, so no additional information is gained from assessing them. Low internal consistency suggests the items may not be assessing the same domain.
	Inter-observer	Evaluates variability between different assessors on the same subject	There may be systematic errors, specific to a particular group of assessors, and this parameter may not be generalizable when the tool is used by a different group of assessors.
	Intra-observer	Evaluates variability within a single assessor on a single subject	Commonly evaluated by the same assessor scoring video recordings of their own assessments. This is not essential unless there is low inter-observer reliability
	Test-retest	Evaluates variability within the subject (influenced by random factors such as familiarity with items and mood)	Difficult to interpret in early childhood when changes in development occur over a short time. Usually the repeat assessment should be carried out within 2 weeks of the first test.
Validity	**Content**	Experts in the field make consensus agreement on whether the individual item and the range of items	Subjective measure that cannot be used in isolation to evaluate validity.
	Criterion	Ideally assessed by comparison to an established "gold standard" test assessing the same construct	Usually "gold standard" tests are not available so the comparison is typically against another recognised test regularly used in the same population and thought to measure the same domain.
	Discriminant/ Convergent	Evaluates expected positive and negative correlations between scores in different domains or between different tests of the same of differing underlying construct.	Scores from two independent tests (e.g. one using report method the other a direct test) of one domain should correlate where neither test is considered a 'gold-standard'. To ensure the test is not overlapping with constructs not of interest, the scores evaluating different constructs should poorly correlate, e.g. test scores on 'fine motor' should correlate poorly with 'social emotional'.
	Construct	Statistical evaluation to see whether values of observed data fit a theoretical model of the constructs (confirmatory) or to explore a possible model of the 'underlying traits' being measured.	Large numbers of assessments are required to evaluate this.

Table 1.9 The mandatory and ideal criteria used for choosing the tool and criteria fulfilled by Bayley III

Mandatory	Bayley III	Ideal	Bayley III
Local senior clinician with experience on the tool or previous versions.	✔	Can measure up to 7 years of age	✖
Can be administered by paediatrician	✔	Easy to train	✖
Must give detail on 2-3 year old where high prevalence of HFMD	✔	Can be used to give immediate feedback to carer/parent	✔
Assessment tool	✔	Low cost of licences/translation fee	✖
Cognitive, language and motor	✔	Internationally recognised	✔
Engages parents	✔		
Direct test	✔		
Evidence of high degree of reliability and validity in country of construct	✔		
Large normative sample in country of original construct with normed scores that can compare changes over time/groups	✔		

1.9 Aims of thesis

Severe HFMD has emerged as a significant threat to the health of young children across the Asia Pacific region. Major outbreaks have overwhelmed healthcare systems in several resource-limited regions. For example in China, most regions have observed a progressive increase in the numbers of cases of HFMD annually; in Guangdong province alone there were 48917, 93067, 226622, 274006, 330621, and 358068 HFMD cases reported each year from 2008 to 2013.[340] Similarly the 2011 outbreak in Vietnam resulted in over 100,000 cases being reported across the country, with 166 deaths.[91]

As reviewed earlier, the age group predominantly affected is children under 5 years old, with younger age groups (< 24 months) at risk of more severe disease. Development of a number of clinical classification systems, together with the introduction of management guidelines for supportive care according to the disease grade, has undoubtedly improved survival but there are still no reliable early predictors of likely disease progression or poor outcome. Evidence based therapeutic interventions are also limited, with supportive treatment remaining the mainstay of care. Vaccine development appears promising but is still a distant prospect for practical application.

The stereotypic involvement of the brainstem seen on MR imaging and at autopsy raises significant concerns regarding long-term neurodevelopmental outcomes in these children, with the potential for several of the major domains of development to be influenced. The current literature suggests that language development is most likely to be adversely affected, particularly in children less than 24 months of age at illness onset, but methodological issues such as the absence of a suitable control group, and/or lack of validation of standardised tests have hindered

interpretation of study findings to date. Finally most of the focus on outcomes has been on cases with virological confirmation of EV-A71 infection. However, in clinical practice, virological confirmation is often not feasible, and a broader understanding of the spectrum of outcomes associated with severe HFMD, irrespective of the specific pathogen causing the disease, should be informative for clinicians managing these children and also give a more accurate picture of the true burden of severe disease from a public health perspective. A prospective observational study is needed to address these questions.

In this thesis I will present the work I have carried out on severe HFMD (Grade 2a, 2b and >2b as per Vietnamese Ministry of Health classification) in Ho Chi Minh City - aiming first to prospectively evaluate the long-term neurological and developmental outcomes in a cohort of Vietnamese children with HFMD, and second, to identify MRI changes that are prognostic indicators associated with poor outcome. To carry out this work I adapted and validated an internationally recognised neurodevelopmental tool, the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley III), for use in this population of Vietnamese children. The specific hypotheses are as follows:

1. Children affected by severe HFMD (grade 2a, 2b and >2b as per Vietnamese Ministry of Health classification) under the age of 4 years have lower cognitive, language and motor scores than a comparative group of healthy children, as measured by internationally recognised adapted tools and measured a week post discharge and at 6 months after discharge.
2. Children with severe HFMD and MRI changes during the acute phase of the illness predict lower outcome scores at 6 months after discharge compared to severe HFMD children with no MRI changes.

2. Methods

This chapter outlines the study design and procedures. More detailed statistical methods are described in the relevant results chapters.

2.1 Introduction

We designed and conducted an observational cohort study to assess neurodevelopmental outcomes at discharge and at 6 months after discharge in a sample of children aged less than 4 years admitted to the Hospital of Tropical Diseases with severe (≥grade 2a) HFMD. The neurodevelopmental outcomes were compared to a sample of healthy children from District 8, HCMC. Brain MRI from a convenience sample of enrolled HFMD cases was carried out during hospital admission.

2.2 Study collaborators

2.2.1 OUCRU-VN

The Oxford University Clinical Research Unit (OUCRU-VN) is a large-scale clinical and public health research unit with campuses in Ho Chi Minh City and Hanoi in Viet Nam. OUCRU-VN is hosted by the Hospital of Tropical Diseases (HTD) in Ho Chi Minh City, and the National Hospital for Tropical Diseases (NHTD) in Hanoi. As a Wellcome Trust Major Overseas Programme, OUCRU-VN has received considerable support from the Wellcome Trust since its establishment in 1991.

The work of the unit covers clinical and public health research and includes work in immunology, host and pathogen genetics, molecular biology, virology, mathematical modelling, bioinformatics, biostatistics and epidemiology. This work

is supported by both a Clinical Trials Unit and Data Management Centre (compliant with national and international regulations) and a comprehensive Management, Finance and Administrative Centre. OUCRU-VN enjoys the support of the Vietnamese government, and works closely with the Ministry of Health Viet Nam and the Department of Health of Ho Chi Minh City. We have developed strong links with more than 20 Vietnamese hospitals and research institutions including HTD, NHTD, The National Institute of Hygiene and Epidemiology (NIHE), and Hanoi Medical University.

2.2.1.1 Clinical Trials Unit

Our Clinical Trials Unit (CTU) supports governance and operational management of all OUCRU-VN research. The CTU is responsible for ensuring that all research is conducted efficiently, accurately and in compliance with the applicable international and local regulations including the World Medical Association Declaration of Helsinki (2008) and the International Conference on Harmonization guidelines of Good Clinical Practice (1996) (GCP). The CTU is a multi-disciplinary group which functions as a coordinating centre to manage research at dozens of national and international research sites. Ethical and regulatory approval, logistics and human resources management, control of investigational medicinal products and clinical trials monitoring are all activities of focus for the CTU. The CTU works to increase the research capacity in all countries which host OUCRU-VN research by delivering training in protocol procedures, GCP Guidelines, human subjects ethics and research operations.

The Research Ethics Committee of each participating site must approve all clinical research before the research is initiated. The Oxford Tropical Research Ethics

Board additionally reviews OUCRU-VN work internationally. The Ethical Committee of the Viet Nam Ministry of Health must also review clinical drug trials. The relevant ethics committees before use will approve the study protocol, informed consent form, all patient materials and any amendments to these documents.

2.2.1.2 Data Management

OUCRU has a Data Management and Information Technology Group (comprised of an IT Manager, Data Manager, Data Programmers and Data Entry Team) which is responsible for the overall design, implementation, and monitoring of clinical databases for clinical trials. In addition, the group provides technical support for computer installations, internet connections and communications technology. This group is experienced at managing large and complex multidisciplinary datasets to international regulatory standards. The core application we use for managing databases in clinical trials is the *CliRes Data Management System*. This centralised system was developed at OUCRU and is based on international regulatory standards such as ICH Good Clinical Practice and FDA regulations. The system supports both paper-based and electronic data collection methods. The major supporting tools within the system include multiple language interface, data validations, double data entry discrepancy triggers and audit trails for data changes.

2.2.1 Hospital for Tropical Diseases

The Hospital for Tropical Diseases, founded in 1862 was originally a private hospital built from community funds and donated to the Vietnamese government

in 1865. It was originally named Cho Quan Hospital after the local village and a hospital for the treatment of sick prisoners and patients with venereal diseases. In 1904 a Psychiatric wing was added and focused on leprosy and neurological conditions until it was taken over by the army in 1954 and named the Ngo Quyen Institute for Tuberculosis.

Construction of the main hospital block began in 1972 in cooperation with the government of South Korea opening in 1974 as the Vietnamese-Korean Medical Centre (522 beds) comprising wards for contagious disease, leprosy, psychiatry, internal medicine, surgery and paediatrics. In 1975 it was taken over by the Military Managing Committee of Saigon and reverted to the name Cho Quan Hospital. Following a decision by the Ministry of Health it became the first hospital specialising in infectious diseases in Viet Nam and given the responsibility for the prevention and treatment of epidemic infectious diseases for Ho Chi Minh City and the provinces of the old zone (B2). It was named the Centre for Tropical Medicine in 1989 and subsequently named the Hospital for Tropical Diseases under the direction of the Health Service of HCMC. It now has approximately 600 in-patient beds dedicated to infectious diseases, we believe the largest such dedicated hospital in the world. The Hospital continues to have responsibility for advice on all aspects of infectious diseases for the south of Viet Nam a catchment population of over 40 million people.

2.2.2 Children's Hospital 1 (CH1)

Children's Hospital 1 (CH1) is one of two major paediatric centres in the city. The hospital opened in 1956 with 268 beds, but has expanded considerably over the years to meet increasing need. It now has 1000 in-patient beds on 17 clinical

wards and employs over 1200 staff; in excess of 3500 children are seen each day in the 35 outpatient clinics on site. Together with Children's Hospital 2, the hospital offers specialist paediatric care to the 10 million children of southern Viet Nam, covering the full range of medical and surgical disorders seen in the region, as well as providing leadership and training in paediatric diagnosis and management throughout the southern provinces via well developed outreach programmes. The hospital has a long tradition of involvement in clinical research, and over the last 10 years we have developed a very successful series of collaborations focused on common and serious local problems such as dengue, encephalitis and influenza. The research programme was recently consolidated with the opening of a Clinical Trials Unit on site at CH1 enabling the execution of major research studies, in particular intervention studies, in the future.

2.2.2.1 Dr Thanh: Retired Head of Psychology department (CH 1)

Dr Thanh, a paediatrician and psychologist, set up the Psychology department at Children's Hospital 1. She has collaborated with several international institutions in research and also set up a Masters course in Psychology at the Pham Ngoc Thach Medical University, HCMC. She has prior experience using the Bayley scales of infant development 2nd edition (BSID II) through verbal translation. Dr Thanh has led translation and adaptation of other child developmental tests including the A Developmental NEuroPSYchological Assessment (NEPSY II). Dr Thanh has also translated parental guidance books from French and English into Vietnamese. She presents at International Conferences in Thailand on child psychology in HIV children. She is widely respected as an expert in the field in Vietnam.

2.2.3 Hung Vuong Maternity Hospital

Hung Vuong Hospital was built in 1900 as a 60 bed maternity hospital, which has expanded to 800 bed capacity. It is now the regional tertiary-level referral centre for obstetrics with over 200 deliveries a day. It has the highest technical facilities for 24 districts in HCMC. It has a history of international collaboration and is a centre for postgraduate medical study and specialisation.

2.2.4 District 8 Preventive Medical Centre

The Preventive medical centre (PMC) in District 8, HCMC was set up in 2007 superseding the previous District 8 Health centre. Its aim is to prevent HIV, sexually transmitted diseases, communicable diseases in the community. It supports health communication in the community, including schools and kindergartens. It is under the control of the People's Committee of district 8.

2.2.5 Medic MRI centre

Medic centre was established in 1990. Its main focus is on diagnostic imaging with X-ray, ultrasound, computerised tomography (CT) scans and MRI machines. The Toshiba Access LPT6.0 open MR Imager was installed in 1996 and was the first MR Imager installed in Vietnam. The centre carries out approximately 50 MRI scans a day with a team of 16 doctors, 5 nurses, 3 technicians and 2 transportation staff.

2.3 Ethical approval

The Oxford Tropical Research Ethics Committee and the Institutional Review Boards of The Hospital for Tropical diseases and Children's Hospital 1, Ho Chi

Minh City (HCMC), approved this study. ClinicalTrials.gov Identifier: NCT02066714

2.4 Participants

2.4.1 Pilot study

Prior to the main study, a pilot study was carried out on a convenience sample of 42 children whose parents worked at the Oxford University Clinical Research Unit, HCMC. This was to evaluate the adaptation of Bayley III as per guidance by the International Test commission.[341] The parents volunteered and gave consent for a video to be recorded during the assessment for training and for reliability evaluation. Children were aged <4 years, had no prior known chronic medical problems (e.g. congenital heart disease, epilepsy, HIV), had no prior learning disability or developmental regression and were believed to be developing normally.

2.4.2 HFMD study

Two cohorts were recruited to the main study. The first was the HFMD group and the second was the healthy group in HCMC. They were recruited in parallel.

2.4.2.1 HFMD cohort

The HFMD cohort was recruited from the Hospital of Tropical Diseases (HTD). Children were HFMD classified (Vietnamese Ministry of Health Guidelines Table 1.4) by the admitting clinician. The children with Grade 2a disease are admitted to paediatric ward C and those with Grade 2b or more are admitted to the Paediatric Intensive Care Unit (PICU). All children aged less than 4 years were approached

about the study. Those who were ex-premature (<37 weeks gestation), or had previous PICU admission irrespective of ventilation, chronic illness requiring medication or repeated hospitalisation (congenital heart disease, epilepsy, HIV), or prior learning disability or developmental regression, were excluded from the study.

All enrolled HFMD cases were approached about participating in a research brain MRI scan. The children had a history of more than day 5 of illness (defined from fever onset), judged by the treating physician to be stable for transfer to another unit for the MRI and had no known history of complications or reactions to sedation.

2.4.2.2 Deciding where to recruit Healthy cohort

HTD is a tertiary level referral hospital for infectious diseases for southern Vietnam and we needed to identify a suitable population from which we could ask volunteers as healthy cohort. HTD routinely collects demographic data on all admissions in a centralised database. Due to the high volume of HFMD cases in 2011, a task force of clinicians was assembled to target schools, kindergartens or areas with high numbers of HFMD cases. The task force identified that rapid transmission occurred within informal nurseries. These are unregulated child-care provisions, usually in a home with high number of children and poor sanitation facilities. Most of these were located in District 8, HCMC, close to HTD. Using the information from this database, we chose to recruit the healthy cohort from district 8, where the most frequent admissions to HTD with HFMD occurred.

2.5 Study sites

2.5.1 Pilot study

The Pilot study recruited volunteers from friends and family who had affiliations with OUCRU. Assessments were done following consent at OUCRU.

2.5.2 HFMD cohort

The HFMD cohort was recruited from admissions to the ward or PICU with HFMD from July 2013 till December 2014 as per inclusion criteria.

2.5.3 Healthy cohort

2.5.3.1 Kindergartens district 8

The PMC selected 3 kindergartens where the study could be conducted on the basis of good relationships with the administrative teams. Two were government-run, Tuoi Ngoc and 19 Thang 5, and the third was private, Hoang Mai. Tuoi Ngoc is a government school of 700 children from 18 months to 5 years old. 19 thang 5 kindergarten has 600 children up to the age of 6 years. Hoang Mai kindergarten is a private kindergarten with 587 children aged 24 - 60 months. At all three kindergartens, volunteers were chosen by the school nurse or principal. Assessments took place at the kindergartens, unless the school was closed for holidays and then assessments took place at OUCRU.

2.5.3.2 Hung Vuong Hospital Birth Cohort

Oxford University Clinical Research Unit (OUCRU) collaborates with Hung Vuong Hospital to enrol mothers delivering and babies born in a birth cohort. The cohort inclusion criteria include residing in District 8, HCMC. There are regular study visits for blood sampling at 4, 9 and 12 and 18 months after birth plus access to

study medical care whenever the child is unwell. The birth cohort study records sibling data of those enrolled. At the birth cohort study visits, information about the neurodevelopmental study was discussed with the parent/guardian. They were asked if they were willing to consider for any of their children to participate in the neurodevelopmental study. All assessments took place at OUCRU.

2.5.3.3 District 8 ward clinics

The ward clinics are the government primary care services for the region including vaccination. These are run by the Preventive Medical Centre in District 8. The PMC selected three ward clinics for us to approach for study participants, based on previously working together with OUCRU and having good leadership systems that could manage the additional workload of the study. We visited the clinics on immunisation days and spoke to parents about the study. Parents who were interested wrote down their details to be contacted by the study team. Assessments took place at the ward clinic, unless rooms were in use and then participants came to OUCRU.

2.6 Outcome measures used in the study

2.6.1 Bayley Scales of Infant and Toddler Development

The Bayley Scales of Infant and Toddler Development, 3rd Edition (Bayley III) has 6 subtests; cognitive (91 items), receptive language, expressive language, fine motor and gross motor plus adaptive behaviour (241 items). Each item is scored as 1 or 0, and the item should be observed by the assessor and not from the history of the parents. Start points are set according to 14 age categories. These were calculated from the US standardization sample, where the start item had a 95% pass rate for the age group. This was done to limit extensive testing.[336] The child needs to pass the first three items following the start point to continue,

otherwise the child goes back to the previous start point and again follows the rule of passing the first three items to continue. The child continues till the child fails 5 consecutive items, which is considered the ceiling of the child's abilities. Raw scores are converted to standard scores based on US norms, which have a mean of 10 and standard deviation of 3. The study team translated and adapted the tool as per international test commission guidance.[341] Cognitive, motor and language domains were evaluated at two time points, six months apart.

2.6.2 Amiel-Tison neurological Examination (HFMD cohort)

The Amiel-Tison is a comprehensive neurological examination which evaluates the Passive muscle tone, motor activity, primitive reflexes and gross motor skills up to the age of 6 years.[338] It was designed to evaluate neurological maturation of infants from birth and stratify risk of cerebral palsy and hence following a birth or prenatal insult. It has been used in research to evaluate premature, low-birth weight infants and those with hypoxic ischaemic events.[342-344] Even when used for research in premature infants, the tool has been modified. Leroux et al. modified the tool to evaluate preterm infants and reported: "we have combined some items into a single item (i.e. head circumference, anterior fontanels, squamous sutures and other sutures were combined into cranial morphology). Moreover, seven items were not analysed because they were previously described as poorly informative owing to their rarity (ocular signs, seizures, Moro reflex and fasciculation of the tongue), lack of relevance in preterm infants (high arched palate) or poor interobserver reproducibility (palmar grasp and asymmetric tonic neck)"[344]

This study evaluated children at risk of a motor impairment due to an insult mainly after the neonatal period. Hence, the Amiel-Tison was modified to remove items

more specific to neonatal insults such as head circumference growth, high arched palate and ocular signs. The motor milestones included in the Amiel-Tison assessment were unnecessary as the Bayley's motor domain is an objective measure.

When researching neurological assessment tools for early childhood, I discussed options and experience with Professor Marlow, who led the EPIcure study.[333] The main concern was finding a detailed tool that could monitor children up to the age of 6 years. It was only the Amiel-Tison that had this facility and hence, with exclusion of items specific to neonatal insults, the scoring system was the same. Table 2.1 compares the original scoring system to the new scoring system, highlighting omission of some sections with expansion of objective assessments (Tables 2.2-2.4).

I taught the Amiel-Tison to the hospital team and one doctor, interested in neurology, carried out the neurological assessments with me.

Table 2.1 Amiel-Tison scoring system and modified scoring system for comparison, colour coded for age group

	Original scoring			Modified scoring		
Sections	**Severe deficit**	**Moderate Deficit**	**Minor Abnormality**	**Severe deficit**	**Moderate Deficit**	**Minor Abnormality**
Head growth (insufficient HC growth; all sutures abnormal or an isolated abnormality with squamous sutures)	**0-3 months**			**Not assessed**		
	2	1				
	4-6 months					
	2	1				
	7-9 months					
	2	1				
	10-60 months					
	2	1 or 2	0 or 1			
Social interaction (alertness and attention, visual tracking, excitability)	**0-3 months**					
	2	1				
	4-6 months					
	2	1				
	7-9 months					
	2	1				
	10-60 months					
	2	1	0			
Passive muscle tone (limbs and trunk)	**0-3 months**			**Expanded in Table 2.1b**		
	2	1				
	4-6 months					
	2	1				
	7-9 months					
	2	1				
	10-60 months			**Expanded in Table 2.1c & d**		
Trunk imbalance	2	1	1			
Stretch reflex in limbs	2	1 or 2	1			
Hypotonia or rigidity	2	0	0			
Involuntary movements	2	1	0			
Parachute reaction (scored according to age of acquisition)	2	1	0			

Motor activity (quality and quantity)	**0-3 months**			**Expanded Table 2.1b**
	2	1		
	4-6 months			
	2	1		
	7-9 months			
	2	1		
Primitive reflexes (sucking)	**0-3 months**			
	2	1		
	4-6 months			
	2	1		
Head control (according to age of acquisition)	**7-9 months**			**Not assessed**
	2	1		
	10-60 months			
	2	1	0	
Evident Asymmetric Tonic Neck Reflex	**7-9 months**			**Expanded in Table 2.1b**
	2	0		
Sitting position	**10-60 months**			**Expanded in Table 2.1c & d**
	2	1	0	
Independent walking	1	1	0-1	

	Severe deficit	**Moderate deficit**	**Minor abnormality**	**Severe deficit**	**Moderate deficit**	**Minor abnormality**
0-3 months	A score of 2 in at least four out of five sections	Mostly scores of 1, some scores of 2 acceptable		score of 2 in at least three of seven sections	score of 1 in at least three of seven sections and up to two 2 in any section	a score of 1 in at least two of seven sections
4-6 months	A score of 2 in at least four out of five sections	Mostly scores of 1, some scores of 2 acceptable				
7-9 months	A score of 2 in at least four out of six sections	A score of 1 in at least four out of six sections, some scores of 2 acceptable				
10-60 months	A score of 2 in at least six of seven sections (including inability to walk)	A score of 1 in at least five of seven sections, some scores of 2 acceptable	A score of 1 in at least three of seven sections	score of 2 in at least four of eight sections	score of 1 in at least three of seven sections and up to two 2 in any section	a score of 1 in at least two of seven sections

Table 2.2 Expanded definition of neurological abnormalities age 3-9 months

Sections	Score 2	Score 1	Score 0
A) Passive muscle tone Lower limbs			
• Adductors	No resistance	Age dependent angles	Age dependent
• popliteal angle right	No resistance	Age dependent angles	Age dependent
• popliteal angle left	No resistance	Age dependent angles	Age dependent
Slow angle • Dorsiflexion of foot right	>=110	90-100	=<80
• Dorsiflexion of foot left	>=110	90-100	=<80
Rapid angle • Dorsiflexion of foot right	Tonic stretch	Phasic stretch	Identical
• Dorsiflexion of foot left	Tonic stretch	Phasic stretch	Identical
Upper limbs • Hand right	Inactive thumb/constantly closed hand	-	Finger movements present
• Hand left	Inactive thumb/constantly closed hand	-	Finger movements present
• Scarf sign right	Position1 (age>6m) No resistance	Position1 (age3-6m)-	Position 2/3
• Scarf sign left	Position1 (age>6m) No resistance	Position1 (age3-6m)-	Position 2/3
B) Comparison of left and right side	-	right side /left side more tonic	none
C) Body axis			
• Dorsal extension	excessive	-	Moderate/minimal
• Ventral flexion	excessive	absent	Moderate
• Comparison of curvatures	rag doll	flexion<extension	extension=<flexion
D) Diffuse rigidity	Lead pipe resistance	-	none
E) Motor activity			
• Face	-	insufficient	Varied and symmetrical

Sections	Score 2	Score 1	Score 0
• Facial paralysis	Present	-	Absent
• Fasciculation of tongue	Present	-	Absent
• Limbs			
• Spontaneous movements	Barely present or uncoordinated	Insufficient or uncoordinated	Coordinated and varied
• Involuntary movements	Present	-	Absent
• Dystonia	Present	-	Absent
F) Deep tendon and cutaneous reflexes			
• Bicipital reflex (R/L)	Absent/clonus	Very brisk	Normal
• Knee reflex	Absent/clonus	Very brisk	Normal
G) Primitive reflexes:			
• Asymmetric tonic neck reflex	Present (>6m)	-	Absent
• sucking	Absent or inadequate	Insufficient	present
• Moro reflex	Present (>6m)	-	Absent
• grasping reflex	Present (>6m)	-	Absent
• automatic walking reflex	Present (>6m)	-	Absent
H) Gross motor function			
• Sitting position	-	Falls backwards (>6m)	No abnormality
• Standing position	Excessive extension	-	No abnormality
• Lower limb deformities	Scissoring of legs	-	No abnormality

Total 8 sections of which score 2 exists in 7 sections and score 1 in 7 sections.

Severe deficit: score of 2 in at least 3 of 7 sections

Moderate deficit: score of 1 in at least 3 of 7 sections and up to two 2 in any section.

Mild abnormality: a score of 1 in at least 2 of 7 sections

Table 2.3 Definition of neurological abnormalities age 10-24 months

Sections	Score 2	Score 1	Score 0
A) Passive muscle tone Lower limbs			
• Adductors	=<70	80-100	>=110
• popliteal angle right	=<80	90-100	>=110
• popliteal angle left	=<80	90-100	>=110
Slow angle • Dorsiflexion of foot right	>=110	90-100	=<80
• Dorsiflexion of foot left	>=110	90-100	=<80
Rapid angle			
• Dorsiflexion of foot right	Tonic stretch	Phasic stretch	Identical
• Dorsiflexion of foot left	Tonic stretch	Phasic stretch	Identical
Upper limbs			
• Hand right	Inactive thumb/constantly closed hand	-	Finger movements present
• Hand left	Inactive thumb/constantly closed hand	-	Finger movements present
• Scarf sign right	Position1	-	Position 2/3
• Scarf sign left	Position1	-	Position 2/3
B) Comparison of left and right side	-	right side /left side more tonic	none
C) Body axis			
• Dorsal extension	excessive	-	Moderate/minimal
• Ventral flexion	excessive	-	Moderate/minimal
• Comparison of curvatures	rag doll	flexion<extension	extension=<flexion
D) Diffuse rigidity	Lead pipe resistance	-	none
E) Motor activity			
• Face	-	insufficient	Varied and symmetrical

Sections	Score 2	Score 1	Score 0
• Drooling	-	Present	Absent
• Facial paralysis	Present	-	Absent
• Fasciculations of tongue	Present	-	Absent
• Limbs			
• Spontaneous movements	Barely present or uncoordinated	Insufficient or uncoordinated	Coordinated and varied
• Involuntary movements	Present	-	Absent
• Dystonia	Present	-	Absent
F) Deep tendon and cutaneous reflexes			
• Bicipital reflex (R/L)	Absent/clonus	Very brisk	Normal
• Knee reflex	Absent/clonus	Very brisk	Normal
• Cutaneous reflex	Extension (after 12 months)	-	flexion
G) Primitive reflexes: Asymmetric tonic neck reflex	Present	-	Absent
H) Postural Reactions			
• Lateral propping	Absent	Incomplete	Present
• Parachute reaction	-	Absent/incomplete	Present
I) Gross motor function			
• Sitting position	-	Falls forward/backwards	No abnormality
• Standing position	Excessive extension	-	No abnormality
• Lower limb deformities	Scissoring of legs	-	No abnormality

Total 9 sections of which score 2 exists in 8 section and score 1 in 7 sections.

Severe deficit: score of 2 in at least 4of 8 sections

Moderate deficit: score of 1 in at least 3 of 7 sections and up to two 2 in any section.

Mild abnormality: a score of 1 in at least 2 of 7 sections

Table 2.4 Definition of neurological abnormalities age 24-60 months

Sections	Score 2	Score 1	Score 0
A) Passive muscle tone Lower limbs			
• Adductors	=<30 or no resistance	40-90	>100
• popliteal angle right	=<90 or no resistance	90-100	120-160
• popliteal angle left •	=<90 or no resistance	90-100	120-160
Slow angle • Dorsiflexion of foot right	>=110	90-100	=<80
• Dorsiflexion of foot left	>=110	90-100	=<80
Rapid angle			
• Dorsiflexion of foot right	Tonic stretch	Phasic stretch	Identical
• Dorsiflexion of foot left	Tonic stretch	Phasic stretch	Identical
Upper limbs			
• Hand right	Inactive thumb/constantly closed hand	-	Finger movements present
• Hand left	Inactive thumb/constantly closed hand	-	Finger movements present
• Scarf sign right	No resistance	Position1	Position 2/3
• Scarf sign left	No resistance	Position1	Position 2/3
B) Comparison of left and right side	-	right side /left side more tonic	none
C) Body axis			
• Dorsal extension	excessive	-	Moderate/minimal
• Ventral flexion	excessive	-	Moderate/minimal
• Comparison of curvatures	rag doll	flexion<extension	extension=<flexion
D) Diffuse rigidity	Lead pipe resistance	-	none
E) Motor activity			
Face			
• Facial expressions	-	insufficient	Varied and symmetrical
• Drooling	Present	-	Absent

Sections	Score 2	Score 1	Score 0
• Facial paralysis	Present	-	Absent
• Fasciculations of tongue	Present	-	Absent
Limbs			
• Spontaneous movements	Barely present or uncoordinated	Insufficient or uncoordinated	Coordinated and varied
• Involuntary movements	Present	-	Absent
• Dystonia	Present	-	Absent
F) Deep tendon and cutaneous reflexes			
• Bicipital reflex (R/L)	Absent/clonus	Very brisk	Normal
• Knee reflex	Absent/clonus	Very brisk	Normal
• Cutaneous reflex	Extension (after 12 months)	-	flexion
G) Primitive reflexes: Asymmetric tonic neck reflex	Present evident	Present elicited (> 5years)	Absent
H) Postural Reactions			
• Lateral propping	Absent	Incomplete	Present
• Parachute reaction	-	Absent/incomplete	Present
I) Gross motor function			
• Holding head behind the axis	Abnormality present	-	Abnormality absent
• Poorly maintained head control due to fatigue	Abnormality present	-	Abnormality absent
• Sitting position	-	Falls forward/backwards	No abnormality
• Standing position	Excessive extension	-	No abnormality
• Lower limb deformities	Scissoring of legs	-	No abnormality

Total 9 sections of which score 2 exists in 8 section and score 1 in 7 sections.

Severe deficit: score of 2 in at least 4 of 8 sections

Moderate deficit: score of 1 in at least 3 of 7 sections and up to two 2 in any section.

Mild abnormality: a score of 1 in at least 2 of 7 sections

2.6.3 Brain MRI scans (HFMD cohort)

The MRI centre was 5km from the hospital. Each child had a 1.5 Tesla MRI with as much of the following sequence which was feasible: Sagittal T1WI, Axial T2 WI, Axial T2 FLAIR and Axial diffusion weighted scans. Cuts were made at 5-5.5mm/1.5mm.

2.6.4 Virological samples (HFMD cohort)

Throat and rectal swabs were collected in viral transport medium, divided into three aliquots and stored at -80 ^{0}C until analysis. Thanh et al. describe primers and probes for enterovirus (EV) and enterovirus-A71 (EV-A71).[345] 140 µl of culture supernatant/throat/rectal swabs in viral transport medium was mixed with 20 µl of EAV (a non-human internal control virus) and total nucleic acid was extracted using the QIAamp Viral RNA Mini Kit (QIAgen GmbH, Hilden, Germany), eluted in 100 µl elution buffer and stored at -80 ^{0}C.

Real-time RT-PCR was done using the SuperScript^{R} III One-Step qRT-PCR system with Platinum^{R} Taq DNA Polymerase (Invitrogen, Carlsbad, CA, USA) and was performed in a LightCycler 480 II machine (Roche Diagnostics GmbH, Mannheim, Germany). [345]

To maximise yield of detection, throat swabs were initially screened for EVs/EV-A71 and only the cases with a negative throat swab had the rectal swab tested.

2.7 Procedures

2.7.1 HFMD cohort

Children who met the Vietnamese Ministry of Health Grade 2 criteria were admitted to hospital. Grade 2a were admitted to Children's Ward C (Nhi C) for observation. If they progressed to features of Grade 2b then they were transferred to the Paediatric Intensive care unit (PICU).

In ward C, standard of care consists of 4-hourly observations of heart-rate, respiratory rate and temperature. Day of illness is defined as from start of fever. Children were observed for up to day 7 of illness.

If the disease severity of a child was grade 2b or higher, children were admitted to PICU. The children received intravenous phenobarbital daily (dose: 10mg/kg) and had continuous non-invasive monitoring of heart rate, respiratory rate and continuous pulse oximetry monitoring oxygen saturations. Arterial lines are inserted according to guidance on normal and abnormal manual blood pressure measurements and clinical concern of disease progression.

Grade 2b cases receive one or two doses of intravenous immunoglobulin if the child has one or more of the following; irregular breathing pattern, hypertension, cranial nerve palsies, limb weakness, sustained observed myoclonus.

Grade 3 and 4 children receive supportive care as necessary, including; intravenous milrinone with or without intravenous magnesium sulphate (as part of an on-going intervention trial) to treat hypertension, nasal continuous positive airway pressure or mechanical ventilation for apnoea or respiratory insufficiency, or haemofiltration for persistent hyperpyrexia or hypertension unresponsive to first line medication (milrinone and magnesium sulphate).

2.7.1.1 Approaching participants: recruitment by ward doctors during normal working hours

Grade 2a and 2b participants were approached as soon as possible after admission. Grade 3 and 4 cases are approached during recovery phase. This is the most appropriate time for the doctors to discuss long-term follow-up of these cases.

2.7.1.2 Consent for HFMD participants

HFMD participants enrolled consented to virology rectal and throat swabs, completion of the neurological examination and neurodevelopment schedule. Participating in the MRI sub-study was optional.

2.7.1.3 Virology samples

Throat and rectal swabs for EV and EV-A71 RT-PCR[345] were collected in 1ml viral transport medium (VTM) on the day of enrolment from all participants as part of the study. Both swabs were analysed as described previously in section 2.5.4. If either throat or rectal swab was virus positive, the child was classified as positive for the specific virus identified. All EV positive samples were further characterised and typed by VP1 and/or whole genome sequencing.[346] Routine clinical diagnostic EV and EV-A71 RT-PCR is carried out on all cases >Grade 2b as part of standard of care. These results were also collected in the case record form (CRF). Patients with grade 3 and 4 disease were enrolled to the study when they were in stable condition, often more than a week after admission. Both research RT-PCR results and hospital RT-PCR were taken into account for final

aetiological diagnosis and discrepant results were independently scored by two senior researchers.

2.7.1.4 MRI of HFMD participants

This aspect of the study was additional and optional. Scans were carried out when the responsible clinician deemed the patient at very low risk of progression of disease (> day 5 illness). There is no MRI scanner at HTD. The nearest private facility is 5 km away.

PICU doctors trained in paediatric resuscitation and a nurse accompanied up to two children to the MRI centre in an ambulance. I organised a resuscitation kit for transportation and the child was monitored en route to scan and back with a small portable oxygen saturation and heart rate monitor.

The doctor at the MRI centre currently uses the following intravenous regimes to sedate patients:

- ≤3 year: Thiopental sodium (dose up to 5mg/kg)
- ≥3 year: Propofol (2mg/kg of 0.5%)
- Consider midazolam (dose 0.1mg/kg) as necessary

The patient was attached to a non-invasive saturation monitor and a clinician trained in resuscitation observes the patient during the scan.

The child was monitored by clinical observation and an MRI compatible pulse oximeter. If the child was not cooperative, the scan was abandoned and not repeated. After the scan the child was observed for 30 minutes to 1 hour as

needed before being transferred back to the hospital by ambulance, accompanied by the doctor and nurse.

2.7.1.4.1 Interpretation of brain MRI scans

The Medic centre has radiologists which report scans immediately. These results were fed back to parents and clinically acted upon as appropriate. For the study, a consultant paediatric neuroradiologist, Dr Kling Chong at Great Ormond Street Hospital in the United Kingdom, reviewed the scans. Dr. Chong was informed of the age of the child but was not aware of the severity of the child's illness. A proforma (Table 2.2), which included specific regions listed according to the literature, was used to document MRI changes.

Table 2.5 Proforma to document Brain MRI changes

Regions with abnormality	MRI sequence			
	T1W	T2W	FLAIR	DWI
Posterior aspect of medulla oblongata, dorsal/caudal, left or right				
Posterior aspect of pons				
Ventricle dilatation or abnormality				
Periventricular region				
Midbrain: specify location				
dentate nuclei of cerebellum				
Globus pallidus				
Anterior commissure				
Hypothalamus				
Thalamus				
Putamen				
Cervical spinal cord				
Deep White Matter changes				
Subcortical white matter				
Diffuse changes				
Other comments				

2.7.1.5 Case record form for HFMD participants

A case record form (CRF) (Appendix 1) recorded clinical and demographic data of the enrolled child from enrolment, daily observation and neurological review and discharge. The HFMD grade was determined by the admitting clinician (Table 1.4). The CRF does not specify the criteria from Table 1.4 for the clinician to allocate the grade, to avoid retrospective bias of interpreting documentation from clinical notes. The clinical features listed on the CRF are from enrolment. The day of enrolment may not be the day of admission and features identified on admission may not persist till enrolment.

Daily neurological examination included evaluation of consciousness using the Blantyre coma scale. Blantyre coma scale is a modification of the Glasgow coma scale and is suitable to use in preverbal children. The scale uses motor and crying responses to pain and includes the ability to watch. It was developed for children with cerebral malaria in Malawi.[347] (Table 2.6) It has been used in assessing and monitoring coma in children due to meningitis and encephalitis in LMICs.[348-350]

Table 2.6 Blantyre Coma Scale

Response	Findings	Scores
Best motor response	Localises painful stimulus (pressure with blunt end of pencil on sternum or supraorbital ridge)	2
	Withdraws limb from painful stimulus (pressure with horizontal pencil on nail bed of finger or toe)	1
	No response or inappropriate response	0
Best verbal response	Cries appropriately with painful stimulus or if verbal speaks	2
	Moan or abnormal cry with painful stimulus	1
	No vocal response to painful stimulus	0
Eye movement	Watches or follows (e.g. mother's face)	1
	Fails to watch or follow	0

The study nurse completed the socioeconomic aspects of the CRF. Further details of data collected are described in 2.7.2.5.

2.7.1.6: First follow-up at 7-10 days post discharge

Patients were requested to come back for the first review 7-10 days after discharge. This period after discharge was to ensure the effect of phenobarbital (half-life 20-80 hours depending on the age)[351] does not affect performance on the neurodevelopmental examination.

The neurological examination and neurodevelopmental assessments were carried out in a research clinic held above the PICU (Figure 2.1). The room had blinds for privacy, in a quiet area and had child friendly table and chairs. There were stairs and space outside to evaluate gross motor skills.

Figure 2.1 Picture of assessment room in clinic above PICU

2.7.1.7 Six month follow-up visits

Six month follow-up appointments were arranged with a dedicated study nurse who also supported families regarding general health advice and concerns about their child. Neurodevelopmental and neurological examinations were carried out at the 6 month visit.

Parents or carers were present during the assessment. Allied health workers were not informed of the results but the parents were informed of areas of strength and weakness. Children with concerns were reviewed by a Vietnamese paediatrician and psychologist, and referred to available resources as necessary.

2.7.2 Healthy cohort

2.7.2.1 Kindergartens

The study team carried out talks to all parents and teachers at the kindergartens. Leaflets about the study were handed out (Appendix 2). The principal and nurse at each school collected a list of names of volunteers and passed the contact details to the study team. Assessments were carried out in a private room at each kindergarten.

2.7.2.2 Hung Vuong hospital

Birth cohort study nurses approached participants, informing them of the neurodevelopmental study. The study nurses asked mothers whether they would be willing to be contacted for further information about participation in this second study. If they agreed, the details of the child, parents' contact details and any siblings' details under 4 years were passed weekly to the neurodevelopmental study team. Hung Vuong participants came to OUCRU for assessments.

2.7.2.3 Ward clinics

The ward clinics staff both phoned the parent, or hand delivered letters to the home to inform of the study and arranged a meeting of invited parents with the study team at the clinic. This meeting gave the study team time to discuss the project and answer questions and distribute leaflets. Any one who agreed to participate was approached about the study.

2.7.2.4 Consent for Healthy participants

Healthy participants enrolled consented to completion of the neurodevelopment schedule and demographic and socioeconomic information in the CRF.

2.7.2.5 Case Record Form for Healthy participants

A case record form (CRF) recorded demographic data of the enrolled child from enrolment. The study nurse completed the socioeconomic aspects of the CRF, which included maternal education levels. Johnson et al. highlight higher socioeconomic status is known to improve academic attainment in infants in western studies.[352] Hillemeier et al. found low maternal education was a strong predictor of developmental delay in 7,200 US infants between 24-48 months.[353] Walker et al. review the evidence of the positive effects of higher maternal education in low and middle income countries.[354]

2.7.2.6 First assessment at enrolment

The neurodevelopmental assessments were carried out in a room at OUCRU for the Hung Vuong cohort, at a private room at the ward clinics or kindergartens. There was space outside to evaluate gross motor skills.

2.7.2.7 Six month follow-up visits

Six month follow-up appointments were arranged with a dedicated study nurse who also supported families regarding general health advice and concerns about their child. Neurodevelopmental assessments were carried out at the 6 month visit.

Parents or carers were present during the assessment. Teachers and allied health workers were not informed of the results but the parents were informed of areas of strength and weakness. Children with concerns were reviewed by a Vietnamese paediatrician and psychologist, and referred to available resources as necessary.

2.8 Sample size calculations

Calculation of sample sizes required estimation of the prevalence of severe HFMD grades and the rates of disease progression. Prospective epidemiological data on the prevalence and age ranges from a recent outbreak in Vietnam was used[86].

Sample size was determined based on feasibility considerations and the power calculations outlined below. The ideal sample size to ensure the significance and sample sizes stratified across severity groups described below was up to 250 grade 2a and 2b participants and up to 100 grade 3 and 4 participants recruited.

2.8.1 MRI findings in HFMD

There are limited published data on MRI findings in EV-A71 HFMD. One previous retrospective study of MRI scans in 24 patients, found changes in 13 cases who clinically presented with myoclonus. The equivalent clinical grading would be Grade 2b.[355] This gives a plausible range of 30-70% MRI abnormalities in the population of grade 2b children (95% confidence interval for observed proportion 24/13 (54%) in a population of 24 is 32%-74%). The data from Children's Hospital 1 showed that amongst patients who reached Grade 2b, 25% subsequently progressed to Grade 3 or 4.[86]

A sample size of 100 children with Grade 2a and 100 children with Grade 2b with MRI scans provides 80% power to detect a difference between the proportion of children with abnormal MRI in grade 2a vs. grade 2b of 14% vs. 30% (the lower estimated range of Grade 2b's with MRI changes) or of 50% vs. 70% (the higher

estimated range of Grade 2b's with MRI changes).

2.8.2 Neurodevelopment

We aimed to include up to 350 children with HFMD grade 2a, 2b, >2b, and 350 healthy children (age range similar to Grade 2a) and follow them up at enrolment and 6 months follow-up.

The Bayley scoring system has a mean score of 100 with a standard deviation (SD) of 15 in healthy children from the US. Based on this, we assumed an SD of approximately 15 in all 5 study populations. As little is known about within-child longitudinal changes in Bayley scores over time, power considerations were based on pair-wise comparisons between groups at each time point separately. With the sample sizes given, there would be 80% power at the two-sided 5% significance level to detect differences in mean scores of - 7.4 or larger between grade 2a or 2b children compared to grade >2b.

2.9 Data analysis

2.9.1 Data management

All participants had allocated study codes and data entered in a dedicated case record form (CRF). This was double data entry into an online secure system standard at OUCRU. The assessor entered Bayley III data into a secure online data entry system. I entered the neurological examinations into the online data management system.

2.9.2 General statistical methods

Standard data summaries such as median (inter-quartile range) for continuous variables and frequency (%) for categorical variable together with graphical summaries (histograms, boxplots and scatterplots) were used for descriptive analyses in all studies. Analysis was undertaken using R version 3.0.1.[356] For binary data, Fisher's exact test was used. For categorical and non-parametric data, the Wilcoxon rank-sum test was used. Results were significant at a level of $p \leq 0.05$.

2.9.3 Analysis plan of Bayley adaption

2.9.3.1 Reliability

The seven trained assessors independently scored 18 videos from the pilot study for evaluation of inter-observer reliability.

Test and retest was carried out in a convenience sample from the main study. Parents recruited in the healthy cohort were asked after the first assessment if they were willing to return within two weeks for a repeat assessment. Since the Bayley III can take up to 90 minutes, in order to limit fatigue and boredom at the re-test, only 2 or 3 of the domains were repeated. As a result to get a minimum of 20 children for each domain, a minimum of 40 children were required to participate. There is no clear guidance for the required sample size to calculate test-retest reliability but the study was limited by the feasibility of parents willing to attend twice, the shortage of available room space for testing and cost. In order to accommodate parents to come at weekends for the re-test, the children were not seen for the re-test by the same examiner and not in the same testing environment as the original, hence not optimal test re-test conditions.

R package ICC[357], was used to calculate the ICC estimates and 95%CI, which are based on mean squares obtained by applying analysis of variance models to the data.[356]

2.9.3.2 Validity

Comparison of a test to a local gold standard evaluates concurrent validity. As a local gold standard may not exist in many LMIC, researchers have looked at relationships with known variables that influence child neurodevelopment, such as maternal education and stunting, (<2 standard deviations length for age z (standardised to the mean) score measurements using WHO growth standards).[320, 358, 359] Univariate and multi-variate regressions using raw scores were used to evaluate the relationship between maternal education and stunting independently on subtest scores.

Structural equational modelling and confirmatory factor analysis were carried out to evaluate whether the test items were measuring the hypothesised underlying traits in the infants assessed. The details are within the relevant results sections.

2.9.3.3 Comparison to US publically available Bayley III data

The mean raw score for each age category from the US Bayley manual was plotted with locally weighted scatterplot smoothing (LOESS) of study data raw scores for each domain. The Vietnam study data was split into 13 age groups (<6 months and then 3 monthly age groups). The mean and 95%CI were calculated for each age group with the mean US raw score for the age groups overlaid. This

was to evaluate whether there is similarity between the US and Vietnam data. Further comparative analysis was limited without full US datasets.

2.9.4 MRI in HFMD

Dr. Kling Chong at Great Ormond Street Hospital reported all MRI scans. Dr. Chong was not aware of the severity of the HFMD cases. The proportions of abnormal MRI's between different grades were compared based on a Fisher's exact test. Regression analysis of subtest scores was performed to see if an abnormality on MRI was an independent risk factor for outcome.

2.9.5 Neurological examination

The proportions of abnormal neurological examinations between different grades were compared based on a Fisher's exact test.

2.9.6 Neurodevelopment

The literature suggests that important covariates such as level of maternal education and stunting influence neurodevelopment.[358] The healthy group was recruited concurrently within the appropriate age range of HFMD cases and hence there is overlap in age and sex distribution between the two groups. The resources needed to generate 1:1 matching for every grade 2a HFMD case concurrently would mean potential wasting of healthy cases and would have been an inefficient use of resources and time. However, the healthy group may not have similar distribution on the covariates of stunting and maternal education, and regression analysis may increase bias of estimated effect when the relationship between the outcome scores to covariates is non-linear, especially if the means and variance of covariates between the groups is large.[360, 361] To 'balance'

covariates between the HFMD and healthy cohorts we analysed the scores by adjusting for covariates based on a propensity score weight in the final outcome model.[362] This method has been shown to be more robust when there are scarce outcomes.[363]

Martens and colleagues used a simulated population to compare regression and propensity score methods and concluded:[364] "

The differences between propensity score methods and linear/logistic regression analysis are systematic and can be substantial, especially when the number of prognostic factors is more than five, the treatment effect is larger than an OR of 1.25 (or smaller than 0.8) or the incidence proportion is between 0.05 and 0.95. This difference is frequently overlooked by analysts in the literature. With respect to the objective to adjust for the imbalance of covariate distributions between treatment groups, we illustrated that the estimate of propensity score methods is in general closer to the true marginal treatment effect (the effect that would be found when both treatment groups had similar distributions of prognostic indicator) than the estimate of linear/logistic regression analysis."

Hence I carried out a) linear regression to compare all HFMD grades to the healthy group and b) propensity score balance to calculate the average treatment effect on the treated (ATT) of having HFMD grade 2a (compare balanced healthy and grade 2a), and having more severe HFMD (comparing grade 2a to 2b and >2b).

2.10 Ethical considerations

The Bayley III is not a diagnostic test in Vietnam. Despite the inclusion and exclusion criteria, if a child was felt by an assessor to have more difficulties than

expected on items, they were offered a review by the lead Vietnamese psychologist. It was explained to the parents that the review was offered as a second opportunity for the child to demonstrate skills, as children were very shy and not cooperative until well into the testing process.

2.11 Summary

The prospective study adapted an appropriate child development tool to assess cognitive, language and motor domains and neurological function in Vietnamese children with severe HFMD at two time points over 6 months. Additionally MRI scans were carried out during the acute admission to evaluate clinical and possible prognostic indicators of outcome.

The following results chapters describe firstly the reliability and validity of the adapted Bayley III for Vietnam, secondly, the adjusted regression and propensity score analysis of Bayley III scores by HFMD severity compared to the healthy group and lastly the descriptive changes in brain MRI in relation to Bayley III scores.

3. Adaptation and validation of Bayley III for Vietnam

There is no internationally recognised valid child developmental assessment tool in use in Vietnam. For the purpose of this and future studies in Vietnam we adapted the Bayley III. The adaptation process had to be robust enough to ensure accurate and reliable group comparisons to compare data between different studies in the same or different countries and to evaluate children longitudinally as they use different tests over time. In this study, I compared two groups from the same setting. I deconstructed the Bayley III to the underlying traits that it is designed to measure and looked at whether these traits are also measured in the Vietnamese adaptation. This is an important prerequisite in order to interpret differences between the Bayley III scores of children from US and Vietnam.

This chapter presents data which:

a) Assesses the psychometric properties of the Vietnam Bayley III by evaluating reliability and validity in a healthy sample of urban Vietnamese children

b) Compares study data to US publically available data to evaluate whether US scaled scores are appropriate for Vietnamese children.

Additionally we hypothesised:

i) that the same underlying structure of traits or latent factors evaluated by the original Bayley is the best-fit model for the Vietnamese sample

ii) that the tool was evaluating the same underlying traits in males and females in the Vietnamese sample.

3.1 Training and adaptation

3.1.1 Training on original Bayley III

Dr. Thanh, the local lead psychologist and myself attended training on Bayley III by an official trainer, Betty Hutchson. Ambiguous items were clarified by Betty Hutchson or Gloria Maccow, both of whom are experts on Bayley III employed by the publisher, Pearsons.

3.1.2 Adaptation procedures

Adaptation of the cognitive, language and motor domains followed the guidance from the international test commission and additional publications in reducing cultural bias.[341, 365-367] An initial direct translation of the Bayley III record form was closely scrutinised by local psychologists for ambiguity. Pilot testing while training psychologists on Bayley III, was used to modify items in addition to language advice from a post-doctoral language expert based at the University of Hawaii.[368]. None of the manuals were translated so study assessors needed to have a good standard of reading and understanding English.

An independent postdoctoral scientist carried out back translation. Discrepancies were reviewed and amended by two further independent post-doctoral scientists, with children and with experience living in the US. During piloting, further adaptations were made to make items less ambiguous and ensure language was appropriate for the target audience.

After several revisions, a version was then back translated to English. Discrepancies were reviewed by a second team consisting of two postdoctoral Vietnamese scientists, with children who had spent more than 2 years with their

children residing and working in the US. Items were clarified and a consensus reached with the psychological team about changes. This led to the final version used in the study population.

The socio-emotional and adaptive domains were not adapted, as too many items were not easily adaptable. These include items reporting "playing make-believe where the story makes sense", discriminating this from "imitates familiar make-believe" and "describes his or her feelings to explain why he or she is doing something", "makes plurals by adding an –s", "states time and day of favourite television shows", "uses wall switch to turn on and off lights", "puts own dirty cup or plate in the sink or dishwasher". Many items focus on independent tasks by young children are not necessarily encouraged in Vietnamese society and so children may receive incorrectly low scores on an adaptation of the socio-emotional-adaptive domains. Changing many items on the test would alter the fundamental structure of the test.

If a child was not cooperative or shy we gave the child time to settle and used toys not included in the Bayley kit for the child to play with. During this time, no scoring was done. Children also started on the fine motor domain before progressing to cognitive. However, the receptive language was always tested before expressive language. Modifications are listed in Table 3.1.

The tonal Vietnamese language has regional dialects, split to north, middle and south. Hence separate minor modifications are needed for application regionally. The study sample may not be demographically representative of Vietnam and further sampling would be ideal.

Table 3.1 Modifications to Bayley III.

Domain	Item No	English version	Vietnamese version
Cognitive	59	Introduce dog's name	
		This was not mentioned.	Let's say: *The dog's name is Clifford* before telling story
Cognitive	84	Spinning top picture	Ask the child: *Look, tell me What is this?* And Using the child's answer.
Cognitive	69-71	If the child feared doll and bear	
		This was not mentioned.	Get 0 score
Receptive	24	Discriminate between "tai" and "tay"	
		In English: Ear = tai Hand=tay	*Tai* and *tay* have similar sound in Vietnamese, so we need to say: " lo tai". "ban tay"
Expressive	27	Difference language	
		This was not mentioned.	If the child can understand assessor's question, but they answer correct by other language such as: Chinese, English. ➔ Get 1 score
Expressive	31-35-37	Picture Washing by machine/ Vacuuming replaced with hand wash and sweeping	 
Expressive	46-47-48	Picture page 165 (snow image but is one of several pictures that can be used)	Not Using as children not familiar
Translation of language during instruction During assessment, flexibility as several different words used with young children for the following items.		Block	Block = khoi, cuc.. So, ask parent use *khoi*, *cuc* or any other synonym word at home.
		Apple	Apple = tao, bom. So, ask parent use *tao, bom* or synonym word at home

Domain	Item No	English version	Vietnamese version
		Big	Big= to, lon, bu. So, ask parent use *to, lon, bu* or any other synonym word at home
		Small	Small= nho, be So, ask parent use *nho, be* or any other synonym word at home
		Cup	Cup= ly, tach So, ask parent use *ly, tach* or any other synonym word at home
		Ball	Ball= bong, banh So, ask parent use *bong, banh* or any other synonym word at home

3.1.3 Training on Vietnamese Bayley III

Child development and infant psychology are not established as a specialty in Vietnam. I ran an introductory course on child development, which included an overview of the Bayley III. Psychologists, special educators and paediatricians were invited to participate. Seven assessors who had either a degree in psychology or special education were recruited. They underwent 6 months of intensive training with practice on volunteers, which was recorded on video followed by constructive feedback in video training sessions. Items that were not clear or a source of confusion were queried with the publisher's assessment training consultant. Ongoing video training occurs during the study to maintain consistency of scoring.

3.2 Sample characteristics

3.2.1 Comparison to census data

There were 267 healthy control children aged less than 43 months enrolled in the study. 191 (72%), 54 (20%), 22 (8%) were recruited from Hung Vuong Hospital birth cohort, kindergartens and ward clinics respectively (Table 4.14). Each child underwent one assessment and 206 children had two assessments and 3 had three assessments over a six-month period, resulting in a total of 476 assessments.

We compared the latest publically available census data from 2011 with the study cohort. (Table 3.2 and 3.3)

Applying Fisher's exact test, there were significant differences in proportions of stunted children (OR 0.66, 95%CI 0.48-0.99, p-value = 0.037) and levels of maternal education (No school /primary OR 0.24, 95%CI 0.0029-0.89, p-value < 2.2e-16, secondary OR 0.64, 95%CI 0.5-0.83, p-value < 2.2e-16 and higher OR 2.62, 95%CI 2-3.43, p-value = 4.263e-12) between the study and census data. There were also significant differences in proportions of families owning refrigerators (OR 49.3, 95%CI 19.06-182.62, p-value < 2.2e-16), using air-conditioning (OR 10.5, 95%CI 8.12-13.72, p-value < 2.2e-16), owning a motorbike (OR 17.97, 95%CI 6.08-87.6, p-value <2.2e-16) and having access to better quality water (OR 1.52, 95%CI 1.15-2.01, p-value 0.002) and sanitation facilities (OR 7.36 95%CI 3.12-22.17, p-value 6.271e-10) compared to 2009 census data. The study sample was not designed to be representative of the general population, but that of urban Vietnam. There are no directly comparable population level data for urban population within Vietnam in the last 5 years.

Table 3.2 Characteristics of the validation sample and the Multiple Indicator Cluster Survey 2011, which sampled 11,614 households across the six regions of Viet Nam.[369]

	Healthy Cohort Study Population			*Multiple Cluster Survey 2011*			
				National Prevalence stunting N=3678			Urban: N=983
Study N= 267	**Male n=147**	**Female n=120**	**Both sexes n=267**	**Male N=1821**	**Female N=1751**	**Both sexes N=3678**	**Both Sexes N=112**
Age at enrolment in months Median (IQR)	15.87 (16.16)	15.97 (12.16)					
Z scores: length for age (all data) Mean (SD)	-0.93 (1.45)	-0.76 (1.44)					
Stunted (<-2SD z scores: length for age according to WHO guidelines)	30 (20%)	13 (11%)	43 (16%)	432 (23.7%)	378 (21.6%)	835 (22.7%)	112 (11.4%)
Maternal Education							
				Rural and urban both sexes N=3678			
No School or Primary	21 (14%)	22 (18%)	43 (16%)	865 (23.5%)			
Secondary School	71 (48%)	55 (46%)	126 (47%)	2149 (58.4%)			
Higher Education	55 (37%)	43 (36%)	98 (37%)	664 (18.1%)			

Table 3.3 Household Assets compared to 2009 Vietnam Census[370]

Urban Households	Study n=267	Urban Census 2009
Refrigerator use	263 (99%)	3,986,771 (57.4%)
Air conditioner use	178 (67%)	1,125,186 (16.2%)
Motorcycle use	267 (99%)	5,778,734 (83.2%)
Drinking water		
Piped	193 (72%)	4,410,452 (63.5%)
Borehole	0	2,111,460 (30.4%)
Rain	0	166,694(2.4%)
Natural Spring	0	256,987 (3.7%)
Bottle	74(28%)	NA
Sanitation		
Sanitary toilet	263 (98%)	6,098,231 (87.8%)

Urban population in 2009: 25,374,262
Urban households in 2009: 6,945,594

3.3 Reliability of Vietnamese Bayley III

3.3.1 Internal consistency of subtest items

Internal consistency (how well related items are measuring the same underlying trait) of the subsets with all ages combined was very good with Cronbach's alpha 0.95 to 0.97.[319] When this was split into four age groups, the fine motor domain at age group 18-24 months and receptive language aged less than 12 months had a Cronbach's alpha of <0.7, below the acceptable cut-off described by Cicchetti.[319] (Table 3.4)

Table 3.4 Internal consistency: Cronbach's alpha by age group and all ages combined

	Ages in Months according to Bayley entry				All ages
	=<12	>12 =<18	>18 =<24	>24 =<43	
Domains					
N	119	113	95	149	476
Cognitive	0.91	0.82	0.77	0.90	0.97
Receptive	0.62	0.85	0.88	0.90	0.96
Expressive	0.79	0.87	0.92	0.89	0.97
Fine Motor	0.89	0.73	0.58	0.83	0.95
Gross Motor	0.93	0.86	0.72	0.85	0.97

3.3.2 Inter-observer reliability

Seven raters scored up to 20 videos for each domain. Intra-class-correlation (ICC) and 95% confidence interval were calculated for agreement within these specific raters using the raw scores. ICC were very good (>0.90) in all domains (Table 3.5).[319]

Table 3.5 Interobserver reliability: 7 assessors

Domain	Cognitive N=20	Receptive N=21	Expressive N=18	Fine Motor=19	Gross Motor=20
ICC	0.99	0.978	0.97	0.99	0.99
CI	0.99-0.99	0.96-0.99	0.94-0.99	0.98-0.99	0.97-0.99

3.3.3 Test-retest reliability

Test-retest reliability was evaluated in up to 29 children using Pearson's correlation. Correlations on raw scores were high in all domains (Table 3.6).

Table 3.6 Test-retest: raw scores

Domain	Median (Range) Days difference between Testing	Pearson correlation
Cognitive N=29	9 (2.1 to 31.2)	0.97 (0.94-0.99)**
Receptive Language N=28	9(2.1 to 30.0)	0.96 (0.95-0.99)**
Expressive Language N=27	9 (3 to 30)	0.97 (0.94-0.99)**
Fine motor N=29	9(2.1 to 31.2)	0.97 (0.96-0.99)**
Gross motor N=25	8.1 (3 to 16.8)	0.96 (0.91-0.98)**

**p value<0.01

3.4 Distribution of raw scores

To evaluate relationships between subtests, the raw scores need to be standardised as the raw score subtests are effectively of different metrics. The sample was split into 13 age groups consisting of 19 to 70 children. The youngest infant was 4.6 months, hence the groups started at <6 months and then at 3 monthly intervals (Table 3.7). Z scores (standardised scores in relation to mean score for the age group) were calculated for each subtest within these age groups. The Z scores were used in the correlation analysis and factor analysis. Standardising the score in this manner allows a more stable measure of the underlying constructs or factors being measured by the observed test scores.[371]

The raw scores were not normally distributed for any domain. (Figure 3.1, 3.2). Distribution improved with calculation of Z scores. (Table 3.7, Figure 3.3).

Figure 3.1 Histogram of subset raw scores

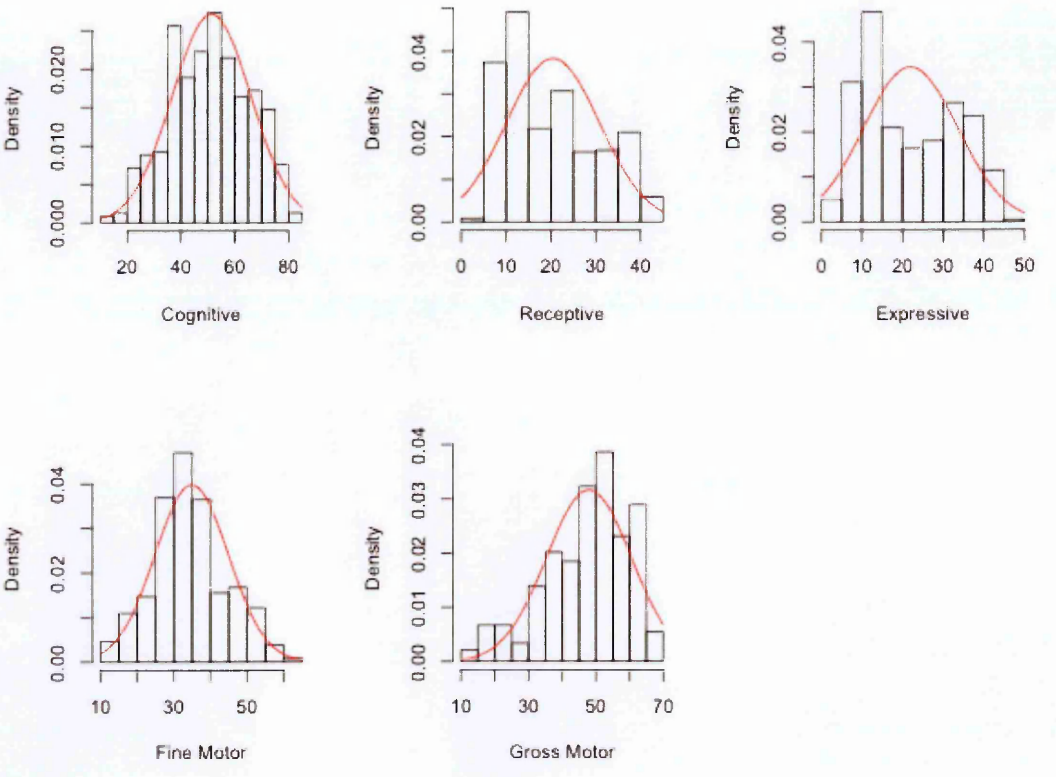


Figure 3.2 Q-Q plot of subtest raw scores:

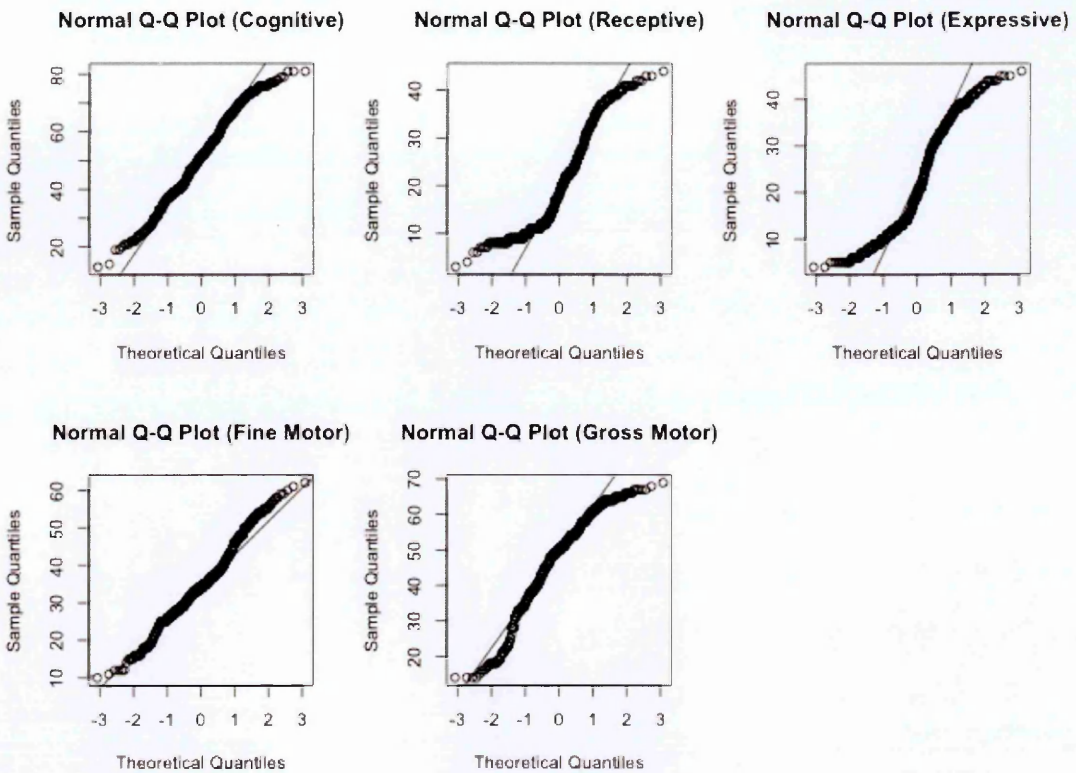
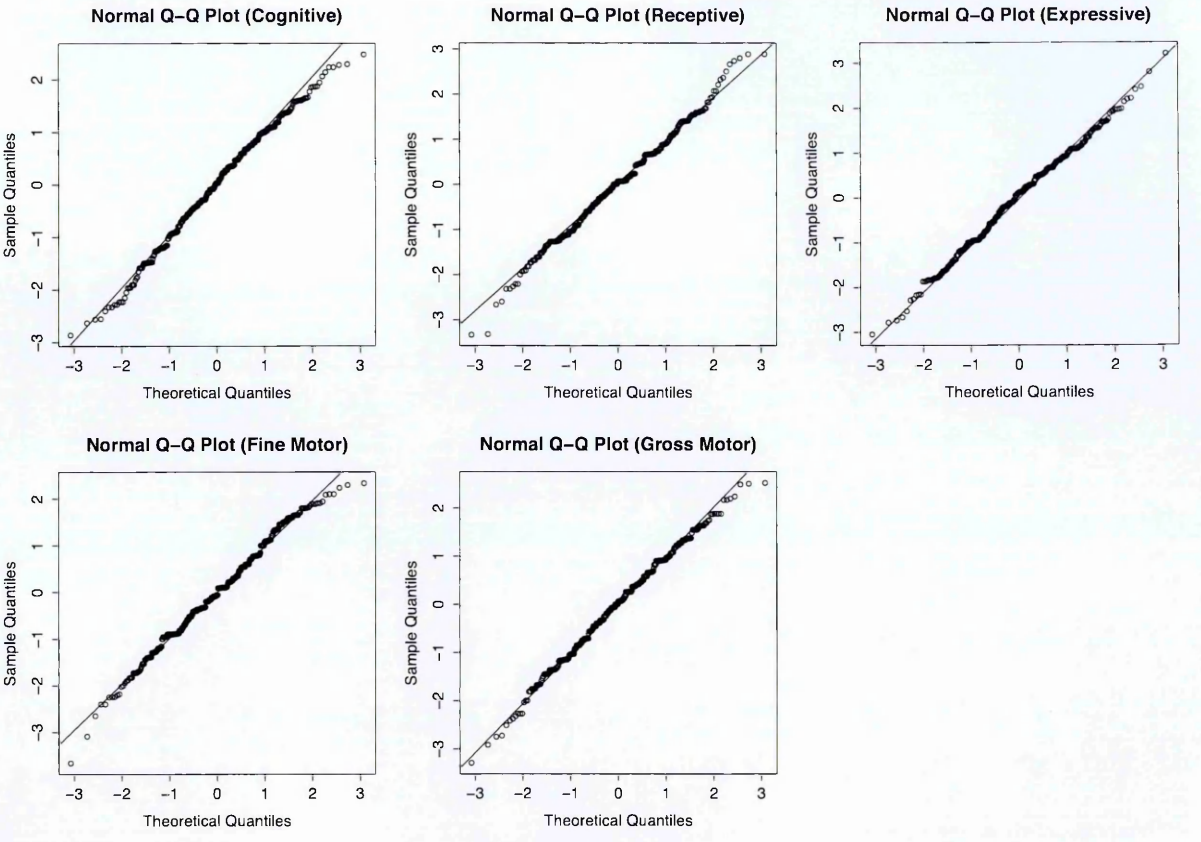


Table 3.7 Mean and standard deviation of raw scores per age group

Age group in months	N 476	CS mean	CS SD	RC mean	RC SD	EC mean	EC SD	FM mean	FM SD	GM mean	GM SD
<6	30	24.0	4.3	8.0	1.5	6.8	1.9	16.4	3.4	19.5	3.8
6-9	19	28.6	3.7	9.3	1.1	8.6	2.2	20.2	2.5	25.8	5.3
9-12	70	37.9	3.1	10.9	1.5	11.4	2.4	26.8	2.0	36.8	3.3
12-15	47	42.4	4.3	11.8	2.5	12.6	3.0	29.2	2.3	42.3	4.1
15-18	66	48.6	4.0	16.0	4.4	16.6	4.8	32.8	2.2	48.2	3.1
18-21	53	52.2	3.5	18.9	4.7	21.3	5.7	34.6	1.8	51.0	2.2
21-24	42	57.3	3.6	23.5	3.4	27.7	6.0	37.4	2.0	53.2	3.1
24-27	31	60.1	4.0	26.1	4.1	30.8	4.2	39.4	2.4	56.8	2.4
27-30	30	66.4	4.8	30.3	4.1	33.5	5.3	42.2	3.4	58.6	3.2
30-33	20	67.7	3.9	33.4	4.7	36.4	3.2	45.6	3.4	61.3	2.5
33-36	20	70.0	3.4	36.5	2.8	38.4	4.9	48.3	3.7	61.7	2.8
36-39	23	71.9	4.0	37.4	3.8	38.3	2.9	51.5	4.7	63.8	2.4
39-43	25	74.8	4.3	38.3	3.4	39.8	3.9	53.6	4.0	63.8	3.2

Figure 3.3 Q-Q plot of subtest z scores



3.5 Validity

3.5.1 Relationship with known risk factors for poor child development

In all domains, scores increased with age. Male sex was predictive of lower cognitive, receptive and expressive scores after adjusting for age. Higher maternal education predicted better scores in all domains in univariate analysis, but only improved scores in cognitive and receptive scores following adjustment for age and sex. Stunting was not predictive of scores following adjustment for age and sex (Table 3.8).

Table 3.8 Univariate and Multiple Regression of raw scores by covariates

Raw scores	Cognitive		Receptive		Expressive	
	Coefficient (95%CI); p-value		Coefficient (95%CI); p-value		Coefficient (95%CI); p-value	
Variable	**Univariate**	**Multiple Regression adjusted for age**	**Univariate**	**Multiple Regression adjusted for age**	**Univariate**	**Multiple Regression adjusted for age**
Sex male	-0.44 (-3.1 to 2.22); 0.75	-0.78 (-1.4 to -0.14); 0.017 *	-0.39 (-2.27 to 1.50); 0.69	-1.17 (-1.77 to -0.56); 0.0001*	-0.97 (-3.07 to 1.12); 0.36	-1.63 (-2.35to -0.9); 1.10e-05*
Maternal Education$^{\Phi}$:		**Multiple Regression adjusted for age or age and gender**		**Multiple Regression adjusted for age or age and gender**		**Multiple Regression adjusted for age or age and gender**
Secondary	3.47 (-0.37 to 7.31); 0.08	0.71(-0.21 to 1.63); 0.13	2.64 (-0.06 to 5.35); 0.06	0.79 (0.04 to 2.27); 0.074	1.93 (-0.08 to 1.66); 0.21	0.10 (-0.95 to 1.15); 0.85
Higher	6.75 (2.8 to 10.69); 0.0008**	1.33 (0.38 to 2.29); 0.006**	5.48 (2.70 to 8.27); 0.0001**	1.54 (0.35 to 2.69); 0.0008**	4.67 (0.63 to 2.44); 0.003**	0.64 (-0.45 to 1.73); 0.25
Stunting	-4.26 (-7.86 to -0.661); 0.021*	-0.31 (-1.19 to 0.56); 0.48	-4.38 (-7.74 to -1.01); 0.011*	-0.84 (-1.90 to 0.22); 0.12	-3.11(-5.66 to -0.56); 0.017*	-0.19 (-1.02 to 0.65); 0.66

Raw scores	Fine Motor		Gross Motor	
	Coefficient (95%CI); p-value		Coefficient (95%CI); p-value	
Variable	**Univariate**	**Multiple Regression adjusted for age**	**Univariate**	**Multiple Regression adjusted for age**
Sex male	0.06 (-1.75 to 1.87); 0.95	-0.4 (-0.87 to 0.07); 0.1	-0.73 (-3.0 to 1.54); 0.53	-0.48 (-0.55 to 0.027); 0.06
Maternal Education $^{\Phi}$:		**Multiple Regression adjusted for age or age and gender**		**Multiple Regression adjusted for age or age and gender**
Secondary	2.59 (-0.029 to 5.21); 0.05	0.38 (-0.31 to 1.06); 0.27	2.30 (-1.01 to 5.61); 0.17	0.041 (-070 to 0.79); 0.91
Higher	4.47 (1.77 to 7.16); 0.001*	0.60 (-0.22 to 1.20); 0.49	3.84 (0.43 to 7.24); 0.03*	-0.45 (-1.22 to 0.33); 0.26
Stunting	-2.4 (-4.85 to -0.06); 0.056	0.21 (-0.44 to 0.85); 0.53	-5.01 (-9.65 to -0.37); 0.03*	0.09 (-1.22 to 0.33); 0.86

*p<0.05
**p<0.01
Φ Maternal Education: No school or primary as baseline for regression analysis

3.5.2 Convergent and discriminant validity

Correlations between subtests were evaluated for convergent and divergent validity. It is expected that the two motor subtests correlate higher to each other than with other subtests and similarly with the two language subtests.

The study data using Z scores correlations ranged from 0.3 (RC and FM) to 0.5 (RC and EC, FM and GM). This is similar to the original US correlation between subsets, 0.36 (EC and GM) to 0.53 (RC and EC). Additionally, the correlation between the fine motor and cognitive subtest was high at 0.5, similar to the US manual: 0.51. (Figure 3.4)

Figure 3.4 Correlation matrix between domains using Z scores from 13 age groups.[a] (476 assessments)

	CS	RL	EL	FM	GM
CS					
RL	0.44				
EL	0.43	0.5			
FM	0.5	0.3	0.38		
GM	0.42	0.32	0.3	0.43	

[a]All correlations were significant at $p<0.05$

CS: cognitive, RL: receptive language, EL: expressive language, FM: fine motor, GM: gross motor

3.5.3 Construct validity

Structural equation modelling (SEM) is a statistical method, used to evaluate theoretical models using observed data[372]. The theoretical model identifies the relationship between the observed data, e.g. test scores and the underlying latent trait (factor) being assessed e.g. language. Confirmatory factor analysis (CFA) is

one SEM method which tests specific hypotheses about the structure of the factor loadings (how much the observed variable contributes to the underlying factor), and uses path analysis diagrams to describe these relationships.[373] The factor structure was based on an *a priori* hypothesis that the best-fit structure would be the same as the original US Bayley III, i.e. a 3-factor model (fine and gross motor subtests on the first factor, receptive and expressive language on the second, and cognitive on the third). Deciding adequate sample size for factor analysis, Comrey and Lee are often quoted stating a sample size of "50 is very poor, 100 is poor, 200 is fair, 300 is good, 500 is very good, and 1,000 is excellent."[374] In practice there are several recommendations on adequate sample size for factor analysis, depending on the number of indicators (observed variables) to factors, and the variance of the indicator explained by the factor (communalities). However it is an area of on-going debate.[374]

Several measures of overall model fit are used. Non-significant Chi square statistics at 0.05 level and Root Mean Square Error of Approximation (RMSEA) < 0.05 indicate good fit.[375, 376] The RMSEA also permits a confidence interval (CI) calculation, whereby a good fit has an upper limit of CI <0.08. The Comparative Fit Index (CFI) has acceptable fit at 0.95, and good fit at >0.97.[375, 376] A criterion p-value of 0.05 was used for all analyses (SEM in R using Psych and Lavaan packages).[356, 377, 378]

3.5.3.1 Confirmatory factor analysis

The study followed the procedure of construct validity as described in the original US Bayley III technical manual.[336] The following models were evaluated for goodness of fit. Null model (no common factors), one factor model; (5 subtests on

a general factor), two factor model; (fine and gross motor subtests on first factor (Motor scale) and receptive and expressive language and cognitive on second factor) and three factor model; (fine and gross motor subtests on the first factor, expressive and receptive language on the second and the cognitive on the third). All models assumed that factors are free to intercorrelate and errors are uncorrelated. The best model fit was 3 factors, the same best fit construct structure as US Bayley. There were no modification indices >10 (ML and WLS) and all standardised residuals <2.58 (ML) suggesting no small areas of ill-fit in the model. (Table 3.9)

It is assumed that subtests loading on the same factors have only the underlying construct to explain their relationship and since underlying factors can correlate, any relationship between subtests of different factors are explained by the factor relationship. Standardised solutions define the metric of the latent variables by fixing the variance of one factor to 1.0. (Figure 3.5-3.7).[379]

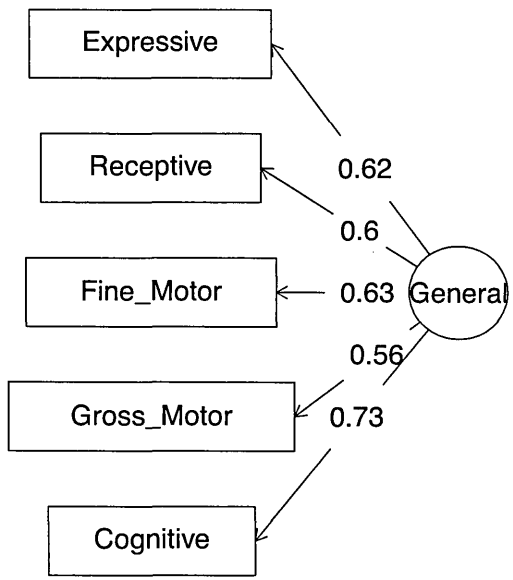
Table 3.9 Goodness-of Fit Statistics for Confirmatory Factor Analysis of subtests.

	N[9]	X^2	Df	p-value	CFI	TLI	RMSEA (CI)
Model 1 Estimator : ML	476	41.59	5	<0.01	0.935	0.871	0.124(0.091 – 0.160)
Model 2 Estimator : ML	476	31.18	4	<0.01	0.952	0.880	0.119(0.083-0.160)
Model 3 Estimator : ML	476	5.98	3	0.11	0.995	0.982	0.046(0.0-0.099)
Model 3 Estimator : WLS	476	6.20	3	0.10	0.983	0.944	0.047(0.0-0.101)

ML: Maximum Likelihood, WLS: Weighted Least Squares, X^2:chi-square, Df: degrees of freedom, CFI: Comparative Fit Index, RMSEA: Root Mean Square of Approximation, TLI: Tucker-Lewis Index, CI: 95% Confidence Interval. [9] Sample includes some children tested twice from test-retest group

Figure 3.5 Model 1: Confirmatory Factor Analysis with unstandardised and standardised estimates

Unstandardised estimates



Standardised Estimates

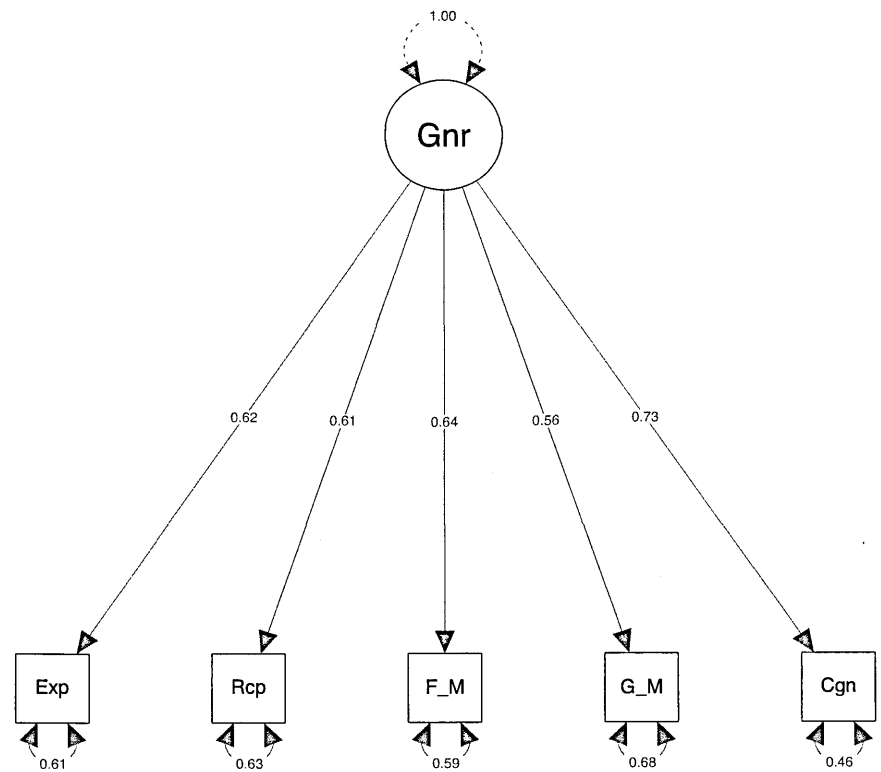
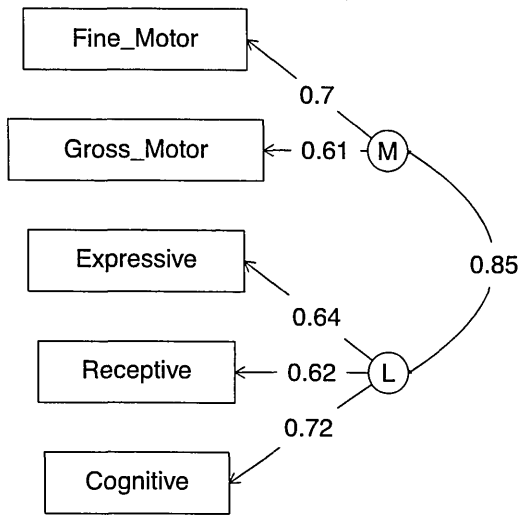


Figure 3.6 Model 2: Confirmatory Factor Analysis

Unstandardised estimates



Standardised Estimates

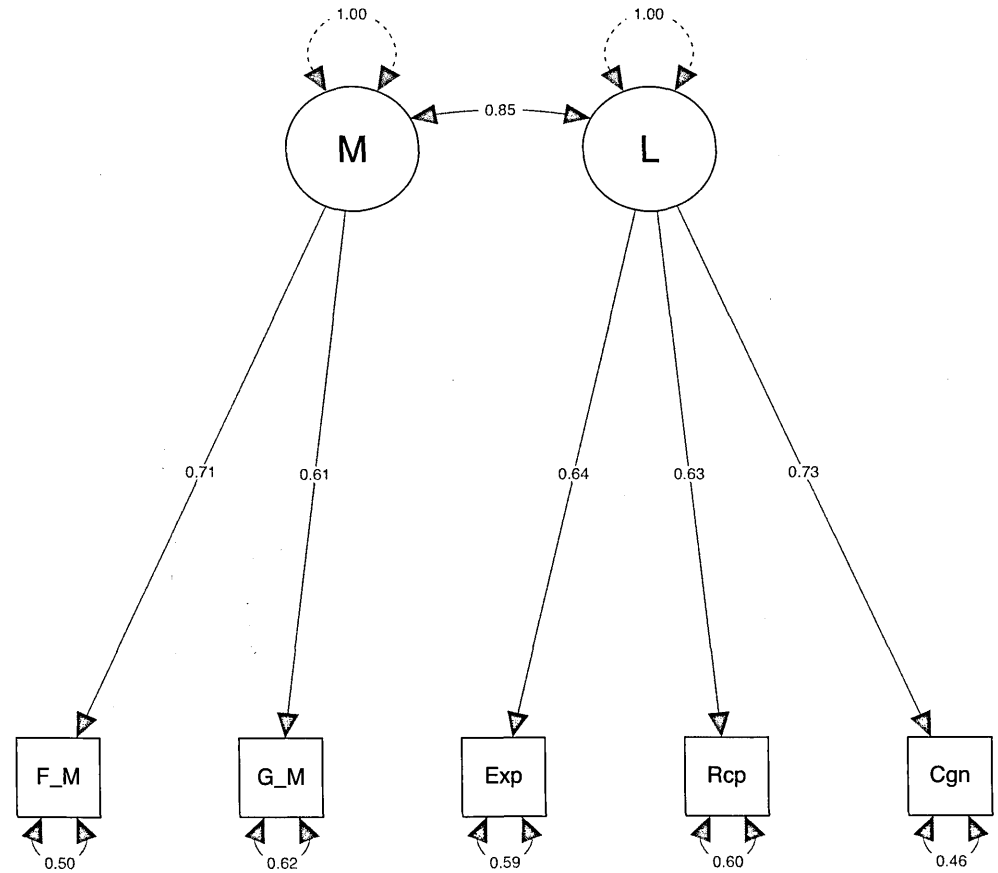
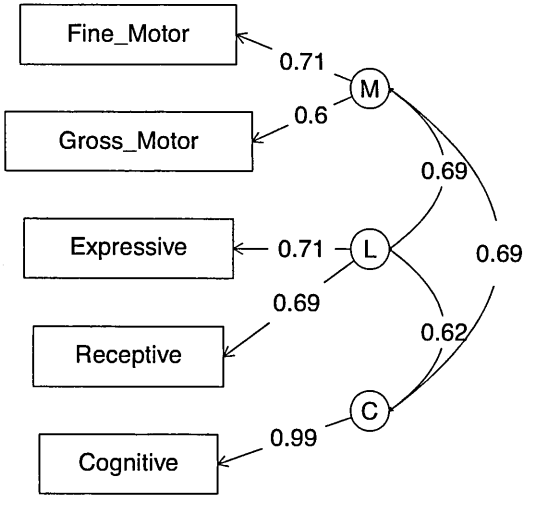
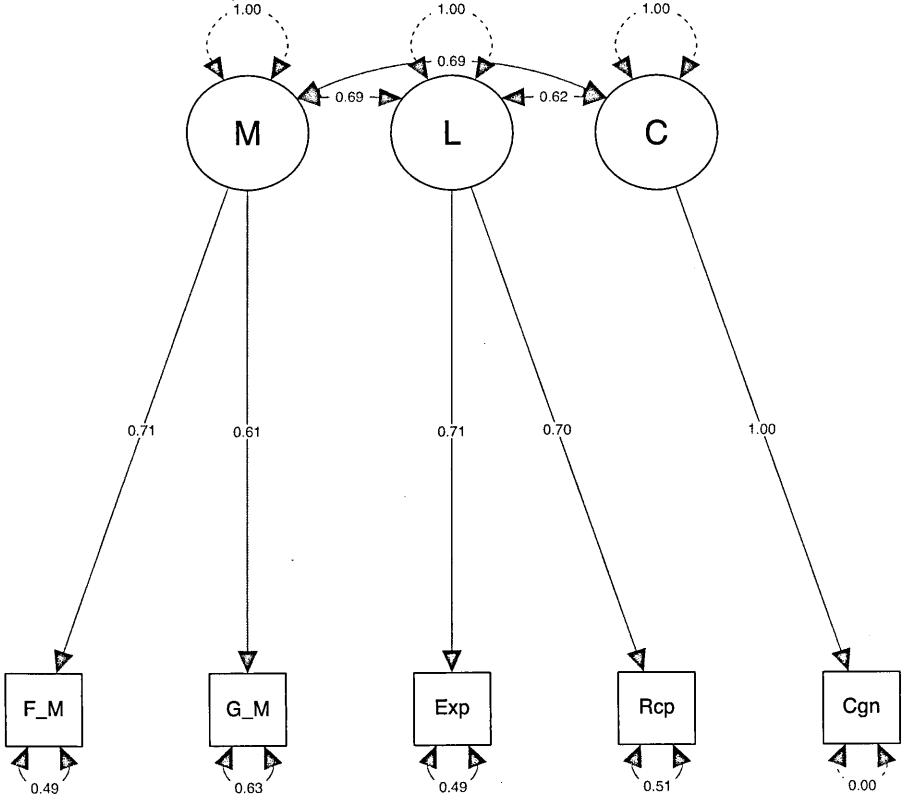


Figure 3.7 Model 3: Confirmatory Factor Analysis

Unstandardised estimates



Standardised estimates



3.5.3.2 Average variance extracted

The average amount of variance in measured variables that a construct is managed to explain should be higher than the shared variance with any other construct, determining that discriminant validity is supported i.e. the items are specific to the construct of interest and the other constructs are sufficiently different. An average variance extracted (AVE) greater than 0.50 suggests that both the validity of the construct and the individual subtests are good.[380] As far as we could determine from the literature, the AVE of Bayley III has never been evaluated before. In this sample, the AVEs for each construct was lower than their shared variance with the other constructs, which demonstrated poor convergent and discriminant construct validity for the Vietnamese Bayley III. (Table 3.10).

Table 3.10 The square roots of AVEs and correlations among latent constructs

	M	L	C
M	(0.66)		
L	0.685**	(0.7)	
C	0.694**	0.616**	(1.00)
AVE	0.44	0.5	1.000

The square roots of AVE on the diagonal in brackets, correlation coefficients on the anti-diagonal.
M: Motor
L: language
C: Cognition
**p-value <0.01

3.5.3.3 Measurement invariance

SEM can additionally evaluate measurement invariance (MI) to determine whether group differences in scores are due to the differences in underlying ability or due to bias or unreliability of the test.[373] Although the Bayley has been used as the "gold standard" for outcome research worldwide, there is no published data on measurement invariance between gender or within different age groups. The major limitation is the requirement of having sufficient sample size to evaluate

factor analysis and hence MI. In this study we evaluated whether there is gender MI.

MI is carried out in a hierarchical process with nested models of "configural", "weak", "strong" and "strict" MI.[381] Configural evaluates whether the number of factors and pattern of loading is similar between groups, indicating participants from different groups conceptualise the constructs in the same way and hence respond to the test in the same way. Weak invariance evaluates whether the magnitude of factor loading is similar between groups (different groups respond to items in the same way) so that a change in one on the test is the same change in unit on the underlying factor for both groups compared. If one unit change in the item score does not result in the same change in the factor score across groups, the regression lines are slopes are unequal; as illustrated in Figure 3.9. Strong or scalar invariance additionally evaluates whether the intercepts are similar between groups (observed scores are related to the latent constructs in the same way irrespective of group membership). If there is no strong invariance, the item-factor relationship is dependent on the group membership as illustrated in figure 3.10-3.11, whereby the same item score gives a higher factor score to females. Strict invariance evaluating the residual variances (same level of systematic measurement error per item for each group) can result in the shift of the item-factor regression lines away from the ideal position (Figure 3.12).[379] In practice, these nested models are evaluated by showing a non-improvement in fit between models.[382] Figures 3.8-3.12 are based on figures from Wu et al.[382]

Strong invariance (or scalar invariance) is required in order to meaningfully compare the means of latent variables across different groups.[383] Wu et al. advise strict invariance to be met before means can be compared.[382] A significant drop in

model fit suggests that the constructs are not measured similarly in the two groups. Changes in the comparative fit index (CFI) >0.01 is commonly used to define whether a more constrained model has a significant decrease in model fit compared to the previous model with fewer constraints.[381]

The analyses were run by means of maximum likelihood (ML) estimation. The use of ML estimation can cause problems when using non-normal data.[375] Other estimation methods for non-normal data include asymptotic distribution free (ADF) (or weighted least squares, WLS). However, Ory et al. identified more consistency between ML and ADF with non-normal data with the ADF estimation providing an overall better goodness-of-fit than ML.[384] For the best-fit model identified by ML, the WLS method was also used to evaluate the potential effect of sample non-normality.

Figure 3.8 Item Factor regression –model test is the same irrespective of gender

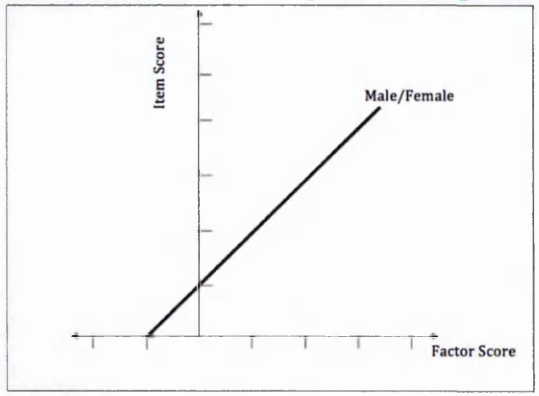


Figure 3.9 Factor loadings are different by gender

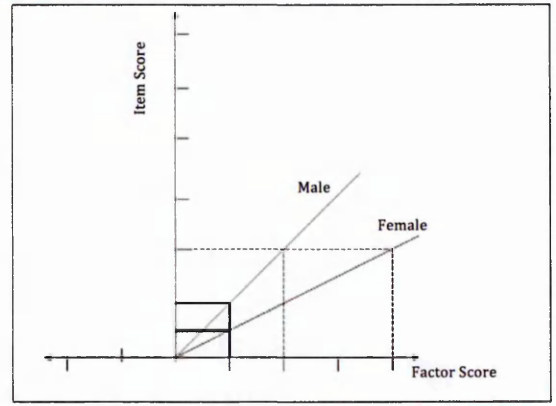


Figure 3.10 Factor Intercepts differ by gender

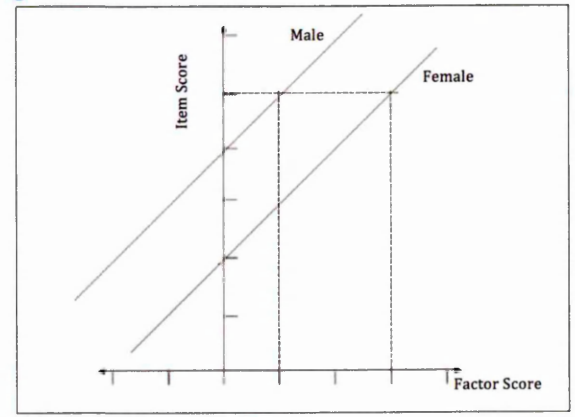


Figure 3.11 Gender factor loading and intercept inequality

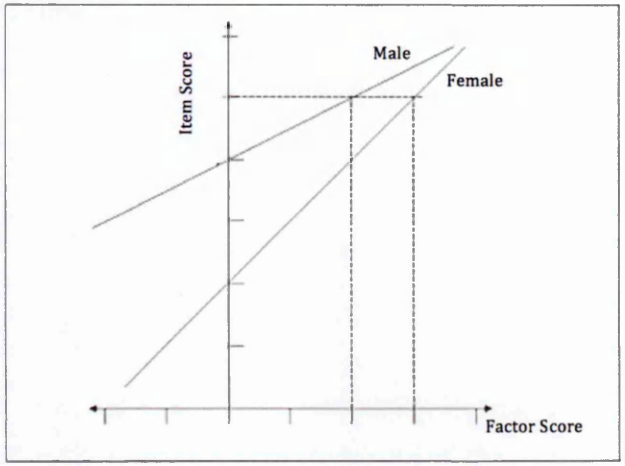
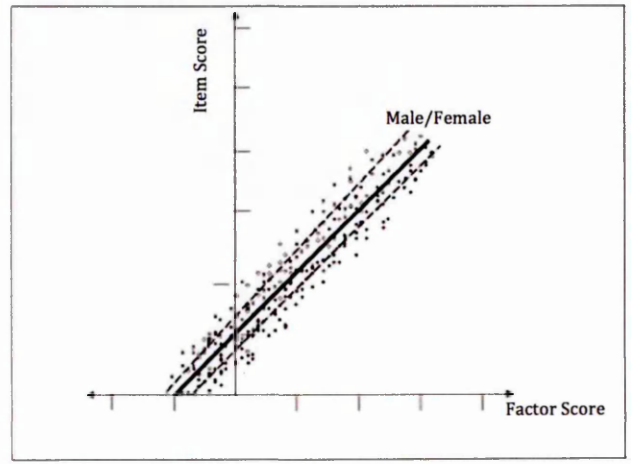


Figure 3.12 Residuals (error) differ by gender



3.5.3.3.1 Gender measurement invariance (MI)

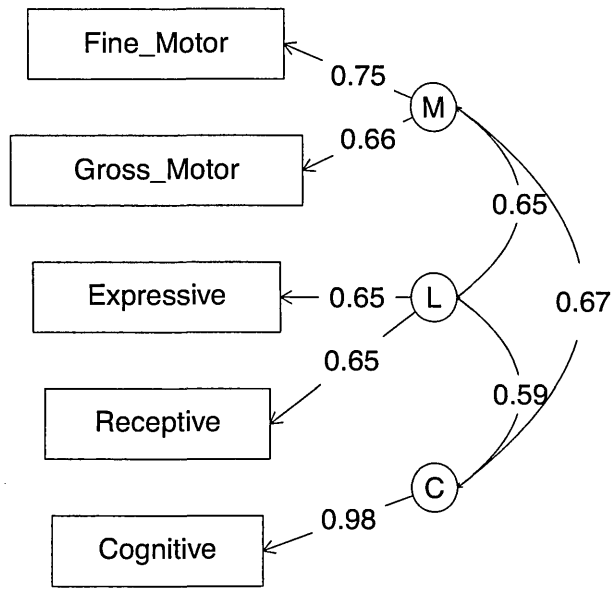
We tested the hypothesis that a change in the underlying latent factor would result in the same change in Z scores irrespective of gender, by evaluating model invariances with restrictions imposed on the model.[385] The goodness of fit indices for male and female subsets suggests that the 3 factor model was a good fit for the observed data at baseline. Factor loadings were significant for both sexes. As restrictions were imposed on the model, goodness of fit indices remained adequate, suggesting the Z scores of the subtests are measuring the same underlying constructs in both males and females. (Tables 3.11, 3.12, Figures 3.13-3.14)

Table 3.11 Evaluating Measurement invariances according to gender.

Estimator: ML	N	X2	df	p-value	CFI	TLI	RMSEA (CI)
Model 3	476	2.686	3	0.443	1.000	1.000	0(0.0-0.074)
Male	256	6.347	3	0.096	0.988	0.962	0.066 (1.0-0.139)
Female	220	3.290	3	0.349	0.999	0.996	0.021 (0.0-0.118)

ML: Maximum Likelihood, WLS: Weighted Least Squares
X^2:chi-square, Df: degrees of freedom
CFI: Comparative Fit Index, RMSEA: Root Mean Square of Approximation
TLI: Tucker-Lewis Index, CI: 95% Confidence Interval

Figure 3.13 Male unstandardised and standardised factor loadings



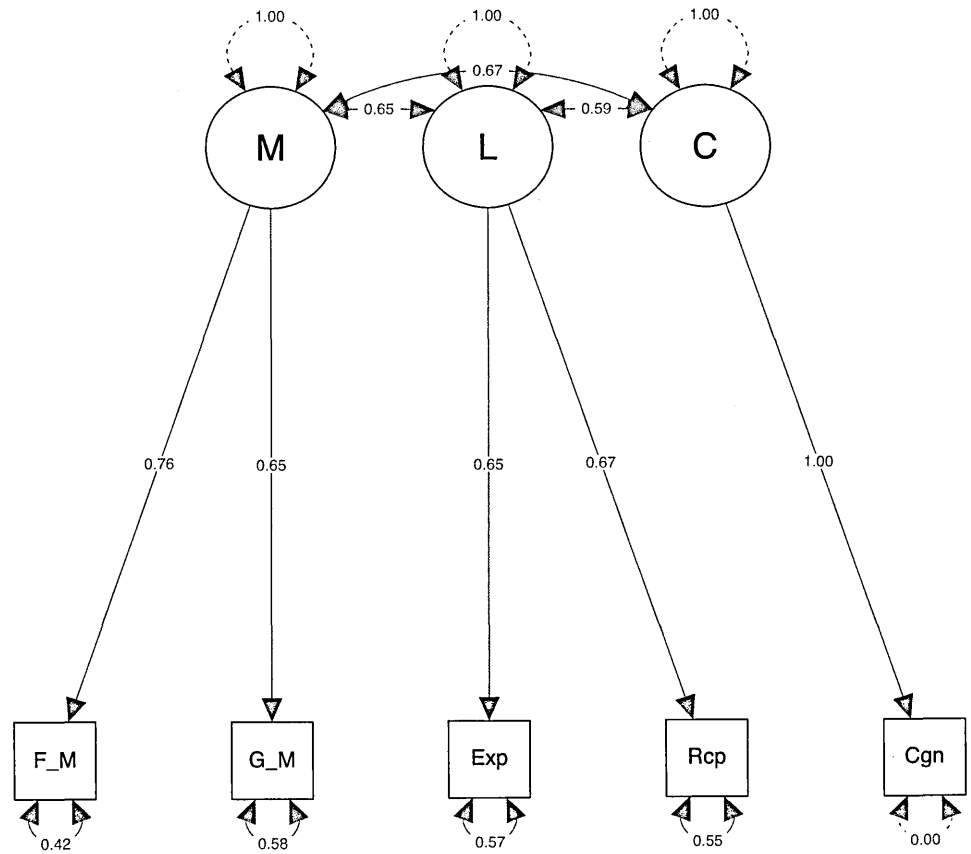


Figure 3.14 Female unstandardised and standardised factor loadings

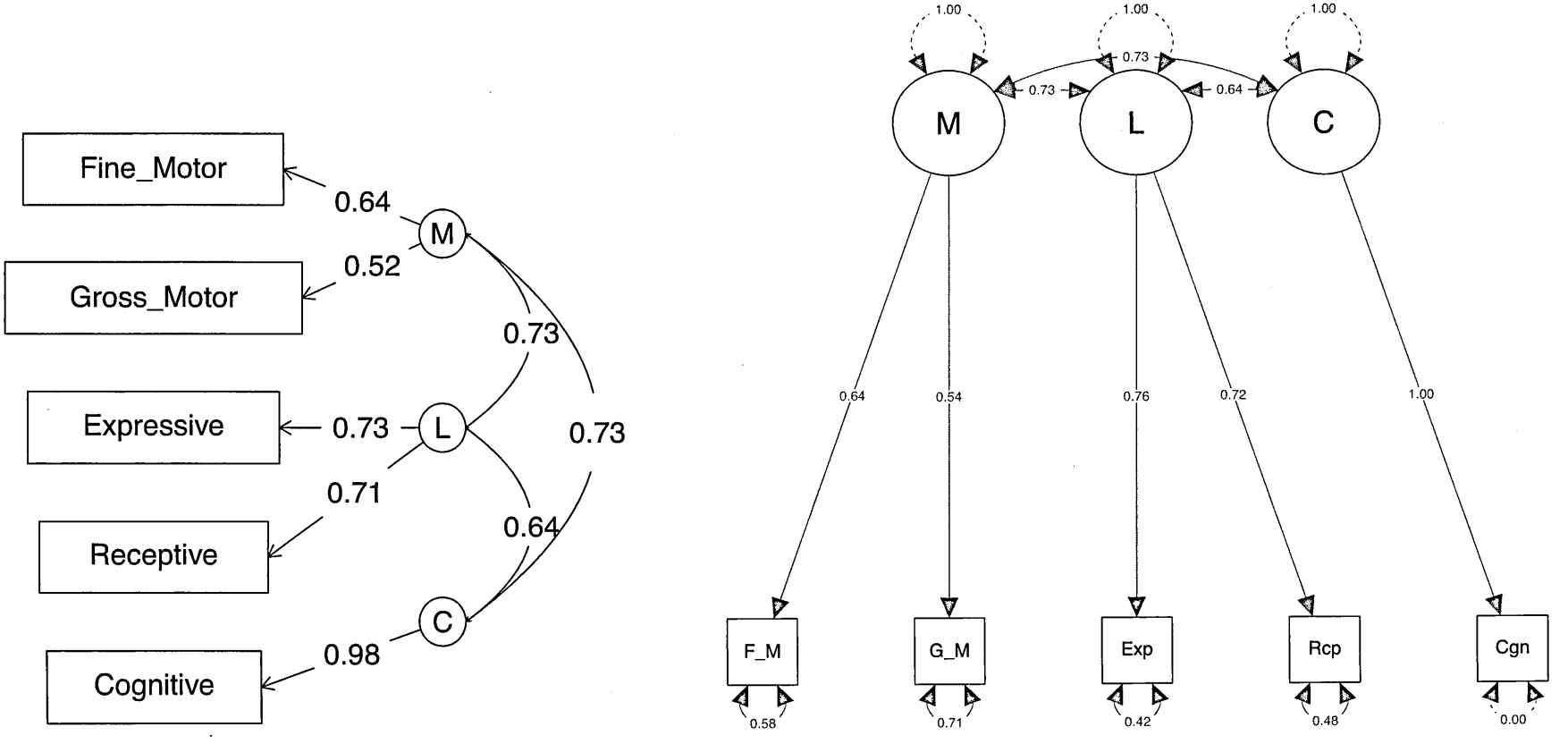


Table 3.12 Measurement invariance by gender: imposing model restrictions

	Df	Chisq	Chisq diff	Df diff	P value	CFI	ΔCFI
Baseline Configural Invariance (equal number of factors)	6	9.637			0.141	0.993	
Metric Invariance (equal loadings)	8	9.806	0.1694	2	0.279	0.997	0.004
Scalar Invariance (equal loadings and intercepts)	10	10.711	0.9050	2	0.380	0.999	0.002
Strict Invariance (equal loadings, intercepts and residuals)	14	17.925	7.2143	4	0.210	0.993	0.006
Strict Invariance +equal means	17	34.05	16.1288	3	0.008	0.969	0.024

Comparison between progressively restrictive models indicated that some aspects of the strict invariance assumption were met which suggested that being male or female did not alter the response to the test items. However, there was a difference in means of the latent variable between males and females, which supports the need to adjust scores for gender.

3.5.4 Comparison to published US Bayley III data

The mean raw score for each age category from the US Bayley manual was plotted with locally weighted scatterplot smoothing (LOESS) of study data raw scores for each domain. The Vietnam study data was split into 13 age groups. The mean and 95%CI was calculated for each age group with the mean US raw score for the age groups overlaid (raw scores equivalent at scale score 10 from the administration manual). This was to evaluate whether there is similarity between the US and Vietnam data. Further comparative analysis was limited without full US datasets. (Figures 3.15, 3.17, 3.19, 3.21, 3.23)

The means and 95% confidence intervals of the raw scores for 13 age groups were plotted. (Figures 3.16, 3.18, 3.20, 3.22, 3.24).

Figure 3.15 Cognitive - US and Vietnam raw scores

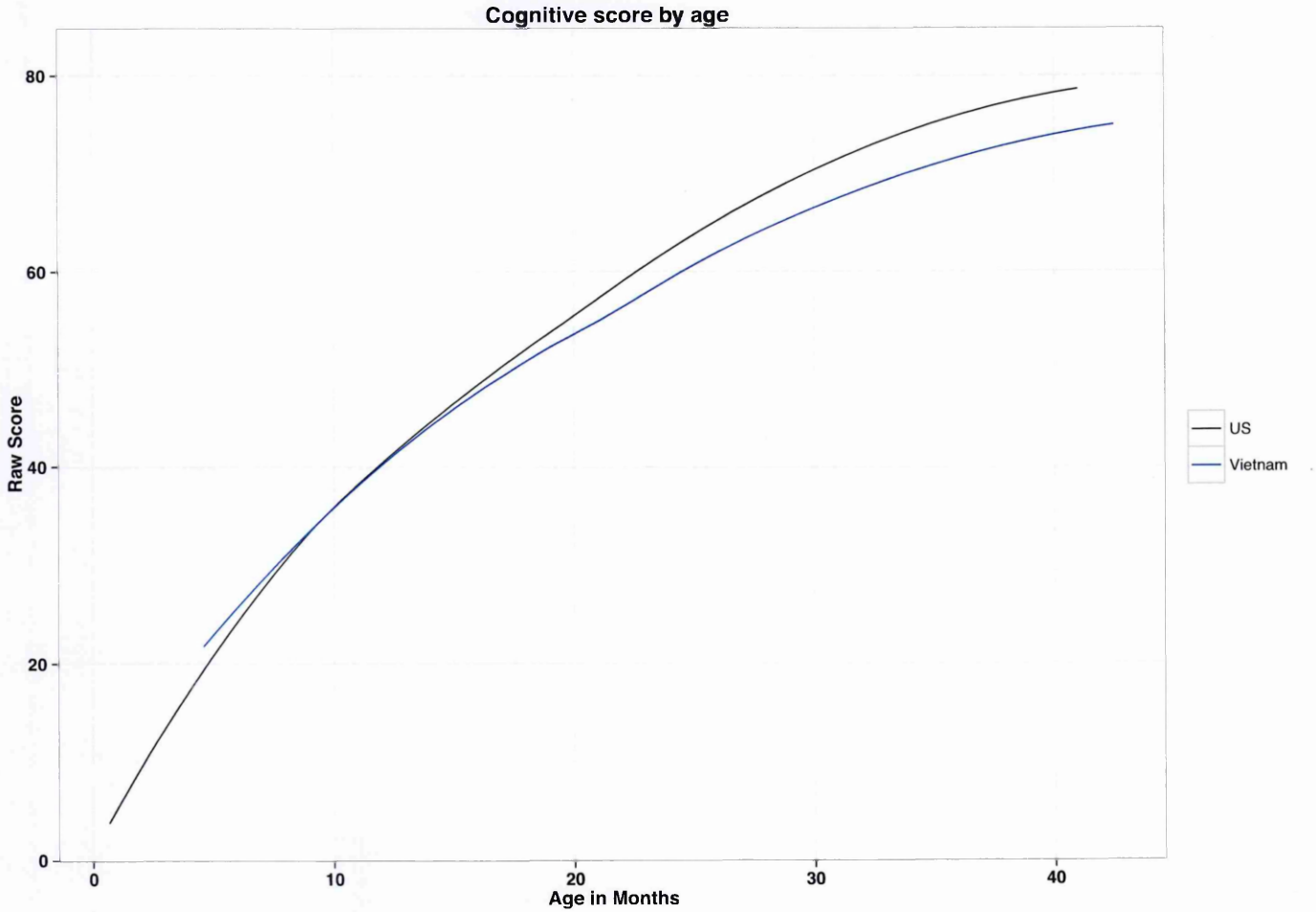


Figure 3.16 Cognitive - US and Vietnam mean raw scores and 95% Confidence Interval

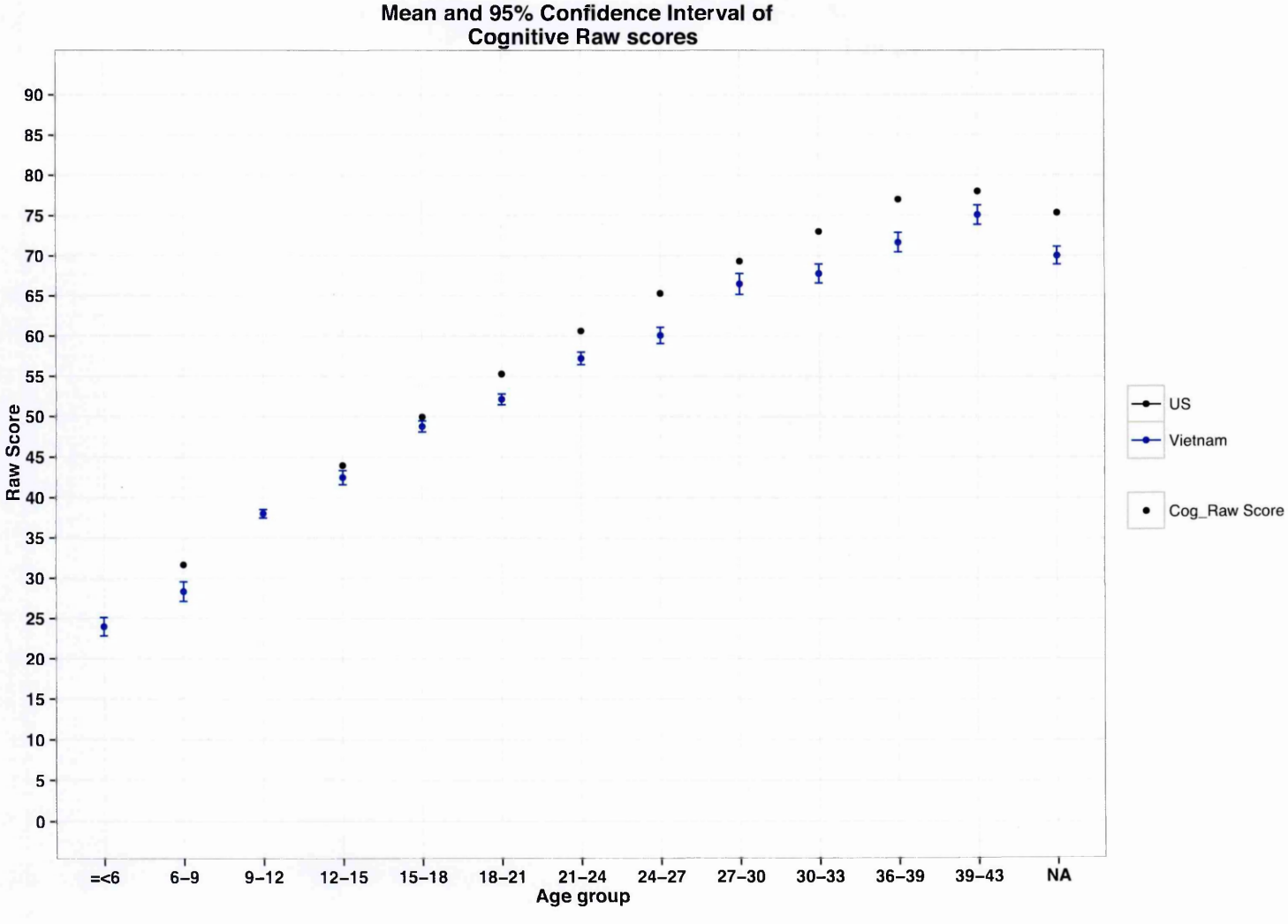


Figure 3.17 Receptive - US and Vietnam raw scores

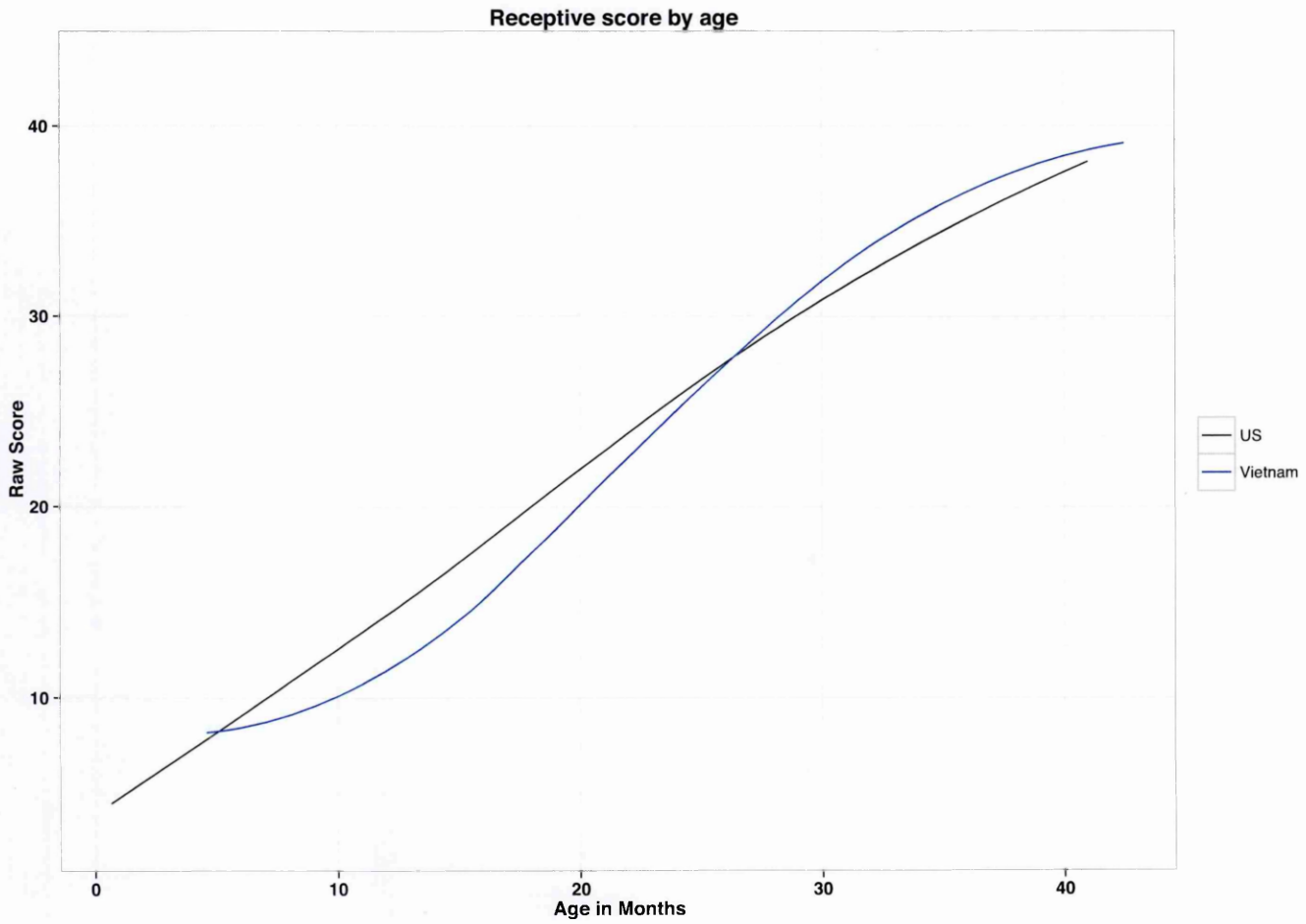


Figure 3.18 Receptive - US and Vietnam mean raw scores and 95% Confidence Interval

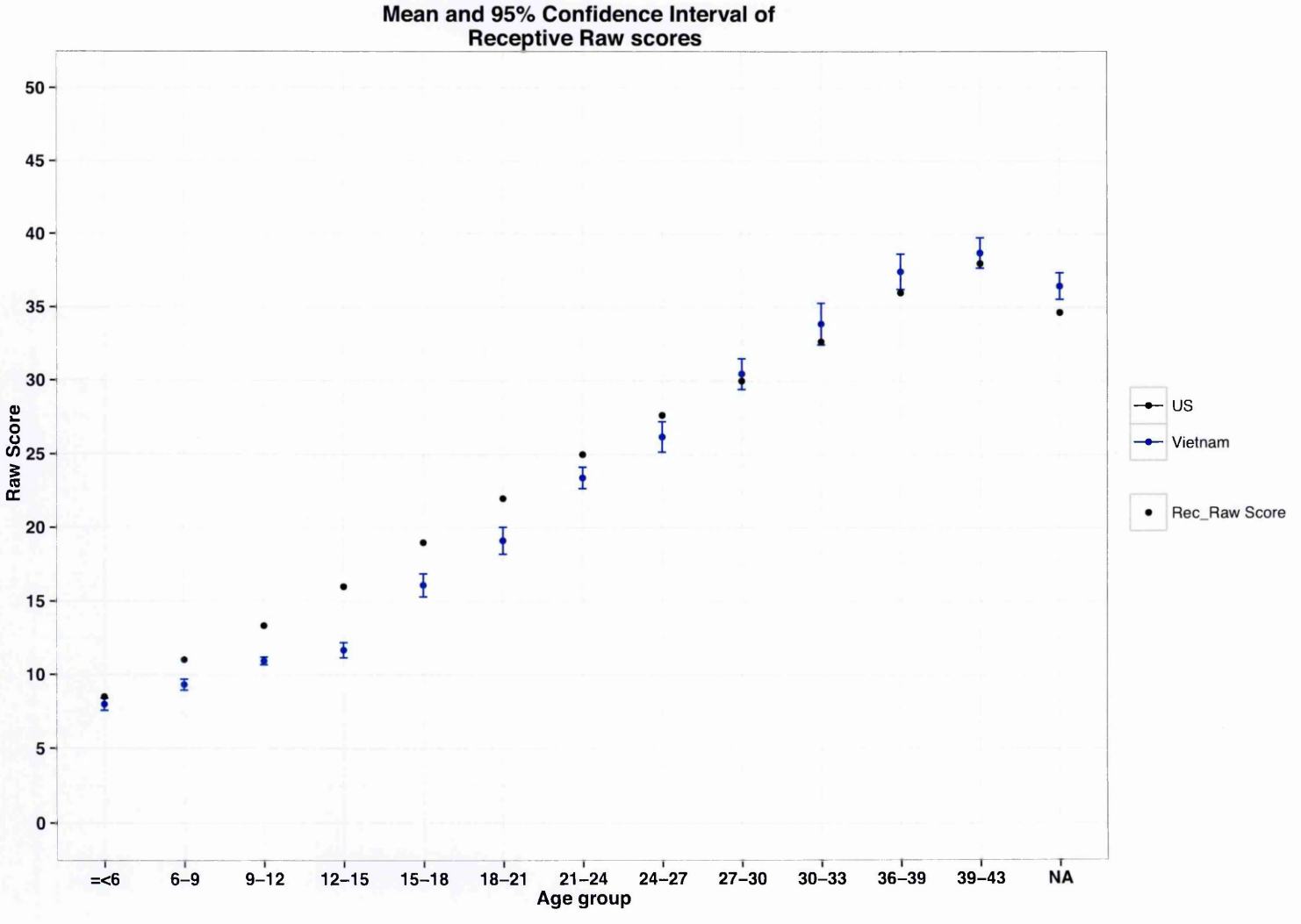


Figure 3.19 Expressive - US and Vietnam raw scores

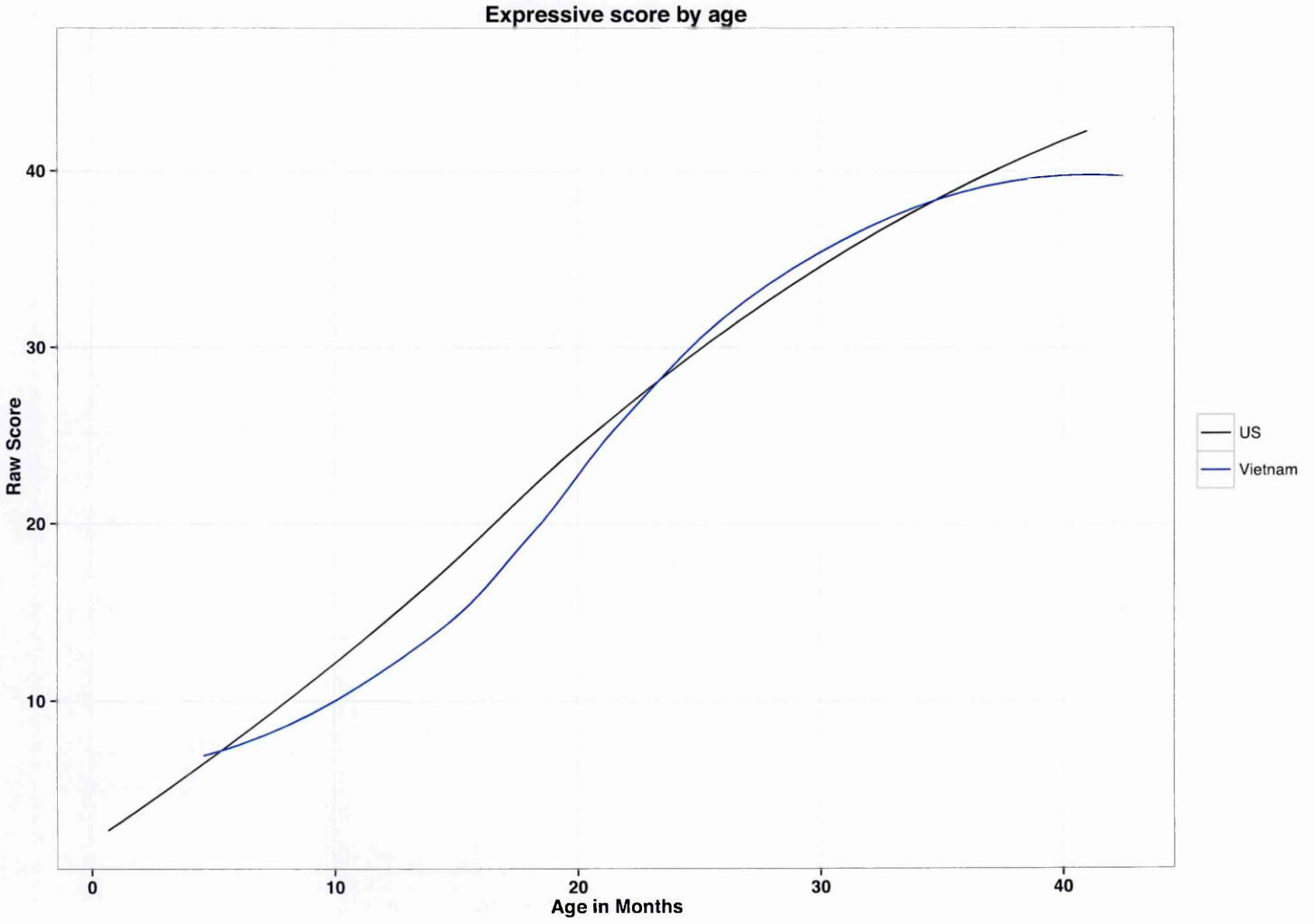


Figure 3.20 Expressive - US and Vietnam mean raw scores and 95% Confidence Interval

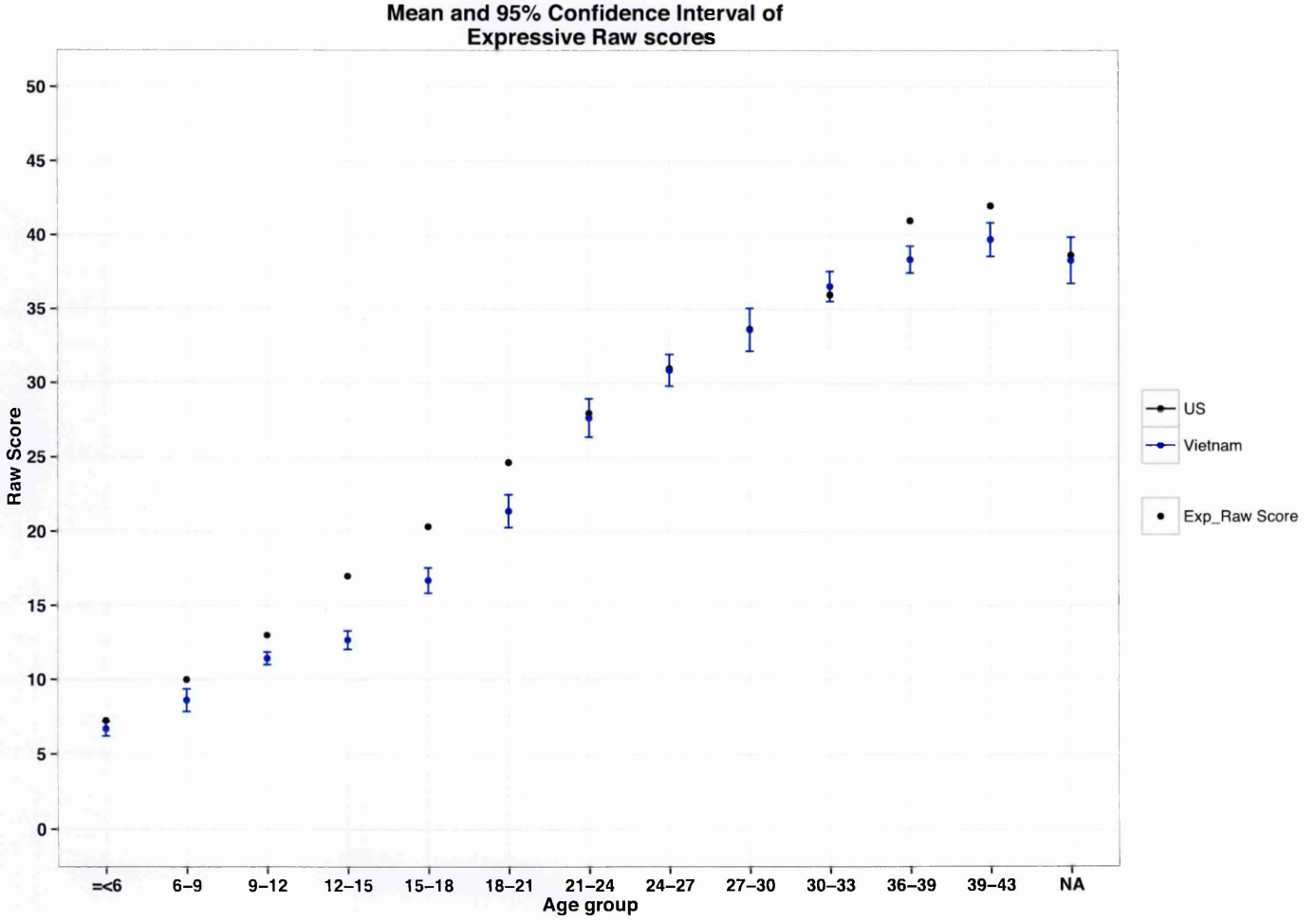


Figure 3.21 Fine Motor - US and Vietnam raw scores

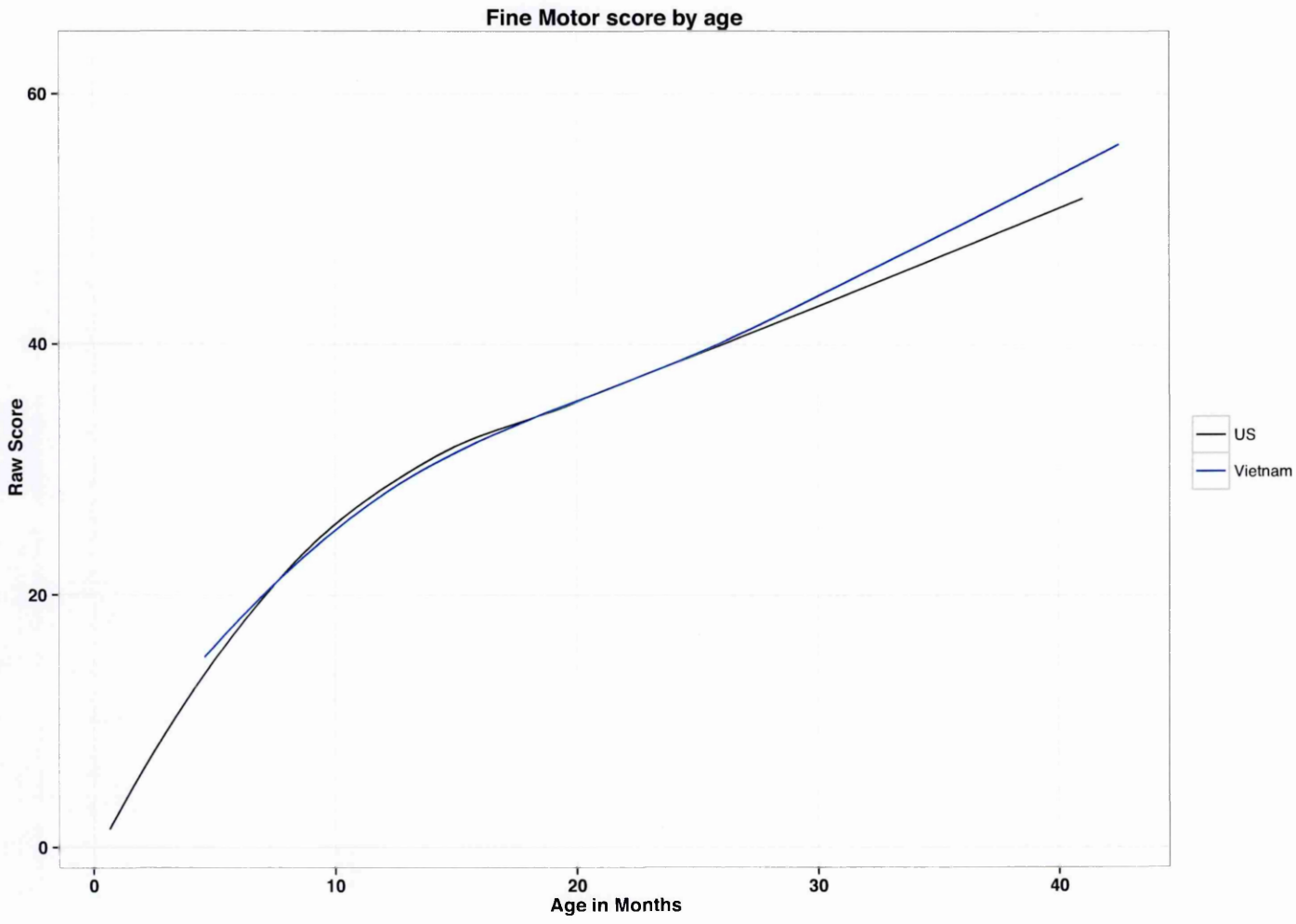


Figure 3.22 Fine Motor - US and Vietnam mean raw scores and 95% Confidence Interval

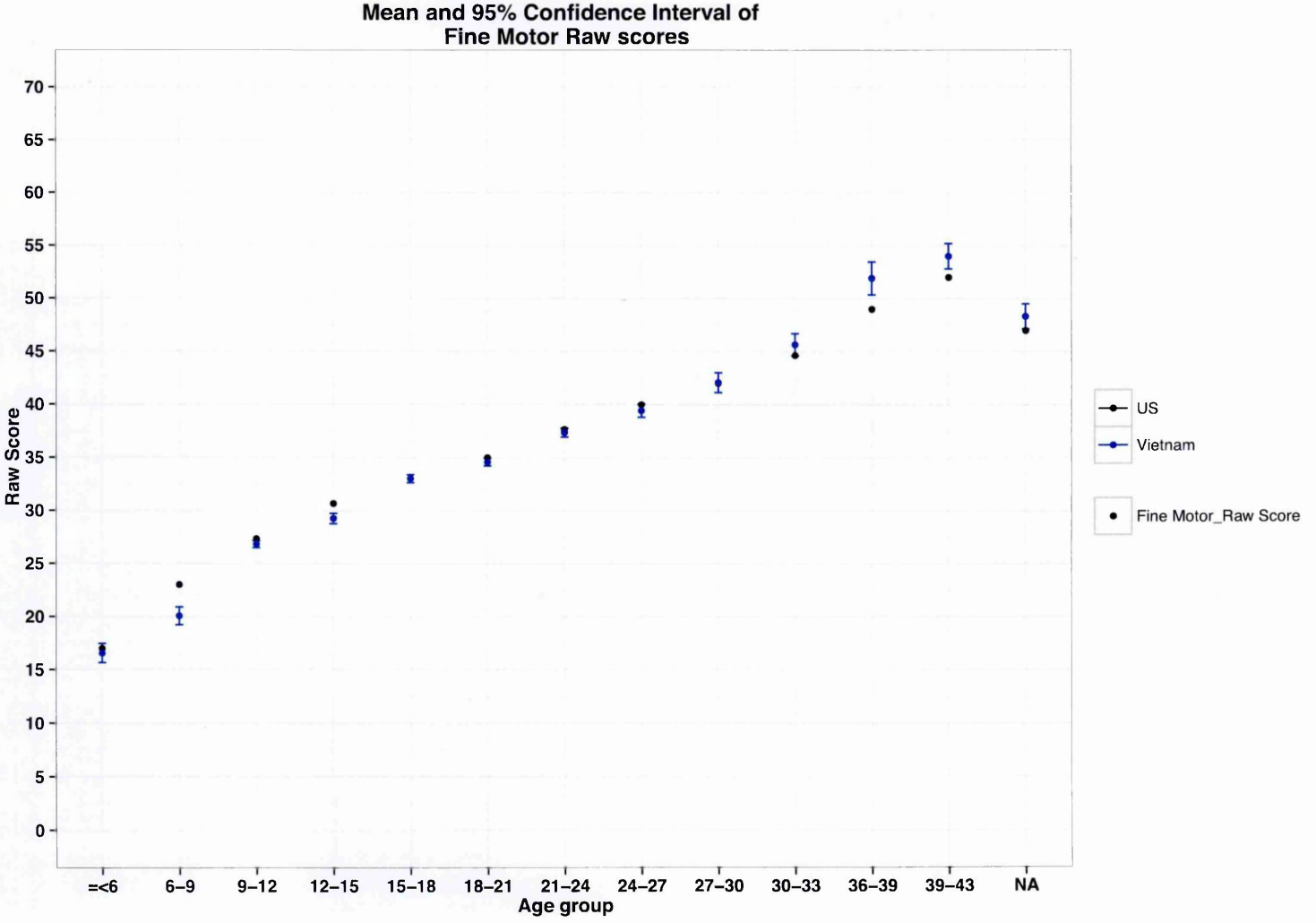


Figure 3.23 Gross Motor - US and Vietnam raw scores

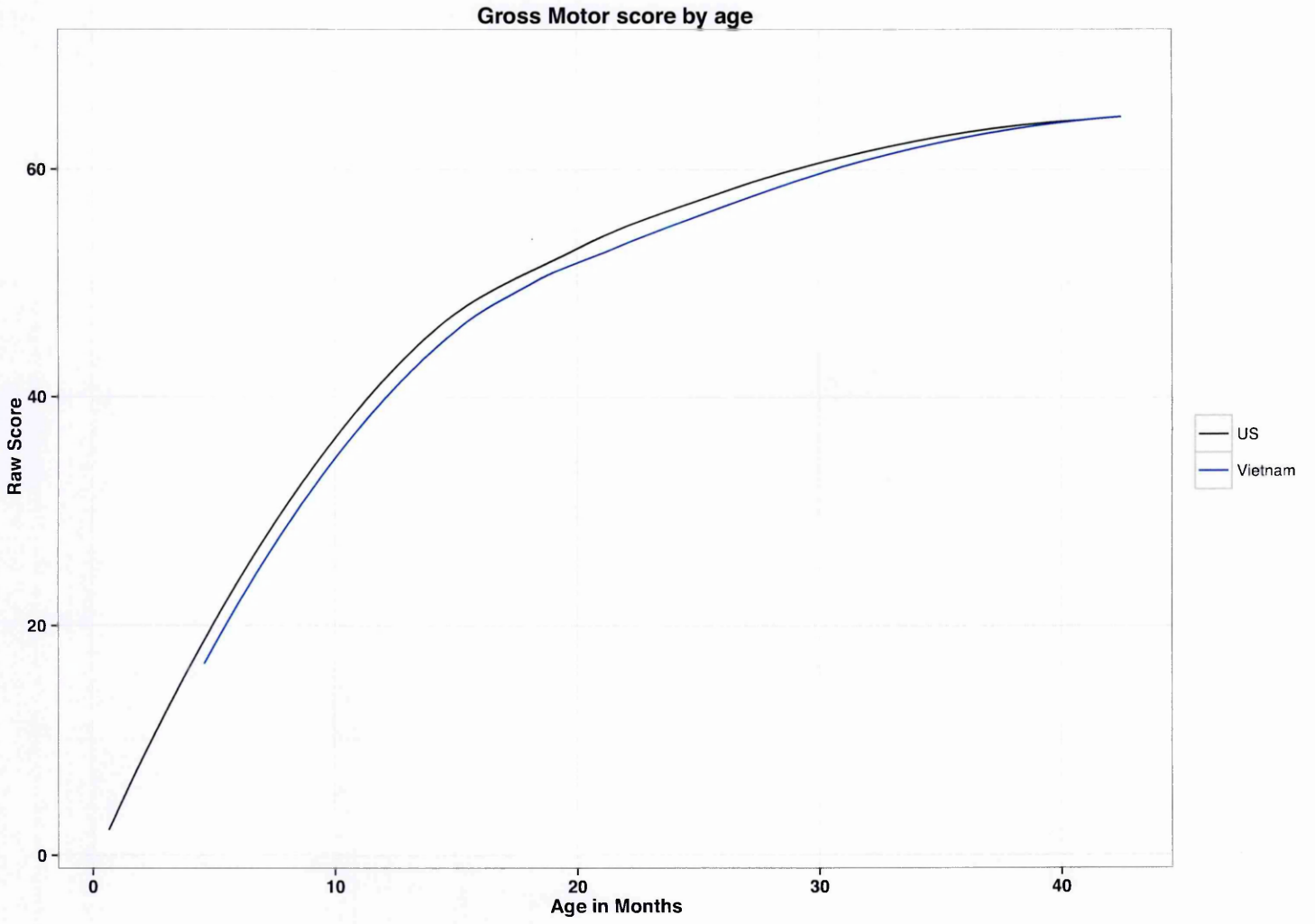
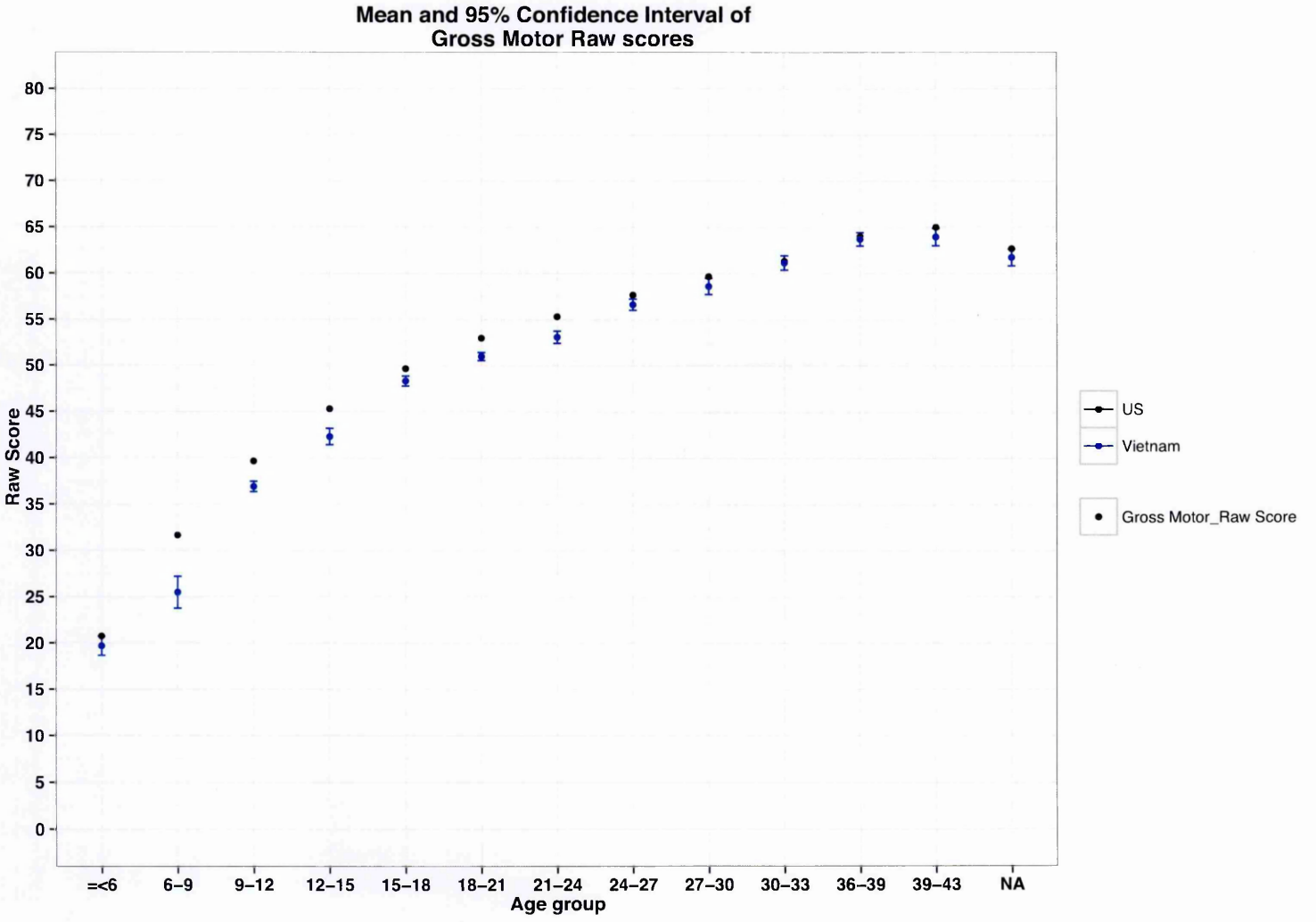


Figure 3.24 Gross Motor - US and Vietnam mean raw scores and 95% Confidence Interval



3.6 Summary

The results demonstrate that the Vietnamese adaptation of Bayley III is reliable and valid on the original properties that were published by the author of Bayley III. Additionally we looked at measurement invariance and found some differences in the underlying abilities measured by the Vietnamese Bayley III between the sexes. This has not been evaluated previously using Bayley III. The process of pilot study, translation and back-translation involving different teams of people from a variety of backgrounds (linguists, scientists and social scientists) has resulted in this successful adaptation.

3.7 Discussion

This chapter demonstrates psychometric methods of testing that are not usually carried out for child development tools. These methods are, however, recognised as important for patient reported outcome measures, and outcome measures in health and allied specialties such as physiotherapy measures.[386] Despite the technical difficulties, the use of free software for analysis should encourage all groups working in child development to explore the underlying structure of the outcome measure and understand psychometric validity.

Our population differs from the latest publicly available census data from 2009 and 2011, with higher levels of maternal education and assets, though comparable levels of stunting. The MICS 2011 data identified stunting prevalent in all levels of maternal education but inversely related, suggesting maternal education may be protective. [369] Stunting is a measure of inadequate linear growth in both the antenatal and postnatal period, which is compounded by infectious diseases and poor environment.[387] Several studies have found

consistent associations between stunting at 2 to 3 years and poorer long term educational and cognitive outcomes compared to children with normal growth, despite adjustment for socioeconomic confounders in LMICs.[388-390] Longitudinal studies also suggest poorer social interaction at school age and attention difficulties.[391, 392] The relationship between stunting and parental education is complex, and different in different settings. Studies in Bangladesh found lower paternal education to be a stronger predictor of stunting but this was not the case in Indonesia.[393] In Indonesia, lower maternal education was a strong predictor of stunting even when adjusted for paternal education, gender, age and weekly per head household expenditure.[393] Higher maternal education may increase knowledge about health issues, increase consumption of healthy foods, change traditional attitudes towards nutrition whilst indirectly challenging gender roles.[394] Research in urban and rural sites in Hanoi, Vietnam from over 1000 children identified that only 12% growth in length was explained by variables in maternal education and household wealth, with more than 80% of variation left unexplained.[395] Our data does not demonstrate stunting as a predictor of lower scores, but the proportion of mothers who did not attend school or had only up to primary education parents was small, and the adverse consequences of stunting may have been mediated by factors associated with higher levels of maternal education.

In this study, the assessors had to be familiar with all items of the Bayley III as the healthy population was enrolled at any age. This required investment in training and monitoring during the study period. We demonstrated that adaptation and application of Bayley III in Vietnam is feasible and investment in this level of training led to good to excellent inter-observer reliability.

Test-retest reliability was not done under ideal conditions (such as same environment), yet reliability was excellent. Mean scores improved in the retest (data not shown) as expected due to some familiarity with items. Vietnam is a filial society and most children are exposed to their main caregiver and immediate family only.[396] They have limited contact with unfamiliar adults and playing with adults before school age is uncommon. The assessors found significant difficulty in getting children to explore unfamiliar toys, to be relaxed and comfortable to speak and cooperate. Since the cognitive domain is the first subtest to be administered, the assessors believed performance on this domain was less good than the performance on other domains that were administered later when the child was more familiar to the situation.

Infants did not cooperate well with items such as sustained play with an unfamiliar object, and had high levels of stranger awareness with some being unfamiliar with western games such as "peek-a-boo" with parents. Identifying parts of the body or clothing in older infants seems to be universally a skill parents teach, and children were familiar being asked about. In the older age groups, nouns and pronouns structures are more complex and depend on relationship of the child to the person or object, so these items are difficult to translate, and actually not age appropriate at the age tested in Bayley III. Despite the challenge in different structure in language and obvious limitations in translation for some items where plurals or verb endings in English are not translatable in the exact format, reliability and validity of the tool has been demonstrated as appropriate for research settings.

We used CFA to evaluate whether the adaptation had the same construct structure as the original Bayley III. Of note, the construct convergent and discriminant validity was not met and this may be due to the large overlap and high correlations between subtest scores. This is not unexpected in view of known overlap of cognitive and language domains in early childhood,[336] and the relationship between fine motor and cognitive skills.[397] Recent work suggests it is the visuospatial coordination (e.g. copying a picture) rather than visual-motor coordination (e.g. tracing over a picture) that relate to cognitive function.[398] Within Bayley, children with fine motor delay may be disadvantaged in scoring adequately on cognitive items requiring fine motor control, especially within timed tasks (e.g. completing a puzzle, pegs in hole). The Bayley start and end point system avoids too many similar items being grouped together, so a child with fine motor difficulties will not be given fine motor related activities consecutively. However, from a clinical perspective it is useful to note when examining children, to differentiate which fine motor aspects a child finds challenging, in order to target intervention.

Multivariate analysis identified that female gender had improved cognitive, receptive and expressive language skills compared to males. In view of gender measurement invariance, this can be interpreted as a true difference in the latent ability being measured by the test. Additionally, we looked at gender MI and found invariance is held irrespective of gender, i.e. the latent construct or factor is measured in the same way between girls and boys but there is a gender difference in latent means. This is in agreement with the multivariate regression analysis on raw scores. Gender differences in these domains are well documented in the literature in early childhood (as reviewed by Ardila et al. (2011)),[399-401] with inconclusive evidence whether early gender differences are

sustained or diminish with age.[401] In view of these findings, gender is an important covariate to adjust for in population or group differences analysis.

Plots of raw scores and means for Vietnam and US show there are discrepancies, where the US mean values do not consistently lie within the Vietnamese 95% CI per age group. This suggests the US scaled scores may not be appropriate for research or clinical use. This was also the case in a Malawian standardization study.[402]

The aim of developing an adapted internationally recognised child development tool was realised, supported by good psychometric properties and a team of assessors who could reliably and consistently assess children. The Bayley III-VN demonstrates the closest raw score pattern to the US from what can be accessed publically and by creating local Z scores from the healthy population, we have useful data for colleagues working in the field assessing children in LMICs.

4. Outcomes following severe HFMD

This chapter presents the Bayley III and neurological examination scores for HFMD cases admitted to HTD over 6 months. These scores were compared to a healthy cohort of children over the same time period. Two methods; linear regression and propensity scoring, were used to evaluate differences in scores between the two groups in order to determine six-month outcomes following severe HFMD in Vietnam.

4.1 Recruitment

Recruitment from HTD and the community started June 2013 and was completed by December 2014. Two hundred and forty two HFMD cases and 291 healthy children were enrolled in the study. Prior to the first neurodevelopmental assessment, 21(9%) HFMD cases withdrew, 13/21 (61%) of these were grade 2a cases. A further 20/242 (9%) withdrew prior to the second assessment at 6 months. Overall, 221/242 (91%) of enrolled HFMD cases attended the first assessment and 201/242 (83%) attended the second assessment. (Figure 4.1 illustrates the enrolled HFMD and the withdrawals.)

The healthy comparison group had 2/291 (<1%) withdrawals prior to the first assessment and 43/291 (15%) before the second. Overall 289/291 (99%) of enrolled healthy group attended the first neurodevelopmental assessment and 248/291 (84%) of enrolled healthy group attended the second assessment.

4.2 Clinical features of HFMD

The HFMD grade on admission was recorded in the CRF. However, clinical features were only determined prospectively from enrolment. This was done to avoid retrospective bias of interpreting documentation from clinical notes. The discharge grade of HFMD, (the most severe grade reached during admission) was used for descriptive analysis (Table 4.1, 4.2). There were no significant differences in age distribution, gender and proportions being cared at home, kindergarten or day care between severity grades. Most of the children in all grades were being looked after at home. There were significant differences in time to enrolment with later enrolment times for more severe children. Longer duration of hospitalization and smaller proportions of children from HCMC were among the more severe disease grades. 3/7 (43%) of grade 4 were from HCMC and proportions of HCMC cases from district 8 were 42/191 (22%). HTD is a regional referral hospital and expected to receive more severe cases from outside HCMC.

Table 4.1 Demographic of HFMD cases (includes those who withdrew prior to first assessment)

	DISCHARGE GRADES OF HFMD					
	Total	**grade 2a**	**grade 2b**	**grade 3**	**grade 4**	**Comparison Estimate (95%CI); p-value**
N	242	147	58	30	7	
Demographics						
Gender (male)	142 (59%)	84 (57%)	36 (62%)	19 (63%)	3 (43%)	0.7078$^{\Psi}$
Age at enrolment (months, median, IQR)	16.2 (10.0)	15.1 (8.7)	17.1 (10.2)	19.7 (16.1)	18.7 (17.2)	KW = 4.22, df = 3, p-value = 0.24
HCMC origin	191 (79%)	122 (83%)	42 (72%)	24 (80%)	3 (43%)	0.043$^{\Psi}$ *
District 8	42 (22%)	29 (24%)	6 (14%)	7 (29%)	0	
Illness day on admission (days) (median, IQR)	1 (1)	1 (1)	1 (1)	1.5 (1)	1 (1.5)	KW = 4.91, df = 3, p-value = 0.18
Illness day on	3 (2)	2 (2)	4 (2)	5 (2)	5 (5.5)	KW = 107.89, df

	DISCHARGE GRADES OF HFMD					
	Total	grade 2a	grade 2b	grade 3	grade 4	Comparison Estimate (95%CI); p-value
enrolment (days) (median, IQR)						= 3, p-value < 2.2e-167**
Duration of hospital stay (days) (median, IQR)	5 (2)	5 (2.5)	6 (2)	6 (2)	7 (9.5)	KW = 18.90 df = 3, p-value = 0.00029**
Attends daycare	35 (14%)	22 (15%)	11 (19%)	2 (7%)	0	
Attends Kindergarten	34 (14%)	21 (14%)	7 (12%)	4 (13%)	2 (29%)	0.62$^{\Psi}$
Looked after at home	173 (71%)	104 (71%)	40 (69%)	24 (80%)	5 (71%)	

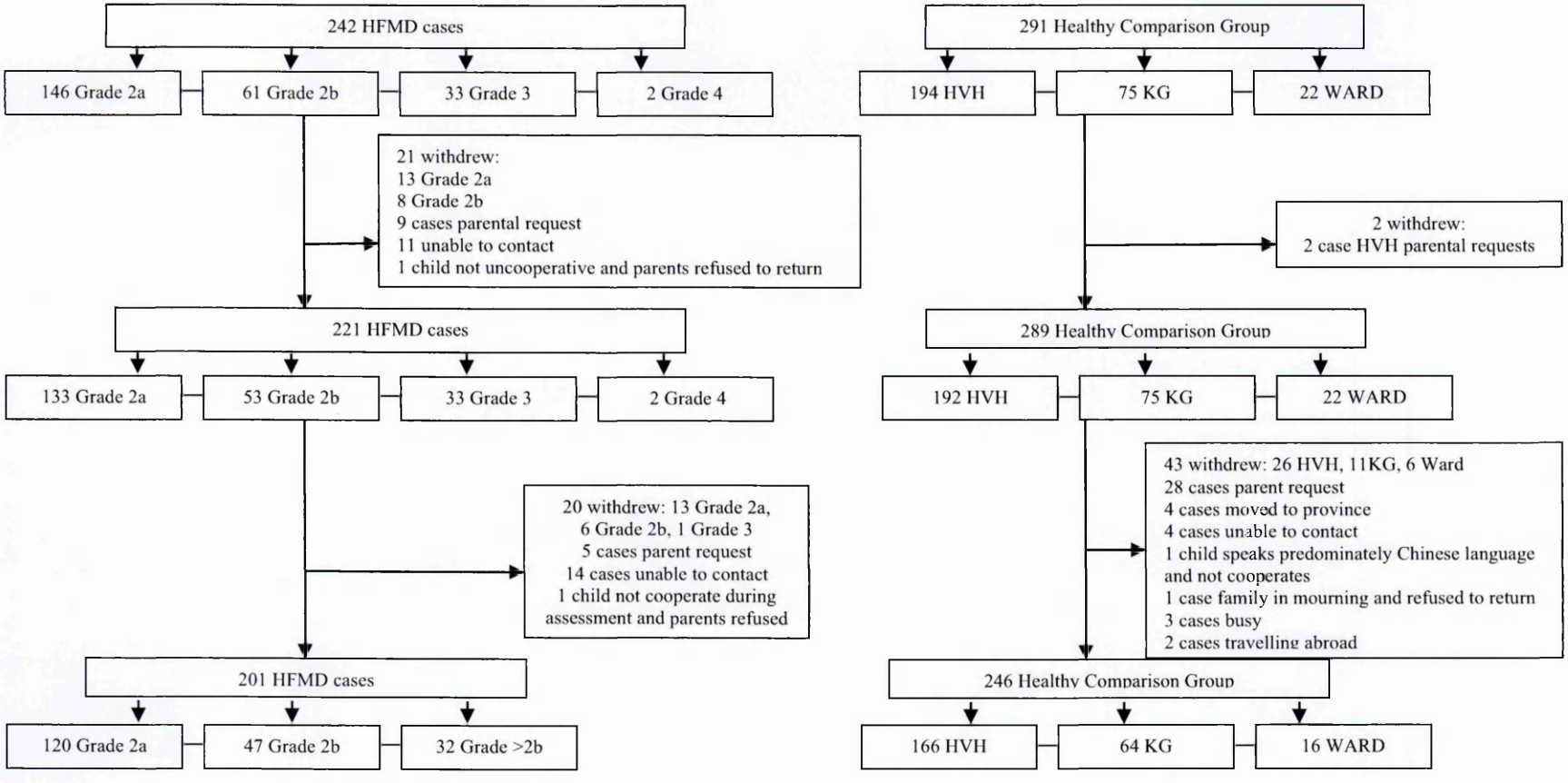
Ψ Fisher's exact test, KW: Kruskal-Wallis chi-squared, *p-value<0.05

There were 171/242 (71%) HFMD cases with grade 2a enrolled, and of these 16 (9%) progressed to grade 2b and 19 (11%) to grade 3 or 4. Of the 55/242 (23%) HFMD cases enrolled with grade 2b, 13 (24%) progressed to grade 3 or 4. No cases were admitted at grade 4. (Table 4.2).

Table 4.2 Admission, Enrolment and Discharge HFMD Grades

	DISCHARGE GRADES OF HFMD				
	Total	grade 2a	grade 2b	grade 3	grade 4
Number of cases	242	147	58	30	7
grade on admission					
grade 1	7	4 (3%)	0	3 (10%)	0
grade 2a	171	136 (93%)	16 (28%)	14 (47%)	5 (71%)
grade 2b	55	1 (1%)	41 (71%)	12 (40%)	1 (14%)
grade 3	2	0	0	1 (3%)	1 (14%)
grade 4	0	0	0	0	0
Not known	7	6 (4%)	1 (2%)	0	0
grade on Enrolment					
grade 1	0	0	0	0	0
grade 2a	146	146 (99%)	0	0	0
grade 2b	61	1 (1%)	58	2 (7%)	0
grade 3	33	0	0	28 (93%)	5 (71%)
grade 4	2	0	0	0	2 (29%)

Figure 4.1 Recruitment of enrolled HFMD grades



4.2.1 Clinical characteristics of HFMD cohort at Enrolment

The median day of enrolment was 3 days after admission (Table 4.1). The clinician following Vietnamese guidelines recorded grading of severity. The CRF did not request specification of why the grading was chosen and recorded the initial classification from the notes. Since clinical features were only determined prospectively from enrolment, some clinical features may have resolved from admission but the grading does not change from the most severe grading during the illness episode.

There were significant differences between severity grades in the reporting of fever, irritability, myoclonus and tremor at enrolment. Children with grade 2a had higher proportions of fever on enrolment whereas the children with grade >2b had higher proportions of irritability, myoclonus and tremor. (Table 4.3) There was a significant difference in proportions of children with a rash, with higher numbers of children with less severe grades presenting with a rash at enrolment, but children with more severe grades presenting with a vesicular rather than a macular rash at enrolment. Additionally, there were significantly higher proportions of children with less severe grades presenting with mouth ulcers at enrolment. (Table 4.4)

All cases had normal Blantyre coma scale score on enrolment, except one child with grade 4 disease. Another child with grade 2b had cranial nerve palsy (not specified and only on admission day) and another child with grade 2b presented with tremor. One grade 4 child had left 6^{th} and 7^{th} lower motor neuron cranial nerve palsy and right lower limb weakness. One child enrolled at grade 4 had a persistent tremor (Table 4.5). As expected, children with more severe grades had more interventions and the two children with grade 4 required ventilation (Table 4.6). All children were recorded as fully recovered by discharge except two

children with grade 4 disease. These two children who did not fully recover at discharge were a 35 month old girl from Dak Nong who had persistent cranial nerve palsy and a 26 month old boy from Binh Dinh who had tremor at discharge. The girl was enterovirus A71 (EV-A71) positive on RT-PCR and the boy had a negative RT-PCR result. EV-A71 was associated with more severe grades and coxsackievirus A6 (CVA6) with less severe grades.

The difference in time between admission and enrolment meant some features that had resulted in the initially classification of HFMD may have resolved by enrolment. However, the child's HFMD grade does not alter despite no longer continuing to fit the criteria, e.g. resolution of myoclonus by enrolment.

Table 4.3 Clinical characteristics of HFMD cohort at enrolment

	DISCHARGE GRADES OF HFMD												
	grade 2a			grade 2b			grade 3			grade 4			
Clinical signs and symptoms	Yes	No	Not known	Yes	No	Not known	Yes	No	Not known	Yes	No	Not known	Fisher's exact test, p-value
Fever	104 (71%)	43 (29%)	0	28 (48%)	30 (52%)	0	11 (37%)	19 (63%)	0	2 (29%)	5 (71%)	0	0.00011**
Headache	0	124	23	0	44	14	0	19	0	0	3	4	NS
Rash	78 (53%)	69 (47%)	0	34 (59%)	24 (42%)	0	17 (57%)	13 (43%)	0	2 (29%)	5 (71%)	0	0.50
Cough	17 (12%)	129 (88%)	1	6 (10%)	52 (90%)	0	2 (7%)	28 (93%)	0	2 (33%)	4 (67%)	1	0.39
Runny nose	15 (10%)	131 (90%)	1	4 (7%)	53 (93%)	1	1 (3%)	29 (97%)	0	2 (33%)	4 (67%)	1	0.13
Vomiting	12 (8%)	134 (92%)	1	0	58%)	0	2 (7%)	28 (93%)	0	1 (14%)	6 (86%)	0	0.21
Diarrhoea	8 (5%)	138 (95%)	1	1 (2%)	57 (98%)	0	1 (3%)	29 (97%)	0	0	6	1	0.74
Drowsiness	0	139	9	0	57	1	0	24	4	0	5	2	NS
Irritability	21 (15%)	122 (85%)	4	2 (3%)	56 (97%)	0	1 (3%)	29 (97%)	0	1 (17%)	5 (83%)	1	0.049*
Myoclonus	6 (4%)	140 (96%)	1	8 (14%)	50 (86%)	0	4 (13%)	26 (87%)	0	1 (14%)	6 (86%)	0	0.031*
Lethargy	0	143	4	0	58	0	4 (13%)	26 (87%)	0	0	7	0	0.00032**
Tremor	0	147	0	2 (3%)	56 (97%)	0	2 (7%)	28 (93%)	0	0	6	1	0.027*
Conjunctivitis	0	146	1	0	58	0	1 (3%)	29 (97%)	0	0	6	1	0.15
Sweating	1 (1%)	145 (99%)	1	0	58	0	0	30	0	0	7	0	NS

Table 4.4 Description and distribution of rash and mouth ulcers

	DISCHARGE GRADES OF HFMD												
	grade 2a			grade 2b			grade 3			grade 4			
Rash	**Yes**	**No**	**Not known**	**Yes**	**No**	**Not known**	**Yes**	**No**	**Not known**	**Yes**	**No**	**Not known**	**Fisher's exact test, p-value**
	78 (53%)	69 (47%)	0	34 (59%)	24 (42%)	0	17 (57%)	13 (43%)	0	2 (29%)	5 (71%)	0	0.50*
Type of rash													
	Mac	**Ves**	**Both**	**Mac**	**Ves**	**Both**	**Mac**	**Ves**	**Both**	**Mac**	**Ves**	**Both**	
	39 (57%)	2 (3%)	28 (41%)	18 (58%)	6 (19%)	7 (23%)	13 (68%)	3 (16%)	2 (11%)	2 (67%)	0	0	0.012* macular 0.51 vesicular 0.022* both 0.050
Mouth Ulcer's													
	Yes	**No**	**Not known**	**Yes**	**No**	**Not known**	**Yes**	**No**	**Not known**	**Yes**	**No**	**Not known**	
	140 (95%)	7 (5%)	0	49 (84%)	9 (16%)	0	17 (59%)	12 (41%)	1	2 (29%)	5 (71%)	0	1.83e-09**

Mac: macular
Ves: Vesicular
Both: Both types of macular and vesicular rash

Table 4.5 Examination at enrolment

	DISCHARGE GRADES OF HFMD												Fisher's exact test, p-value
Blantyre score	**grade 2a**			**grade 2b**			**grade 3**			**grade 4**			
	5	**3-5**	**missing**	**5**	**3-5**	**missing**	**5**	**3-5**	**missing**	**5**	**3-5**	**missing**	
	146 (99%)	1 (1%) *(03-510)*	0	58	0	0	26	0	4	3 (75%)	1(25%) *(03-001)*	3	5.286e-08**
	Yes	**No**	**Not known**	**Yes**	**No**	**Not known**	**Yes**	**No**	**Not known**	**Yes**	**No**	**Not known**	
Cranial Nerve Palsy	0	147	0	1 (2%) (left, CN not specified) *(03-619)*	57 (98%)	0	0	25	5	1 (left) *(03-001)* CN6,7 lower motor neuron)	6	0	5.781e-06**
Limb Weakness	0	147	0	1 (2%) (left lower limb) *(03-077)*	57 (98%)	0	0	25	5	1 (25%) (right lower limb) *(03-001)*	3 (75%)	3	3.97e-09**
Nystagmus	0	147	0	0	58	0	0	26	0	0	4	3	2.901e-07**
Tremor	0	147	0	1 (2%)	57 (98%)	0	0	26	4	0	4	3	3.391e-07**
Ataxia	0	144	0	0	58	0	0	26	4	0	4	3	3.151e-07**

Table 4.6 Management, outcome at discharge and virology

	DISCHARGE GRADES OF HFMD				
	grade 2a	grade 2b	grade 3	grade 4	Fisher's exact test p-value
Total	147	58	30	7	
Nasal Cannula	0	0	22	5	<2.2e-16**
Immunoglobulin	0	5	30	7	<2.2e-16**
Milrinone	0	0	8	1	2.334e-08**
Magnesium	0	0	5	1	1.587e-05**
Phenobarbital IV	1	58	30	7	< 2.2e-16**
Antibiotics	0	0	2	0	0.03676*
Intubation	0	0	0	2	0.00072**
NCPAP	0	0	0	2	0.00072**
Midazolam	0	0	0	2	0.00072**
Abnormal neurology at enrolment (myoclonus, nystagmus, tremor, ataxia, limb weakness, cranial nerve palsies)	1	3	0	1	0.046*

DISCHARGE GRADES OF HFMD					
	grade 2a	grade 2b	grade 3	grade 4	Fisher's exact test p-value
Outcome at discharge					
Full recovery	147	58	30	5 (71%)	0.00072**
PCR					
EVA-71 (n=45)	13 (9%)	10 (17%)	18 (62%)	4 (57%)	1.328e-09**
CVA16 (n=7)	6 (4%)	1 (2%)	0	0	0.69
CVA10 (n=27)	16 (11%)	10 (17%)	0	1 (14%)	0.059
CVA6 (n=44)	32 (22%)	12 (21%)	0	0	0.0076**
EV (n=55)	38 (26%)	12 (21%)	5 (17%)	0	0.38
Untyped & Others$^{\varphi}$ (n=64)	42 (29%)	13 (22%)	7 (23%)	2 (29%)	0.82

*p-value<0.05

**p-value<0.01

$^{\varphi}$CVA4 n=6, CVA8 n=4, CVA12 n=9, CVA2 n=7, CVB1 n=2, CVB4 n=1, CVB5 n=1, E11 n=2, E14 n=1, E30 n=1, E9 n=1, negative RT-CR n=52, PV2 n=1, RhiA n=2.

grade 2a and one at grade 2b. Two progressed to grade 3 and 5 progressed to grade 4. 3/7(43%) had EV-A71 positive RT-PCR, 1/7 (14%) was coxsackievirus A10 (CVA10) positive and the 3/7 (43%) remainder had a negative rRT-PCR result. (Table 4.7)

Table 4.7 Clinical details from the seven children that progressed in disease whilst enrolled in the study

Case	Age at enrolment (months)	Sex	Admission grade	Enrolment grade	Discharge grade	Day illness at admission	Day illness at enrolment	rRT-PCR	Outcomes at discharge	Hospital stay (days)
1	18.4	F	2a	2b	3	1	3	EV-A71	Recover	6
2	19.4	F	2b	2b	3	0	2	Neg	Recover	9
3	26.9	F	2a	3	4	1	3	EV-A71	Recover	6
4	18.7	F	2a	3	4	0	4	CV-A10	Recover	6
5	10.6	M	2a	3	4	2	10	Neg	Recover	12
6	26.9	M	2a	3	4	1	5	Neg	Incomplete (tremor)	7
7	8.8	F	2a	3	4	0	4	EV-A71	Recover	6

4.3 Neurology assessed with the Amiel-Tison tool

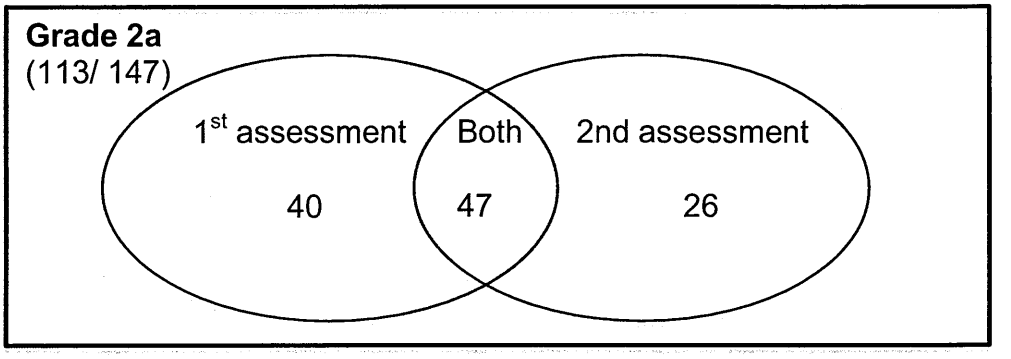
87/147 (59%) children at grade 2a and 34/58 (59%) grade 2b, 34/37 (92%) grade >2b had an Amiel-Tison examination a week after discharge. 73/147 (50%) children at grade 2a, 25/58 (43%) grade 2b, 23/37 (62%) grade >2b had neurology examination at 6 months follow-up. 47/147 (32%) children at grade 2a, 17/58 (29%) grade 2b, 17/37 (46%) grade >2b had both the first examination one week post discharge and the second at 6 months. (Figure 4.2).

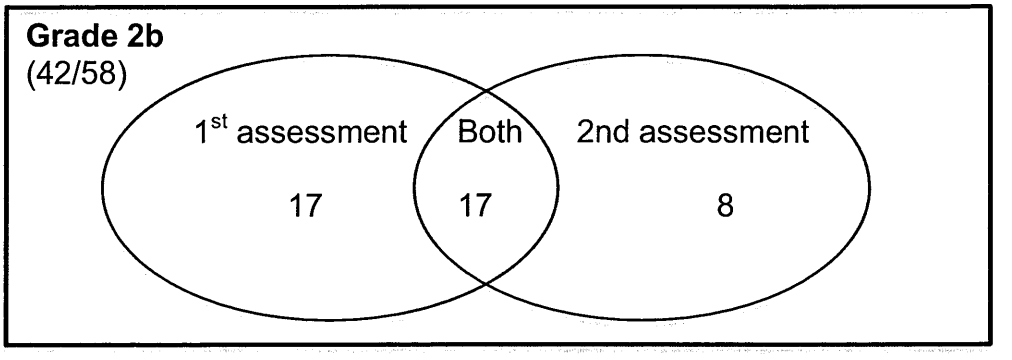
The modified scoring of Amiel-Tison (Table 2.2-2.4) classified 21/160 assessments (13%) of grade 2a children with an abnormal examination in at least one section resulting in 4/113 (4%) children classified with mild abnormality and 3/113 (3%) with moderate abnormalities. 13/58 (22%) assessments of grade 2b children had an abnormality in at least one section resulting in 1/58 (2%) child classified as mild abnormality. 7/37 (19%) assessments of >grade 2b children had an abnormal examination in at least one section resulting in 1/37 (3%) classified with moderate abnormality. The classification is different depending on age groups. All 17 children assessed age 3-9 months had a normal examination. 7/136 (5%) of 10-24 month olds had an abnormal classification. 4/79 (5%) of 24-48 month old children had an abnormal classification (Tables 4.8-4.10). There were 24 children who had the first assessment at 10-24 months and the second at 24-48 months and there were 11 children who had the first assessment at 3-9 months and the second at 10-24 months. There was only one child (code 01) who had persistent abnormal classifications between two assessments (Table 4.10). Child code 01 had grade 4 HFMD and had a persistent lower motor 7th cranial nerve palsy, development of restrictive jaw movements and reported clumsiness when walking and was unable to run. Using the modified criteria, she had

restricted passive movement and abnormal motor activity but could walk, so was classified as moderate abnormality. Three children had mild abnormalities at the first assessment but were classified as normal 6 months later. Two children had moderate abnormalities at the first assessment but were classified as normal 6 months later, and one child (code 586) was classified as normal at the first assessment but had moderate abnormality at the second assessment (Table 4.11). The children who were classified as normal 6 months later may have had difficulty assessing all sections as noted in child 607 who was not co-operative (Table 4.9).

In summary there was no significant difference in prevalence of abnormality between Grades HFMD (Table 4.12).

Figure 4.2 HFMD Discharge Grade cases with Amiel-Tison per visit





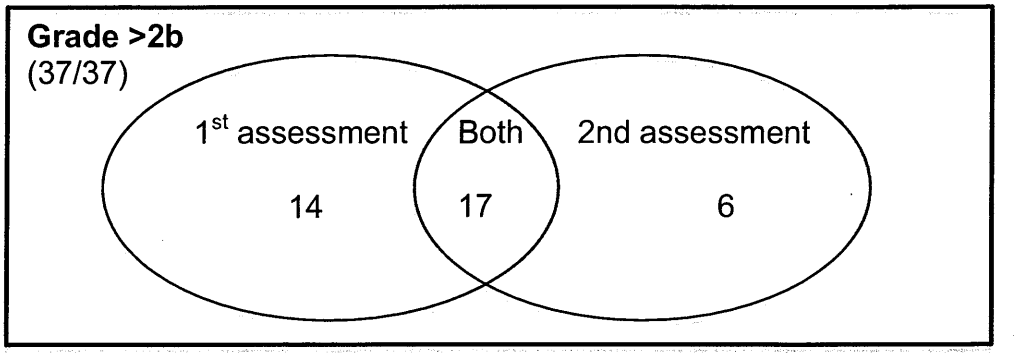


Table 4.8 Amiel-Tison examination abnormality and modified classification age 3-9 months (17 children assessed) with demographic, virological and clinical data.

Code	Grade HFMD	Age (months)	Gender	PCR	Outcome at discharge from CRF
45	2b	7.4	Male	CV-A6	Recover
556	2a	6.8	Female	Neg	Recover
626	2b	8.0	Male	EV	Recover

Code	Grade HFMD	Assessment	Passive Muscle Tone	Comparison of left and right side	Body axis	Diffuse rigidity	Motor activity	Deep tendon reflexes	Primitive reflexes	Gross motor function	Two assessments	Amiel-Tison Classification
45	2b	1st	0	0	0	0	0	1	0	0	No	Normal
556	2a	2nd	0	0	0	0	1	0	0	0	Yes	Normal
626	2b	1st	0	0	0	0	0	1	0	0	Yes	Normal

1= score 1, 2= score 2, N= not examined as child not cooperative. Age is at enrolment. CRF; case record form

Table 4.9 Amiel-Tison examination abnormality and modified classification age 10-24 months (164 assessments on 134 children) with demographic, virological and clinical data.

Code	Grade HFMD	Age (months)	Gender	PCR	Outcome at discharge from CRF
11	2b	13	Male	EV	Recover
56	>2b	10.6	Male	Neg	Recover
77	2b	23.1	Male	EV	Recover
518	2a	9.8	Male	Neg	Recover
583	2a	10.2	Male	Neg	Recover
584	2a	16.9	Female	CV-A6	Recover
597	2b	21.3	Female	Neg	Recover
607	2b	15.2	Male	CV-A10	Recover
611	2a	10.5	Female	Neg	Recover
22	>2b	13.4	Male	EV-A71	Recover
508	2a	21.2	Male	Neg	Recover
511	2a	14.4	Female	Neg	Recover
514	2a	13.7	Female	EV	Recover
555	2a	11.1	Female	Neg	Recover
616	2a	17.6	Male	Neg	Recover
25	2b	9.0	Female	EV-A71	Recover
31	2b	22.4	Male	CV-A6	Recover
49	2b	17.5	Male	CV-A10	Recover
52	>2b	19.4	Female	Neg	Recover
585	2a	11.1	Male	EV	Recover
588	2a	11.5	Male	Neg	Recover
594	2a	10.1	Male	CV-A16	Recover
660	2a	13.5	Female	CV-A10	Recover
521	2a	14.8	Male	Neg	Recover
586	2a	12.4	Male	Neg	Recover
512	2a	10.0	Male	EV	Recover
78	>2b	9.0	Female	EV	Recover
517	2a	11.5	Male	CV-A10	Recover

Code	Grade HFMD	Assessment	Passive Muscle Tone	Comparison of left and right side	Body axis	Diffuse Rigidity	Motor activity	Deep tendon reflexes	Primitive reflexes	Postural reactions	Gross motor function	Two assessments	Amiel-Tison Classification
11	2b	1st	1	0	0	0	0	0	0	0	0	Y	Normal
56	>2b	1st	1	0	0	0	0	0	0	0	0	N	Normal
77	2b	1st	1	0	0	0	0	N	0	0	0	N	Normal
518	2a	2nd	1	0	0	0	1	0	0	0	0	N	Mild
583	2a	2nd	1	0	0	N	0	N	0	N	0	Y	Normal
584	2a	2nd	1	0	0	N	0	0	0	0	0	Y	Normal
597	2b	1st	1	0	0	0	0	0	0	0	0	Y	Normal
607	2b	1st	0	0	0	0	0	0	0	1	1	Y	Mild
607	2b	2nd	1	N	N	N	0	N	0	N	1	Y	Normal
611	2a	1st	1	0	0	0	0	0	0	0	0	N	Normal
22	>2b	2nd	0	0	0	0	1	0	0	0	0	Y	Normal
508	2a	1st	0	0	0	0	1	0	0	0	0	Y	Normal
511	2a	1st	0	0	0	0	1	0	0	0	0	Y	Normal
514	2a	2nd	0	0	N	0	1	0	0	N	0	N	Normal
555	2a	1st	0	0	0	0	1	0	0	1	0	N	Mild
616	2a	1st	0	0	0	0	1	0	0	0	0	Y	Normal
25	2b	1st	N	0	0	N	0	1	0	0	0	Y	Normal
31	2b	1st	0	0	N	N	0	1	0	N	0	Y	Normal
49	2b	1st	0	0	0	0	0	1	0	0	0	Y	Normal
52	>2b	1st	0	0	0	0	0	1	0	0	0	N	Normal
585	2a	2nd	0	0	0	0	0	1	0	0	0	Y	Normal
588	2a	1st	0	0	0	0	0	1	0	0	0	Y	Normal
594	2a	2nd	0	0	0	0	0	1	0	0	0	Y	Normal
660	2a	1st	0	0	0	0	0	1	0	0	0	N	Normal
521	2a	1st	2	0	0	0	0	N	0	1	0	Y	Moderate
586	2a	2nd	0	0	0	0	0	0	0	0	2	Y	Moderate
512	2a	1st	0	0	0	0	0	0	0	1	1	Y	Mild
78	>2b	1st	0	0	0	0	0	0	0	1	0	N	Normal
517	2a	1st	0	0	0	0	0	N	0	1	0	Y	Normal

1= score 1, 2= score 2, N= not examined as child not cooperative. Age is at enrolment. CRF; case record form

Table 4.10 Amiel-Tison examination abnormality and modified classification age 24-48 months (92 assessments on 76 children) with demographic, virological and clinical data.

Code	Grade HFMD	Age (months)	Gender	PCR	Outcome at discharge from CRF
1	>2b	35.4	Female	EV-A71	Incomplete
7	2b	29.0	Male	EV	Recover
523	2a	39.6	Male	EV	Recover
550	2a	28.8	Male	Neg	Recover
591	2a	26.2	Male	CV-A6	Recover
666	2b	28.1	Male	EV -A71	Recover
43	>2b	22.5	Male	Neg	Recover
3	2b	28.2	Female	EV-A71	Recover
20	2b	39.6	Male	EV-A71	Recover
53	>2b	29.5	Male	EV	Recover

Code	Grade HFMD	Assessments	Passive Muscle Tone	Comparison of left and right side	Body axis	Diffuse Rigidity	Motor activity	Deep tendon reflexes	Primitive reflexes	Postural reactions	Gross motor function	Two assessments	Amiel-Tison Classification
1	>2b	1st	1	R	N	0	2	0	N	1	0	Y	Moderate
1	>2b	2nd	1	R	0	0	2	0	0	0	0	Y	Moderate
7	2b	1st	1	0	0	0	0	0	0	0	0	Y	Normal
523	2a	1st	1	0	0	0	0	2	0	0	0	Y	Moderate
550	2a	1st	1	0	0	0	0	1	0	0	0	Y	Mild
591	2a	1st	1	0	0	0	0	0	0	0	0	N	Normal
666	2b	1st	1	0	0	0	0	0	0	0	0	N	Normal
43	>2b	2nd	0	0	0	0	1	0	0	0	0	Y	Normal
3	2b	2nd	0	0	0	0	0	1	0	0	0	N	Normal
20	2b	1st	0	0	0	0	0	1	0	0	0	Y	Normal
53	>2b	1st	0	0	0	0	0	1	0	0	0	N	Normal

1= score 1, 2= score 2, N= not examined as child not cooperative. Age is at enrolment. CRF; case record form.

Table 4.11 Summary of abnormalities classified by modified Amiel-Tison scoring system with demographic, virological and clinical data.

Code	Grade HFMD	Age (months)	Gender	PCR	Outcome at discharge from CRF	1st Assessment Amiel-Tison Classification	2nd Assessment Amiel-Tison Classification
518	2a	9.8	Male	Neg	Recover	Not done	Mild
607	2b	15.2	Male	CV-A10	Recover	Mild	Normal
555	2a	11.1	Female	Neg	Recover	Mild	Not done
521	2a	14.8	Male	Neg	Recover	Moderate	Normal
586	2a	12.4	Male	Neg	Recover	Normal	Moderate
512	2a	10.0	Male	EV	Recover	Mild	Normal
1	>2b	35.4	Female	EV-A71	Incomplete	Moderate	Moderate
523	2a	39.6	Male	EV	Recover	Moderate	Normal
550	2a	28.8	Male	Neg	Recover	Mild	Normal

CRF; case record form

Table 4.12 Number of children with abnormal classification according to modified Amiel-Tison scoring system

Classification	Grade 2a	Grade 2b	Grade >2b	Fisher's exact test p-value
Normal	106	57	36	0.53
Mild	4	1	0	0.57
Moderate	3	0	1	0.50
Total	113	58	37	

Note: Fisher's exact test were done for comparisons between grade 2a, grade 2b and grade >2b (combine grade 3 and 4)

4.4 Distribution of covariates for Bayley III score analysis

4.4.1 HFMD cohort

The numbers of children enrolled at grade 3 and 4 were small and for analysis were combined to form group >2b. Despite the loss to follow-up, there was no significant difference in age distribution, stunting, gender or maternal education between total HFMD cases at enrolment, 1st assessment and 2nd assessment. (Table 4.13).

At enrolment, there were no significant differences in age between HFMD grades (Kruskal-Wallis chi-squared = 4.22, df = 3, p-value = 0.24), gender (p-value = 0.71), stunting (p-value = 0.21) and maternal education levels (p-value = 0.93). There was no significant difference in proportions of severity of HFMD at each assessment (X-squared = 0.69, df = 6, p-value = 0.99).

4.4.2 Healthy cohort and grade 2a HFMD

The age range from the healthy comparison group was limited to same range as the HFMD cases (4.5 months to 42 months) hence 23 healthy cases were excluded. Table 4.14 shows the distribution of covariates for the included healthy comparison group.

There were no significant differences in age between the children enrolled at grade 2a HFMD and the healthy comparison group (Kruskal-Wallis chi-squared = 0.65, df = 1, p-value = 0.42), gender (p-value=0.7476) and stunting (p-value = 0.8528). However, there was a significant differences in the levels of maternal education (p-value < 2.2e-16), with the higher proportions of secondary and higher education in the healthy comparison group compared to grade 2a HFMD.

Table 4.13 Covariates for multivariate linear analysis and propensity matching by discharge HFMD grade

	Enrolment					1st Assessment					2nd Assessment					Comparison p-value€
Discharge grade	**Total**	**grade 2a**	**grade 2b**	**grade 3**	**grade 4**	**Total**	**grade 2a**	**grade 2b**	**grade 3**	**grade 4**	**Total**	**grade 2a**	**grade 2b**	**grade 3**	**grade 4**	
Number	242	147	58	30	7	221	134	50	30	7	201	121	44	29	7	
Age at enrolment months median (IQR)	16.2 (10.0) Range: 5-41 months	15.1 (8.7)	17.1 (10.2)	19.7 (16.1)	18.7 (17.2)	16.3 (10.3)	15.2 (9.1)	17.1 (10.2)	19.7 (16.1)	18.7 (17.2)	16.9 (10.7)	16.2 (9.7)	17.1 (10.0)	19.7 (16.8)	18.7 (17.2)	KW = 0.297, df = 2, p-value = 0.862
Gender Male	142 (59%)	84 (57%)	36 (62%)	19 (63%)	3 (43%)	133 (60%)	79 (59%)	32 (64%)	19 (63%)	3 (43%)	121 (60%)	71 (59%	29 (66%)	18 (62%)	3 (43%)	0.9301
Maternal Education: No school /Primary	86 (36%)	50 (34%)	23 (40%)	10 (33%)	3 (43%)	80 (36%)	48 (36%)	19 (38%)	10 (33%)	3 (43%)	72 (36%)	41 (34%)	18 (41%)	10 (34%)	3 (43%)	X-squared = 0.393, df = 4, p-value = 0.983.
Secondary	130 (54%)	82 (56%)	28 (48%)	17 (57%)	3 (43%)	115 (52%)	71 (53%)	24 (48%)	17 (57%)	3 (43%)	104 (52%)	65 (54%)	20 (45%)	16 (55%)	3 (43%)	
Higher	26 (11%)	15 (10%)	7 (12%)	3 (10%)	1 (14%)	26 (12%)	15 (11%)	7 (14%)	3 (10%)	1 (14%)	25 (12%)	15 (12%)	6 (14%)	3 (10%)	1 (14%)	
Stunt	34θ (14%)	22 (15%)	10θ (18%)	1 (3%)	1 (14%)	32θ (15%)	21 (16%)	9θ (18%)	1 (3%)	1 (14%)	26 (13%)	18 (15%)	6 (14%)	1 (3%)	1 (14%)	0.8917
Missing stunt	1	0	1	0	0	1	0	1	0	0	0	0	0	0	0	

p-value€ Difference in covariates between enrolment, 1st assessment and 2nd assessment tested by Fisher's exact test unless otherwise stated
KW: Kruskal-Wallis chi square
θMissing stunt data for one child enrolled grade 2b who completed the 1st assessment only (03-619)

Table 4.14 Healthy cohort covariates

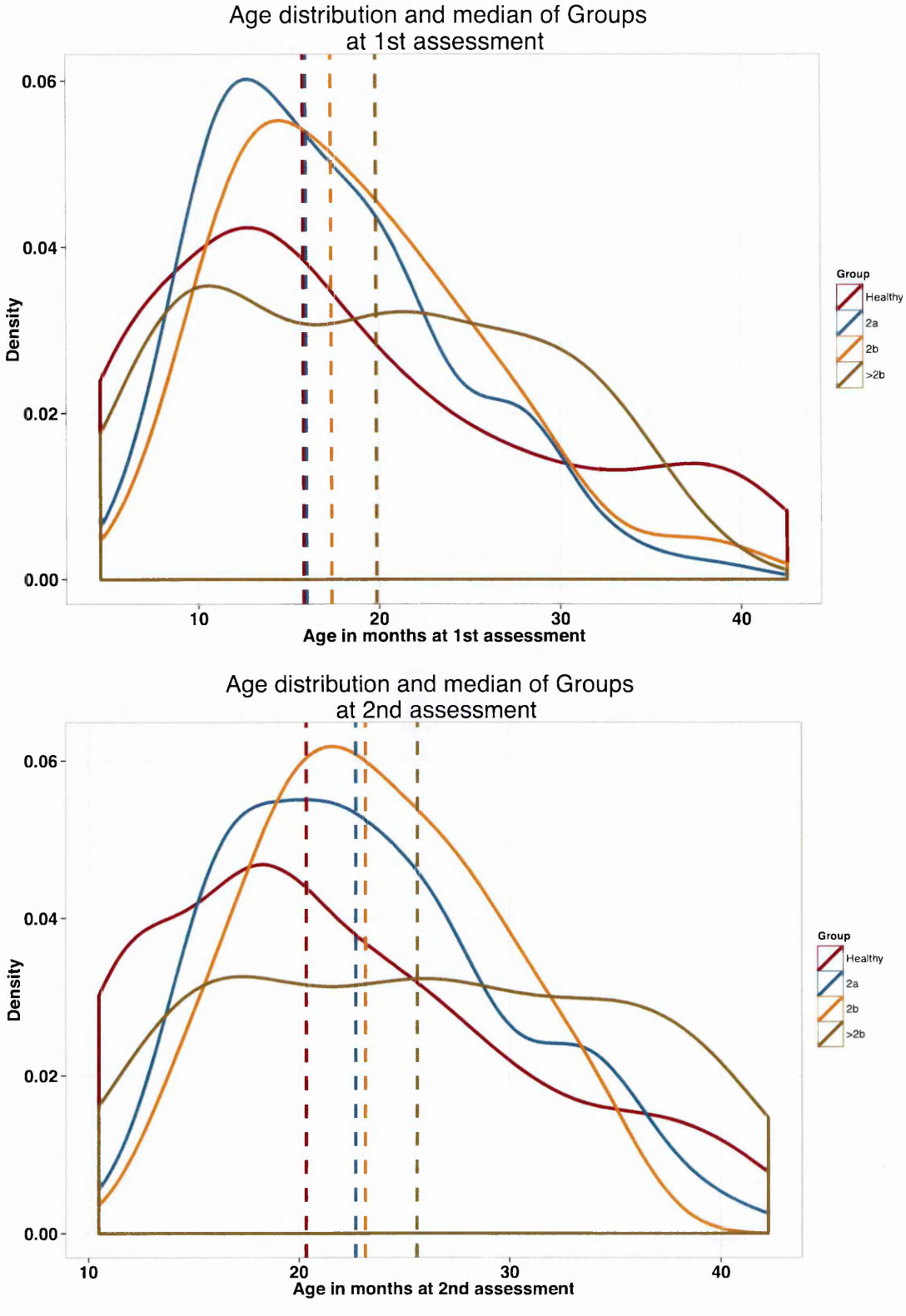
	Enrolment					1st Assessment				2nd Aassessment			
Discharge grade	**Total**	**Limit age <42 months**	**HVH**	**KG**	**Ward**	**Total**	**HVH**	**KG**	**Ward**	**Total**	**HVH**	**KG**	**Ward**
Number	291	268	192	54	22	267	191	54	22	228	165	47	16
Age at enrolment in months median (IQR)	17.1 (17.8)	16.0 (14.5)	13.1 (8.7)	35.5 (9.2)	17.7 (4.4)	16 (14.5)	13.1 (8.8)	35.5 (9.2)	17.7 (4.4)	15.9 (14.5)	12.5 (10.1)	35.5 (9.2)	18.7 (3.5)
Gender Male	158 (54%)	147 (55%)	105 (55%)	33 (61%)	9 (41%)	147 (55%0	105 (55%)	33 (61%)	9 (41%)	123 (54%)	86 (52%)	30 (64%)	7 (44%)
Maternal Education: No school/Primary	43 (15%)	43 (16%)	34 (18%)	2 (4%)	7 (32%)	43 (16%)	34 (18%)	2 (4%)	7 (32%)	33 (14%)	28 (17%)	2 (4%)	3 (19%)
Secondary	138 (48%)	126 (47%)	90 (47%)	22 (42%)	14 (64%)	126 (47%)	90 (47%)	22 (42%)	14 (64%)	107 (47%)	76 (46%)	19 (40%)	12 (75%)
Higher	109 (38%)	98 (37%)	68 (35%)	29 (55%)	1 (5%)	97 (36%)	67 (35%)	29 (55%)	1 (5%)	88 (39%)	61 (37%)	26 (55%)	1 (6%)
Missing Mom Edu	1 (03-901)	1 (03-901)	0	1	0	1	0	1	0	0	0	0	0
Stunt	46 (16%)	46 (17%)	33 (17%)	5 (9%)	8 (36%)	45 (17%)	32 (17%)	5 (9%)	8 (36%)	33 (15%)	25 (15%)	4 (9%)	4 (25%)
Missing stunt	2	1	0	1 (03-713)	0	1	0	1	0	1	0	1	0

4.4.3 Age distribution at first assessment and second assessment

The validation of Bayley in Chapter 3 identified the large influence of age on scores. To adjust for this, Z scores were created by splitting the healthy cohort into 13 age-groups to calculate mean and standard deviation (SD) of raw scores, and from these calculate Z scores of the HFMD children. Further linear regression adjustment was done using the healthy comparison group data. The following plots show the different groups' means and medians of age distribution. (Figure 4.3)

Using the Z scores, further age adjustment by linear regression was carried out. This was to adjust for the fact that there were no significant difference in age distribution between groups (Healthy, grade 2a, grade 2b, and grade >2b) at the first assessment (Kruskal-Wallis chi-squared = 2.1616, df = 3, p-value = 0.5396) but there was a significant difference in age distribution between groups at the second (Kruskal-Wallis chi-squared = 9.6513, df = 3, p-value = 0.02178).

Figure 4.3 Age distributions density plots with medians at assessment by group



4.5 Bayley III scores by domain

4.5.1 Missing Bayley III scores

In total, 201 children with HFMD remained in the study at 6 months follow up. However, despite attending appointments, some children did not cooperate or had incomplete assessments and did not agree to return again to complete assessments. At 6 months, 119/146 (82%) children with grade 2a, 45/61 (74%) grade 2b, 34/37 (92%) >grade 2b (grade 3 and 4) had returned and completed assessments. Two children in grade 4 were ventilated during their admission. The more severe child was in hospital for 42 days. She is from an ethnic minority group that doesn't use Vietnamese as their first language so the language domains could not be assessed. She was too weak to cooperate with the first assessment after discharge. After 6 months she completed the cognitive, fine and gross motor domains only.

Tables 4.15-4.18 evaluate differences in covariates between the children who had an assessment and those who did not for each grade of HFMD and for the healthy cohort.

Table 4.15 Missing Bayley III scores at first and second assessment grade 2a

KW: Kruskal Wallis chi square

grade 2a	1st Assessment			2nd Assessment		
Covariates	**No score**	**Have scores**	**Comparison, p-value**	**No score**	**Have scores**	**Comparison p-value**
Number	1	132		14	119	
Age at enrolment (median, IQR)	16.3	15.15 (9.22)	KW = 0.014, df = 1, p-value 0.91	13.1 (4.74)	16.2 (9.45)	KW = 1.42, df = 1, p-value 0.23
Gender Male	1	78 (59%)		9 (64%)	70 (59%)	OR 1.28, CI: 0.42 Inf, p-value = 0.46
Maternal education: No School/Primary	1	47 (36%)		8 (57%)	40 (34%)	
Secondary	0	70 (53%)		6 (43%)	64 (54%)	p-value = 0.18
Higher	0	15 (11%)		0	15 (13%)	
Stunt: Stunt	0	21 (16%)		3 (21%)	18 (15%)	OR 1.5, CI 0.33 Inf, p-value = 0.39
Not stunted	1	111 (84%)		11 (79%)	101 (85%)	
PCR EV71	0	12 (9%)		2 (14%)	10 (8%)	
CVA10	0	14 (11%)		2 (14%)	12 (10%)	
CVA 16	0	5 (4%)		0	5 (4%)	
CVA 6	0	26 (20%)		2 (14%)	24 (20%)	p-value = 0.84
EV	0	37 (28%)		5 (36%)	32 (27%)	
Negative/ Untypable/others	1	38 (29%)		3 (21%)	36 (30%)	

Table 4.16 Missing Bayley III scores at first and second assessment grade 2b

grade 2b	1st Assessment			2nd Assessment		
Covariates	**No score**	**Have scores**	**p-value**	**No score**	**Have scores**	**p-value**
Number	0	53		8	45	
Age at enrolment (median, IQR)		17.4 (9.9)		23.4 (19.1)	16.9 (8.5)	KW = 2.2937, df = 1, p-value = 0.1299
Gender Male		32 (60%)		5 (62%)	27 (60%)	OR 1.1, CI 0.2391093 Inf, p-value = 0.6085
Maternal education: No School/Primary		20 (38%)		2 (25%)	18 (40%)	p-value = 0.7659
Secondary		26 (49%)		5 (62%)	21 (47%)	
Higher		7 (13%)		1 (12%)	6 (13%)	
Stunt: Stunt		9 (17%)		3 (38%)	6 (14%)	p-value = 0.2616
Not stunted		43 (83%)		5 (62%)	38 (86%)	
Missing stunt		1		0	1	
PCR EV71		10 (19%)		2 (25%)	8 (18%)	p-value = 0.7142
CVA10		8 (15%)		1 (12%)	7 (16%)	
CVA 16		1 (2%)		0	1 (2%)	
CVA 6		13 (25%)		4 (50%)	9 (20%)	
EV		11 (21%)		0	0	
Negative/ Untypable/others		10 (19%)		1 (12%)	9 (20%)	

Table 4.17 Missing Bayley III scores at first and second assessment grade >2b

grade >2b	1st Assessment			2nd Assessment		
Covariates	**No score**	**Have scores**	**p-value**	**No score**	**Have scores[ω]**	**p-value**
Number	1	34		0	34	
Age at enrolment (median, IQR)	35.4	19.7 (16.9)	KW = 2.8337, df = 1, p-value = 0.0923		20.0 (17.6)	
Gender Male	0	22 (65%)		0	21 (62%)	
Maternal education: No School/Primary	1	11 (32%)		0	12 (35%)	
Secondary	0	19 (56%)		0	18 (53%)	
Higher	0	4 (12%)		0	4 (12%)	
Stunt: Stunt	1	1 (3%)		0	2 (6%)	
Not stunted	0	33 (97%)		0	32 (94%)	
PCR EV71	1	20 (61%)		0	20 (61%)	
CVA10	0	1 (3%)		0	1 (3%)	
CVA 16	0	0		0	0	
CVA 6	0	0		0	0	
EV	0	5 (15%)		0	5 (15%)	
Negative/ Untypable/others	0	7 (21%)		0	7 (21%)	

[ω]Lack of language domains for one child

Table 4.18 Missing Bayley III scores at first and second assessment for healthy cohort

Healthy Group	1st Assessment			2nd Assessment		
Covariates	**No score**	**Have scores**	**p-value**	**No score**	**Have scores**	**p-value**
Number	0	270		34	208	
Age at enrolment (median, IQR)		16.1 (14.5)		15.9 (8.8)	14.7 (11.4)	KW = 0.0051, df = 1, p-value = 0.94
Gender Male		149 (55%)		21(62%)	108 (52%)	OR 1.49 CI 0.75 Inf p-value = 0.19
Maternal education: No School/Primary		43 (48%)		10 (29%)	31 (15%)	p-value = 0.02*
Secondary		98 (36%)		18 (53%)	96 (46%)	
Higher		4 (12%)		6 (18%)	81 (39%)	
Stunt: Stunt		44 (16%)		11 (32%)	32 (15%)	OR 2.61 CI 1.2 Inf, p-value = 0.02**
Not stunted		224 (84%)		23 (68%)	176 (85%)	

*p-value<0.05

The data suggest missing Bayley scores were random for the HFMD group but not for the healthy group. The methods for regression analysis and propensity scores require minimal missing covariate data, which the study data satisfy. Dealing with non random missing outcomes in one subset of a dataset requires complex modelling and there is debate whether this process improves precision or bias.[403] For this reason, no imputation for missing outcomes was carried out. Since the HFMD data has missing data at random outcome scores, comparisons between grades, with grade 2a as baseline using the propensity score is useful to determine whether a difference between the more severe groups and the healthy group are biased by limited outcome scores from children from families with lower maternal education and stunted children.

4.5.2 Bayley III score descriptive analysis

Unadjusted median Z scores were lower than healthy group with increasing grade of HFMD for all domains at the first assessment. Median cognitive scores were lower than the healthy group at the second assessment for all grades. There was improvement in receptive and expressive median Z scores between assessments in grade 3 and 4. Grade 3 had improvement in fine and gross motor median Z scores between assessments, while median Z scores of grade 4 got worse. Plots of Z scores by group at each assessment time and changes in Z scores between assessments were similar for all groups. (Table 4.19 and Figure 4.4)

Table 4.19 Unadjusted median and range of Bayley III Z score by domain and time tested

Z scores	Cognitive Z score					
	1st Assessment		2nd Assessment		Difference in scores	
	Median	Range	Median	Range	Median	Range
healthy	0	-2.56 to 2.48	0.03	-2.86 to 2.14	0.041	-2.79 to 3.97
grade 2a	-0.29	-3.12 to 2.23	-0.32	-4.34 to 1.98	-0.29	-4.25 to 3.72
grade 2b	-0.12	-3.97 to 1.66	-0.13	-4.25 to 2.23	-0.29	-2.037 to 2.65
grade 3	-0.093	-3.40 to 1.58	-0.4	-8.13 to 2.23	-0.22	-4.85 to 4.13
grade 4	-0.25	-1.49 to 0.65	-1.12	-1.90 to 0.59	-0.65	-1.93 to 0.21

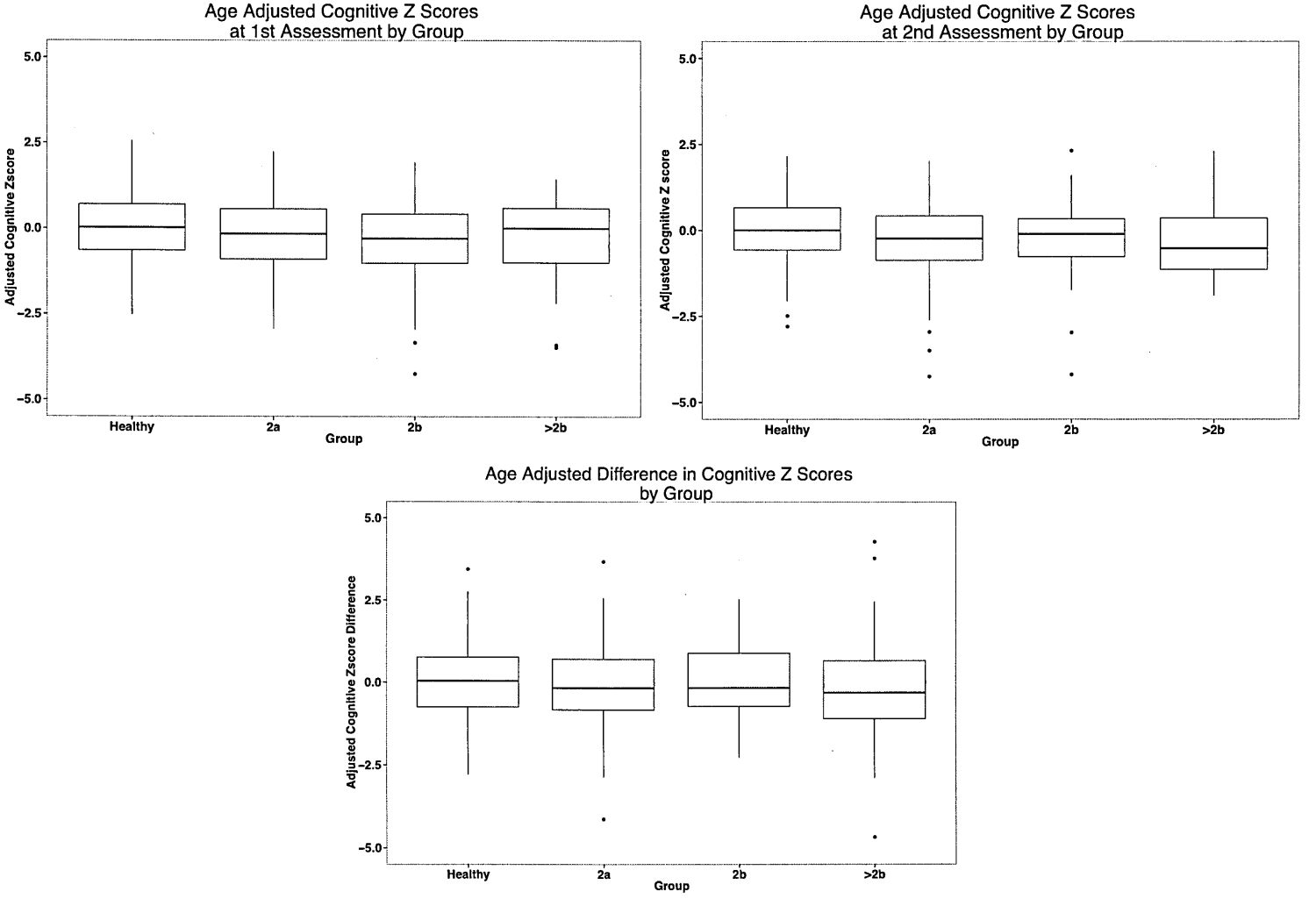
Z scores	Receptive Z score					
	1st Assessment		2nd Assessment		Difference in scores	
	Median	Range	Median	Range	Median	Range
healthy	0	-4.47 to 2.88	0.067	-2.32 to 2.73	-0.11	-2.6 to 3.4
grade 2a	-0.27	-3.49 to 3.68	-0.32	-4.46 to 2.50	-0.17	-3.45 to 3.43
grade 2b	-0.43	-4.85 to 1.55	-0.27	-3.088 to 1.63	-0.013	-2.25 to 3.07
grade 3	-0.66	-4.41 to 1.55	0.16	-5.83 to 2.88	0.34	-3.03 to 4.84
grade 4	-0.94	-2.091 to 0.95	-0.42	-2.95 to 0.13	0.077	-2.35 to 1.77

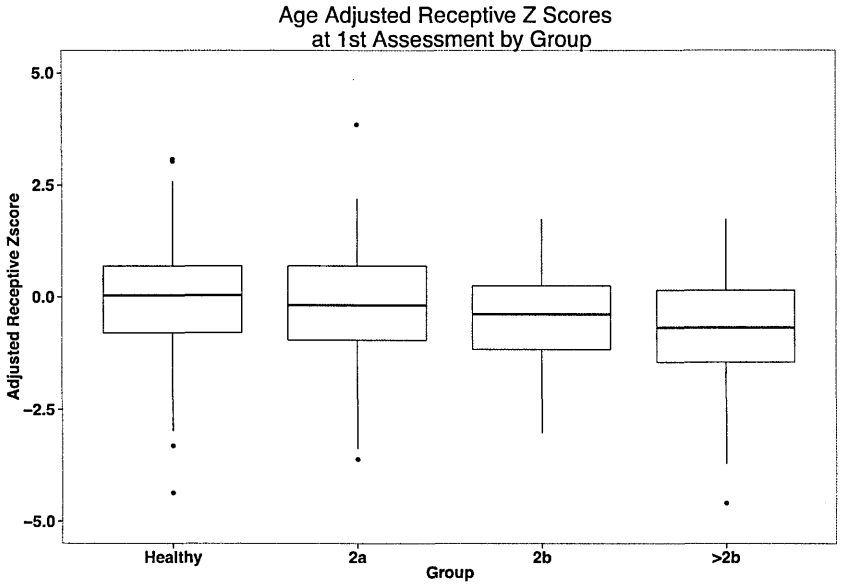
Z scores	Expressive Z score					
	1st Assessment		2nd Assessment		Difference in scores	
	Median	Range	Median	Range	Median	Range
healthy	0.049	-2.74 to 2.80	0.13	-3.048 to 3.21	0.086	-3.88 to 3.79
grade 2a	-0.28	-3.68 to 2.47	-0.21	-3.76 to 2.47	-0.013	-3.12 to 4.10
grade 2b	-0.28	-3.53 to 1.47	-0.12	-1.98 to 1.35	-0.061	-1.075 to 3.38
grade 3	-0.42	-3.53 to 1.35	0.13	-5.44 to 1.71	0.17	-1.91 to 3.47
grade 4	-1.53	-2.25 1.48	-0.54	-1.58 to 0.50	0.47	-0.98 to 1.71

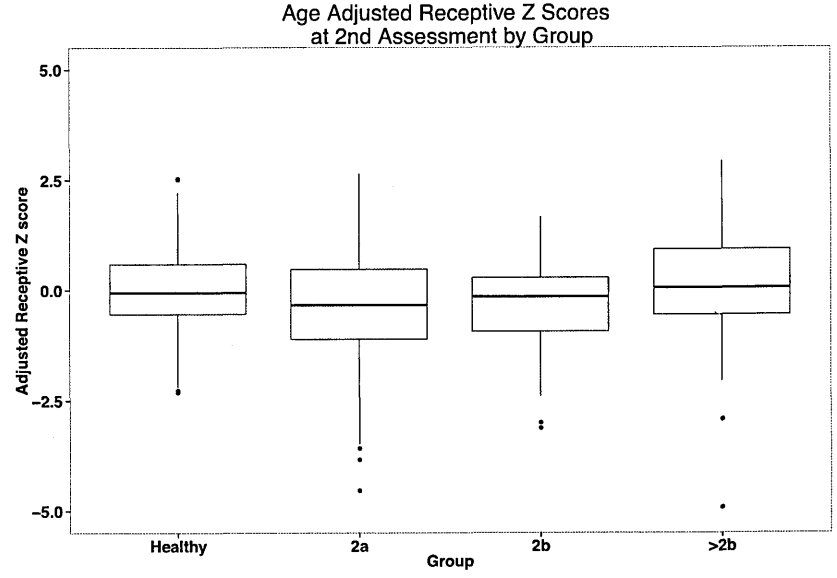
Z scores	Fine Motor Z score					
	1st Assessment		2nd Assessment		Difference in scores	
	Median	Range	Median	Range	Median	Range
healthy	-0.083	-3.09 to 2.33	0.091	-3.67 to 2.10	-0.074	-3.58 to 3.073
grade 2a	-0.35	-2.70 to 2.52	-0.17	-4.22 to 2.33	0.070	-4.58 to 3.78
grade 2b	0.091	-4.78 to 2.10	-0.34	-2.2 to 2.3	-0.059	-3.17 to 2.58
grade 3	-0.73	-3.92 to 1.92	-0.17	-4.29 to 2.09	0.64	-2.19 to 4.49
grade 4	-0.49	-0.90 to 0.67	-0.96	-1.90 to 0.71	-0.89	-1.28 to 1.289216

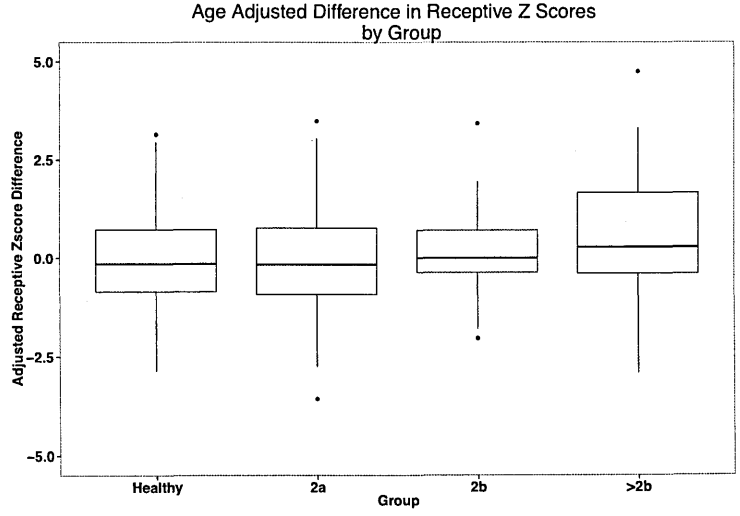
Z scores	Gross Motor Z score					
	1st Assessment		2nd Assessment		Difference in scores	
	Median	Range	Median	Range	Median	Range
healthy	0.061	-3.29 to 2.52	0.061	-2.73 to 2.48	0.0088	-3.23 to 2.68
grade 2a	-0.24	-3.58 to 1.88	-0.28	-4.89 to 1.82	-0.16	-3.78 to 2.91
grade 2b	-0.15	-8.78 to 2.11	-0.065	-2.65 to 1.55	0.133	-2.69 to 6.13
grade 3	-0.48	-3.67 to 2.52	0	-4.52 to 1.69	0.27	-2.27 to 3.51
grade 4	-0.88	-1.15 to 0.50	-0.71	-1.81 to 0.28	-0.0022	-1.02 to 0.44

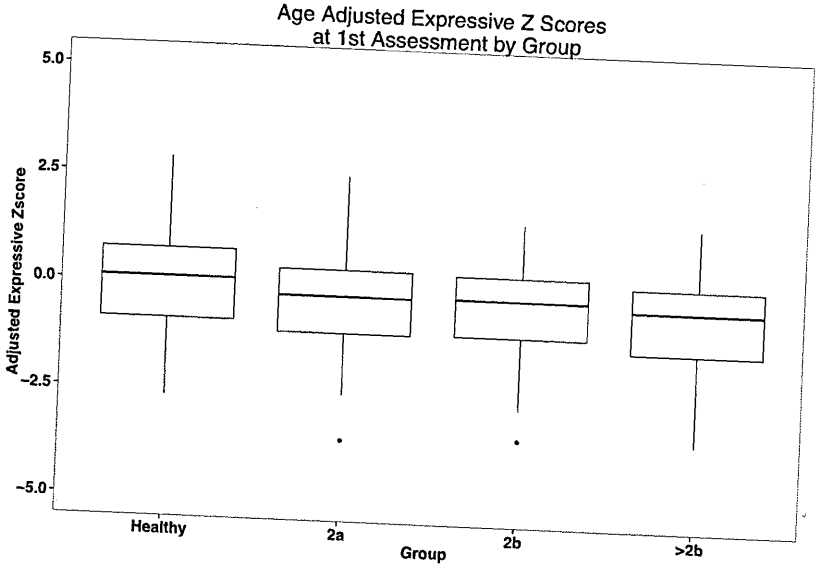
Figure 4.4 Plots of Bayley III Z scores by HFMD grade (grade>2b includes grade 3 and 4) adjusted for age, but not other covariates for each domain and assessment

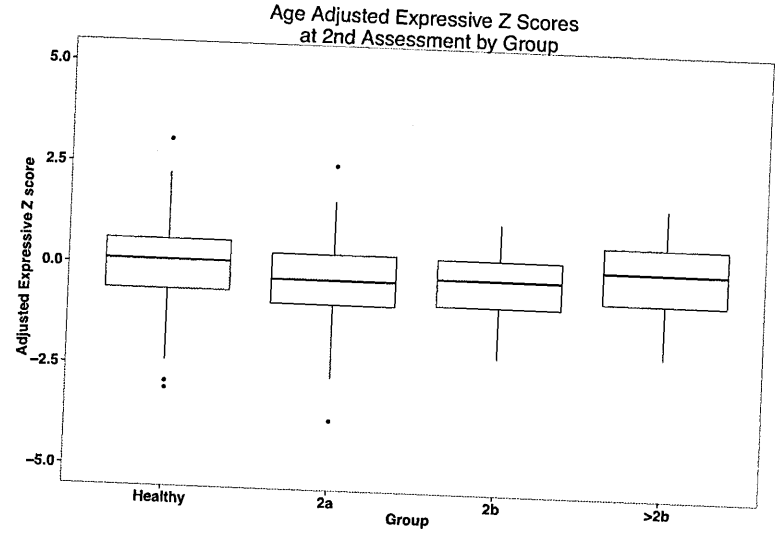


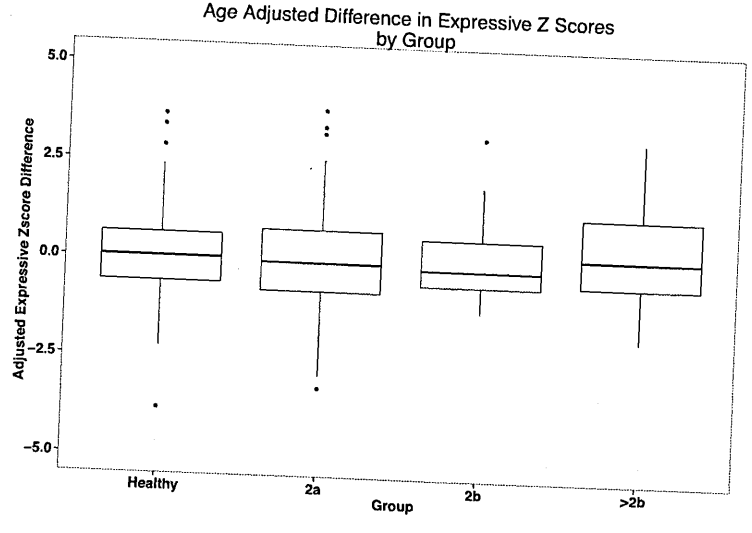

Age Adjusted Receptive Z Scores
at 1st Assessment by Group
Adjusted Receptive Zscore
5.0
2.5
0.0
−2.5
−5.0
Healthy
2a
2b
>2b
Group

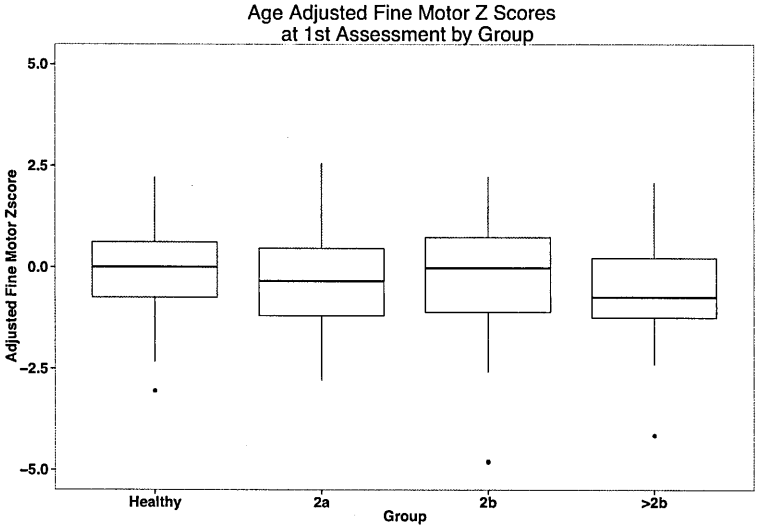

Age Adjusted Receptive Z Scores
at 2nd Assessment by Group
Adjusted Receptive Z score
5.0
2.5
0.0
−2.5
−5.0
Healthy
2a
2b
>2b
Group

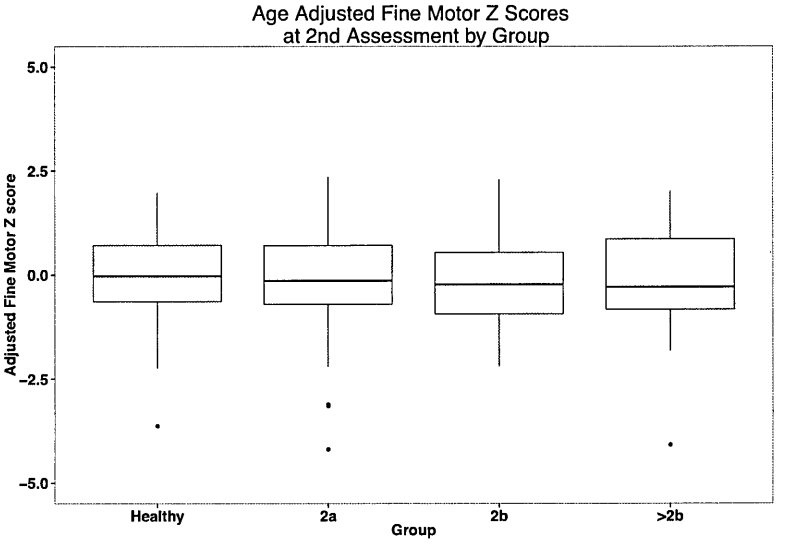

Age Adjusted Difference in Receptive Z Scores
by Group
Adjusted Receptive Zscore Difference
5.0
2.5
0.0
−2.5
−5.0
Healthy
2a
2b
>2b
Group

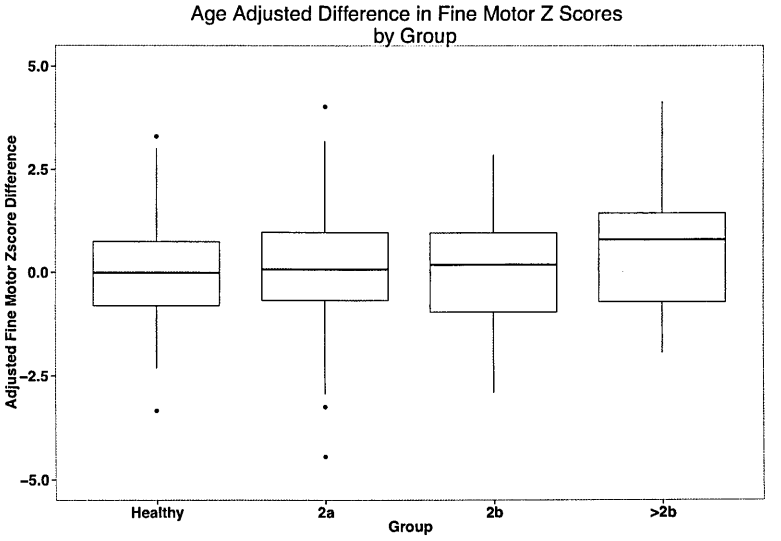

Age Adjusted Expressive Z Scores
at 1st Assessment by Group
Adjusted Expressive Zscore
5.0
2.5
0.0
−2.5
−5.0
Healthy
2a
2b
>2b
Group

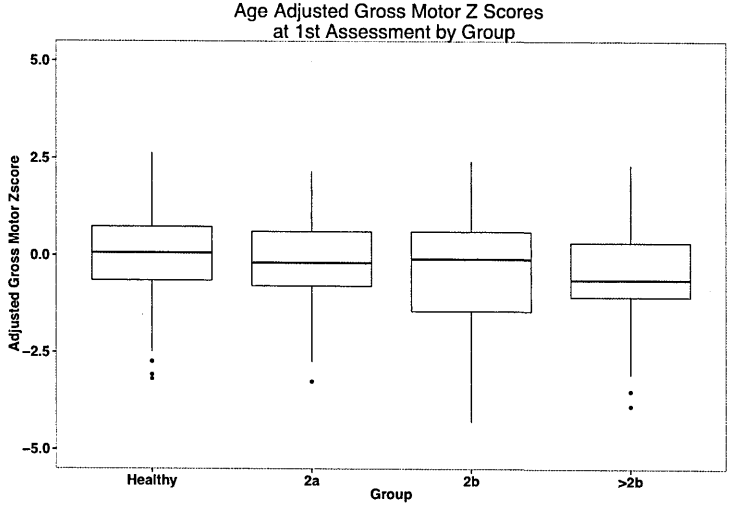

Age Adjusted Expressive Z Scores
at 2nd Assessment by Group
Adjusted Expressive Z score
5.0
2.5
0.0
−2.5
−5.0
Healthy
2a
2b
>2b
Group

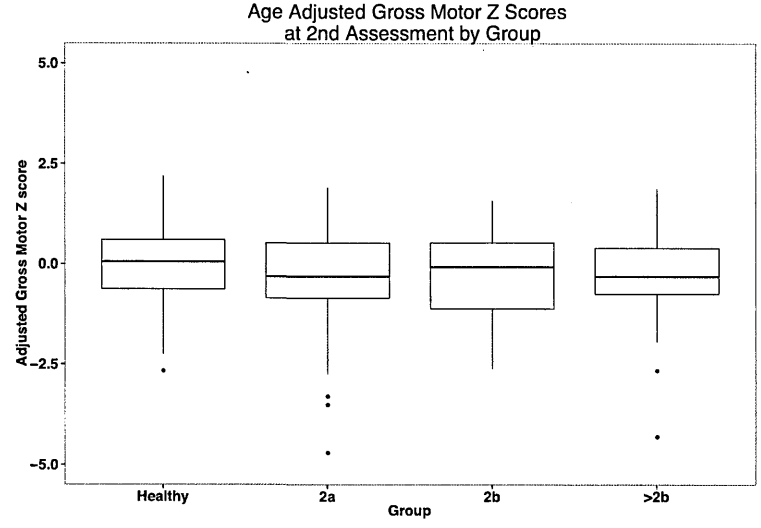

Age Adjusted Difference in Expressive Z Scores
by Group
Adjusted Expressive Zscore Difference
5.0
2.5
0.0
−2.5
−5.0
Healthy
2a
2b
>2b
Group

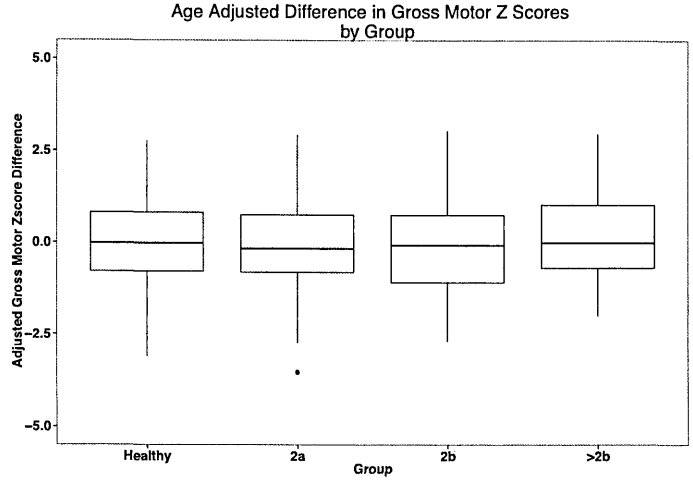

Age Adjusted Fine Motor Z Scores
at 1st Assessment by Group
Adjusted Fine Motor Zscore
5.0
2.5
0.0
−2.5
−5.0
Healthy
2a
2b
>2b
Group


Age Adjusted Fine Motor Z Scores
at 2nd Assessment by Group
Adjusted Fine Motor Z score
5.0
2.5
0.0
−2.5
−5.0
Healthy
2a
2b
>2b
Group


Age Adjusted Difference in Fine Motor Z Scores
by Group
Adjusted Fine Motor Zscore Difference
5.0
2.5
0.0
−2.5
−5.0
Healthy
2a
2b
>2b
Group


Age Adjusted Gross Motor Z Scores
at 1st Assessment by Group
Adjusted Gross Motor Zscore
5.0
2.5
0.0
−2.5
−5.0
Healthy
2a
2b
>2b
Group


Age Adjusted Gross Motor Z Scores
at 2nd Assessment by Group
Adjusted Gross Motor Z score
5.0
2.5
0.0
−2.5
−5.0
Healthy
2a
2b
>2b
Group


Age Adjusted Difference in Gross Motor Z Scores
by Group
Adjusted Gross Motor Zscore Difference
5.0
2.5
0.0
−2.5
−5.0
Healthy
2a
2b
>2b
Group

4.5.3 Linear Regression

Univariate and multiple linear regression were used to adjust the Z scores for age, gender, maternal education and stunting. (Table 4.24) A reduced cognitive Z score at the second assessment was associated with grade 2a and >2b on univariate linear regression alone. A reduced expressive Z score associated with grade 2b and >2b persisted after adjustment for the first assessment but not the second assessment. Grade 2b and >2b were associated with lower fine motor Z scores at the first assessment but not the second. At the first assessment grade 2b were associated with lower gross motor Z scores, but on the second assessment lower Z scores were associated with grade 2a and >2b. There was a positive change in receptive Z scores associated with grade 2b between the two assessments. Linear regression is limited by the assumption that the covariates operate in a linear fashion.

4.5.4 Propensity score analysis

Propensity score matching is a statistical technique where each control is weighted based on the probability of having the same covariate distribution as a HFMD case, i.e. "conditional probability of receiving the treatment rather than being part of the control group given the observed covariates."[404] It is a way of replicating a randomised control experiment with regards to covariate distribution. The healthy child is weighted on a propensity score in relation to the probability of having the same covariates as a HFMD child prior to knowing outcomes. This method assumes the covariates are not dependent on the group selection i.e. HFMD or healthy.[405] For each healthy propensity weighted outcome score, the difference in means in the outcome variable between the HFMD and control gives an estimate of the mean effect of HFMD on the outcome at that propensity score

value (average treatment effect on the treated (ATT)), which results in an unbiased estimate. Using the propensity weighted data to do further covariate adjustment will reduce bias in estimates, as long as certain criteria are fulfilled.[405] Propensity weighting can be done multiple times till the best balance on covariates are achieved.[361]

I used the "Toolkit for weighting and analysis of nonequivalent groups (TWANG)"[406] for propensity score weighting between healthy and grade 2a, and between grade 2a, 2b and >2b. The weighted healthy sample was used to evaluate the effect of grade 2a HFMD cases on scores at enrolment, at 6 months and differences in scores between the two assessments. Then the grade 2a sample was used to evaluate the effect of grade 2b, and >2b HFMD on scores at enrolment and six months later. This was done to evaluate whether >grade 2a HFMD affected scores. No assumptions about outcome were made for children lost to follow-up.

As discussed in section 2.10.6, the benefits of modelling using adjusted propensity weighting over modelling using adjusted linear regression is that the covariates are better matched, reducing bias, particularly if there is poor overlap between covariates on some of the covariates.[407] In this study, more differences between groups were identified with maternal education: an important covariate from the literature of neurodevelopmental outcomes.

4.5.4.1 Balancing covariates

The covariates (age, maternal education, sex, stunting) of the children in the healthy comparison group were weighted to the children in grade 2a and balance

distributions were evaluated.[408]

Balance was achieved with all Kolmogorov–Smirnov distances (KS) <0.06, where 0 suggests the distributions between the weighted healthy covariates, and the grade 2a covariates are the same. (Tables 4.20-4.23) Once balanced, the weights allocated to the healthy children were used to calculate the average affect of HFMD (i.e. average treatment effect on the treated: ATT) on grade 2a children and then, using weights on grade 2a children to match either grade 2b or >2b, the average effect of more severe HFMD on grade 2b and >grade 2b was calculated.

Table 4.20 Balance of covariates in children with grade 2a HFMD and children in healthy group: 270 Healthy children covariates weighted to 133 grade 2a

	Mean of grade 2a	Mean of weighted Healthy group	KS	Pre-weighted Mean of Healthy Group
Statistical notation	**E(Y1$\|$t=1)**	**E(Y0$\|$t=1)**		**E(Y0$\|$t=0)**
Age	17.34	17.08	0.04	17.89
Maternal Education: No School/ Primary	0.35	0.34	0.02	0.16
Secondary	0.53	0.54	0.01	0.47
Higher	0.11	0.12	0.01	0.37
Male	0.59	0.55	0.04	0.54
Female	0.41	0.45	0.04	0.47
Not Stunted	0.84	0.82	0.02	0.83
Stunt	0.16	0.18	0.02	0.17

Table 4.21 Balance of the grade 2a and grade 2b, grade 2a and >grade 2b: 133 grade 2a covariates weighted to 50 grade 2b, 133 grade 2a covariates weighted to 36 grade >2b

	Mean grade 2b	Mean of weighted grade 2a	KS	Pre-weighted Mean grade 2a
Statistical notation	**E(Y1$\|$t=1)**	**E(Y0$\|$t=1)**		**E(Y0$\|$t=0)**
Age	18.77	18.48	0.05	17.34
Maternal Education: No School/ Primary	0.38	0.34	0.04	0.35
Secondary	0.48	0.52	0.04	0.53
Higher	0.14	0.14	0.00	0.11
Male	0.64	0.59	0.05	0.59
Female	0.36	0.41	0.05	0.41
Not Stunted	0.80	0.81	0.01	0.84
Stunt	0.18	0.19	0.01	0.16
Missing Stunt	0.02	0.00	0.02	0.00

grade >2b age limit was 36 months and this limit was applied to grade 2a hence different pre-weighted mean values

	Mean grade >2b	Mean of weighted grade 2a	KS	Pre-weighted Mean grade 2a
Statistical notation	**E(Y1$\|$t=1)**	**E(Y0$\|$t=1)**		**E(Y0$\|$t=0)**
Age	19.48	19.33	0.06	17.03
Maternal Education: No School/ Primary	0.33	0.30	0.03	0.34
Secondary	0.56	0.58	0.03	0.54
Higher	0.11	0.11	0.00	0.12
Male	0.61	0.62	0.01	0.59
Female	0.39	0.38	0.01	0.41
Not Stunted	0.97	0.96	0.01	0.84
Stunt	0.03	0.04	0.01	0.16

Table 4.22 Second assessment: 205 healthy covariates balanced to 119 grade 2a covariates

	Mean of grade 2a	Mean of weighted matched Healthy group	KS	Pre-weighted Mean of Healthy Group
Statistical notation	**E(Y1$\|$t=1)**	**E(Y0$\|$t=1)**		**E(Y0$\|$t=0)**
Age	17.53	17.37	0.06	16.38
Maternal Education: No School/ Primary	0.33	0.29	0.04	0.14
Secondary	0.55	0.56	0.02	0.47
Higher	0.13	0.15	0.02	0.39
Male	0.58	0.53	0.05	0.51
Female	0.42	0.47	0.05	0.49
Not Stunted	0.85	0.84	0.00	0.84
Stunt	0.15	0.15	0.00	0.16

Table 4.23 Balance of the grade 2a and grade 2b, grade 2a and >grade 2b: 119 grade 2a covariates weighted to 42 grade 2b, 118 (age-limited to match grade >2b) grade 2a covariates weighted to 35 grade >2b

	Mean grade 2b	Mean of weighted grade 2a	KS	Pre-weighted Mean grade 2a
Statistical notation	**E(Y1$\|$t=1)**	**E(Y0$\|$t=1)**		**E(Y0$\|$t=0)**
Age	17.76	17.62	0.06	17.53
Maternal Education: No School/ Primary	0.41	0.35	0.06	0.33
Secondary	0.45	0.51	0.06	0.55
Higher	0.14	0.15	0.00	0.13
Male	0.64	0.59	0.05	0.58
Female	0.36	0.41	0.05	0.42
Not Stunted	0.83	0.85	0.01	0.85
Stunt	0.14	0.15	0.01	0.15
Missing stunt	0.02	0.00	0.02	0.00

Age limits of grade 2a was limited to match grade >2b age range

	Mean grade >2b	Mean of weighted grade 2a	KS	Pre-weighted Mean grade 2a
Statistical notation	**E(Y1$\|$t=1)**	**E(Y0$\|$t=1)**		**E(Y0$\|$t=0)**
Age	19.79	19.95	0.06	17.37
Maternal Education: No School/Primary	0.34	0.31	0.04	0.32
Secondary	0.54	0.59	0.04	0.55
Higher	0.11	0.11	0.01	0.13
Male	0.60	0.63	0.03	0.58
Female	0.40	0.37	0.03	0.41
Not Stunted	0.97	0.97	0.00	0.85
Stunt	0.03	0.03	0.00	0.15

Results of propensity score weighted analysis and presented in Table 4.24

Table 4.24 Linear regression of Bayley III Z scores by grade HFMD compared to healthy group and using propensity weights of grade 2a compared to healthy and grade 2b and >2b compared to grade 2a. (Unstandardised beta coefficients, 95% confidence intervals (CI) and p-values). Multivariate linear regression and adjustment by age, gender, maternal education and stunting.

Z scores	Cognitive Z score at 1st assessment (Coefficient (95%CI); p-value)					
Method	Linear regression		Propensity weighted ATT			
	Baseline: healthy		Baseline: healthy		Baseline: grade 2a	
	Univariate	Multivariate		Adjusted		Adjusted
grade 2a	-0.17 (-0.40 to 0.062); 0.15	-0.093 (-0.34 to 0.15); 0.46	grade 2a	-0.18 (-0.44 to 0.077); 0.17		
grade 2b	-0.33 (-0.66 to 0.004); 0.053	-0.27 (-0.61 to 0.078); 0.13			grade 2b	-0.21 (-0.60 to 0.17); 0.28
grade >2b	-0.30 (-0.68 to 0.086); 0.127	-0.21 (-0.60 to 0.19); 0.30			grade >2b	-0.078 (-0.52 to 0.37); 0.73

Z scores	Cognitive Z score difference (Coefficient (95%CI); p-value)					
Method	Linear regression		Propensity weighted ATT			
	Baseline: healthy		Baseline: healthy		Baseline: grade 2a	
	Univariate	Multivariate		Adjusted		Adjusted
grade 2a	-0.19 (-0.47 to 0.083); 0.17	-0.084 (-0.38 to 0.21); 0.58	grade 2a	-0.077 (-0.38 to 0.22); 0.61		
grade 2b	-0.16 (-0.57 to 0.25); 0.43	-0.0045 (-0.43 to 0.43); 0.98			grade 2b	0.022 (-0.45 to 0.49); 0.93
grade >2b	-0.20 (-0.64 to 0.24); 0.37	-0.089 (-0.54 to 0.37); 0.70			grade >2b	0.13 (-0.46 to 0.73); 0.66

Z scores	Cognitive Z score at 2nd assessment (Coefficient (95%CI); p-value)					
Method	Linear regression		Propensity weighted ATT			
	Baseline: healthy		Baseline: healthy		Baseline: grade 2a	
	Univariate	Multivariate		Adjusted		Adjusted
grade 2a	-0.36 (-0.61 to -0.16); 0.0041 **	-0.17 (-0.43 to 0.096); 0.21	grade 2a	-0.23 (-0.51 to 0.054); 0.11		
grade 2b	-0.34 (-0.70 to 0.025); 0.068	-0.11 (-0.49 to 0.26); 0.55			grade 2b	-0.15 (-0.54 to 0.25); 0.46
grade >2b	-0.54 (-0.94 to -0.15); 0.0068 **	-0.31 (-0.71 to 0.089); 0.13			grade >2b	0.0048 (-0.55 to 0.56); 0.99

Z scores	Receptive Z score at 1st assessment					
Method	**Linear regression**		**Propensity weighted ATT**			
Baseline: healthy	**Coefficient (95%CI); p-value**		**Baseline: healthy**		**Baseline: grade 2a**	
	Univariate	**Multivariate**		**Adjusted**		**Adjusted**
grade 2a	-0.19 (-0.43 to 0.044); 0.11	-0.14 (-0.39 to 0.10); 0.26	**grade 2a**	-0.12 (-0.37 to 0.13); 0.35		
grade 2b	-0.54 (-0.88 to -0.20); 0.0021 **	-0.47 (-0.82 to -0.12); 0.0084 **			**grade 2b**	-0.39 (-0.75 to -0.028); 0.036 *
grade >2b	-0.65 (-1.04 to -0.25); 0.0014 **	-0.53 (-0.92 to -0.13); 0.0092**			**grade >2b**	-0.33 (-0.76 to 0.093); 0.13

Z scores	Receptive Z score at 2nd assessment					
Method	**Linear regression**		**Propensity weighted ATT**			
Baseline: healthy	**Coefficient (95%CI); p-value**		**Baseline: healthy**		**Baseline: grade 2a**	
	Univariate	**Multivariate**		**Adjusted**		**Adjusted**
grade 2a	-0.44 (-0.70 to 0.18); 0.00080 ***	-0.24 (-0.51 to 0.028); 0.080	**grade 2a**	-0.21 (-0.51 to 0.079); 0.15		
grade 2b	-0.42 (-0.80 to -0.038); 0.031 *	-0.17 (-0.56 to 0.22); 0.38			**grade 2b**	-0.23 (-0.58 to 0.13); 0.21
grade >2b	-0.24 (-0.65 to 0.16); 0.24	0.024 (-0.39 to 0.43); 0.91			**grade >2b**	0.40 (-0.14 to 0.93); 0.15

Z scores	Receptive Z score difference					
Method	**Linear regression**		**Propensity weighted ATT**			
Baseline: healthy	**Coefficient (95%CI); p-value**		**Baseline: healthy**		**Baseline: grade 2a**	
	Univariate	**Multivariate**		**Adjusted**		**Adjusted**
grade 2a	-0.16 (-0.45 to 0.13); 0.27	-0.029 (-0.34 to 0.28); 0.85	**grade 2a**	-0.016 (-0.33 to 0.29); 0.92		
grade 2b	0.027 (-0.40 to 0.45); 0.90	0.19 (-0.25 to 0.64); 0.39			**grade 2b**	0.12 (-0.30 to 0.54); 0.57
grade >2b	0.47 (0.0098 to 0.93); 0.045 *	0.59 (0.13 to 1.066); 0.0136 *			**grade >2b**	0.74 (0.17 to 1.30): 0.011 *

Z scores	Expressive Z score at 1st assessment					
Method	**Linear regression**		**Propensity weighted ATT**			
Baseline: healthy	**Coefficient (95%CI); p-value**		**Baseline: healthy**		**Baseline: grade 2a**	
	Univariate	**Multivariate**		**Adjusted**		**Adjusted**
grade 2a	-0.31 (-0.53 to -0.086); 0.0066 **	-0.22 (-0.46 to 0.013); 0.064	**grade 2a**	-0.25 (-0.49 to -0.0066); 0.045 *		
grade 2b	-0.46 (-0.78 to -0.14); 0.0052 **	-0.38 (-0.71 to -0.046); 0.026*			**grade 2b**	-0.14 (-0.47 to 0.20); 0.43
grade >2b	-0.59 (-0.96 to -0.22); 0.0018**	-0.41 (-0.78 to -0.031); 0.034 *			**grade >2b**	-0.056 (-0.46 to 0.35); 0.79

Z scores	Expressive Z score at 2nd assessment					
Method	**Linear regression**		**Propensity weighted ATT**			
Baseline: healthy	**Coefficient (95%CI); p-value**		**Baseline: healthy**		**Baseline: grade 2a**	
	Univariate	**Multivariate**		**Adjusted**		**Adjusted**
grade 2a	-0.33 (-0.56 to -0.10); 0.0048 **	-0.22 (-0.46 to 0.022); 0.075	**grade 2a**	-0.22 (-0.48 to 0.047); 0.11		
grade 2b	-0.28 (-0.62 to 0.051); 0.096.	-0.12 (-0.46 to 0.24); 0.53			**grade 2b**	-0.066 (-0.40 to 0.27); 0.70
grade >2b	-0.20 (-0.56 to 0.16); 0.28	-0.031 (-0.40 to 0.34); 0.87			**grade >2b**	0.36 (-0.11 to 0.84); 0.13

Z scores	Expressive Z score difference					
Method	**Linear regression**		**Propensity weighted ATT**			
Baseline: healthy	**Coefficient (95%CI); p-value**		**Baseline: healthy**		**Baseline: grade 2a**	
	Univariate	**Multivariate**		**Adjusted**		**Adjusted**
grade 2a	-0.039 (-0.29 to 0.22); 0.77	-0.011 (-0.29 to 0.26); 0.94	**grade 2a**	-0.043 (-0.33 to 0.24); 0.77		
grade 2b	0.039 (-0.34 to 0.41); 0.84	0.12 (-0.27 to 0.52); 0.54			**grade 2b**	0.016 (-0.37 to 0.40); 0.94
grade >2b	0.36 (-0.041 to 0.77) 0.078	0.35 (-0.070 to 0.77); 0.10			**grade >2b**	0.40 (-0.024 to 0.83); 0.067

Z scores	Fine Motor Z score at 1st assessment					
Method	**Linear regression**		**Propensity weighted ATT**			
Baseline: healthy	**Coefficient (95%CI); p-value**		**Baseline: healthy**		**Baseline: grade 2a**	
	Univariate	**Multivariate**		**Adjusted**		**Adjusted**
grade 2a	-0.26 (-0.49 to -0.037); 0.023 *	-0.19 (-0.44 to 0.60); 0.14	**grade 2a**	-0.25 (-0.50 to -0.0019); 0.049 *		
grade 2b	-0.21 (-0.54 to 0.12); 0.22	-0.15 (-0.50 to 0.20); 0.39			**grade 2b**	0.050 (-0.37 to 0.47); 0.81
grade >2b	-0.59 (-0.97 to -0.21); 0.0025 **	-0.49 (-0.89 to -0.098); 0.015 *			**grade >2b**	-0.37 (-0.79 to 0.055); 0.090

Z scores	Fine Motor Z score 2nd assessment					
Method	**Linear regression**		**Propensity weighted ATT**			
Baseline: healthy	**Coefficient (95%CI); p-value**		**Baseline: healthy**		**Baseline: grade 2a**	
	Univariate	**Multivariate**		**Adjusted**		**Adjusted**
grade 2a	-0.17 (-0.40 to 0.063); 0.15	-0.11 (-0.36 to 0.15); 0.41	**grade 2a**	-0.13 (-0.40 to 0.14); 0.36		
grade 2b	-0.28 (-0.62 to 0.060); 0.11	-0.16 (-0.52 to 0.21); 0.39			**grade 2b**	-0.065 (-0.45 to 0.32); 0.74
grade >2b	-0.19 (-0.56 to 0.18); 0.32	-0.12 (-0.50 to 0.27); 0.55			**grade >2b**	0.12 (-0.32 to 0.57); 0.60

Z scores	Fine Motor Z score difference					
Method	**Linear regression**		**Propensity weighted ATT**			
Baseline: healthy	**Coefficient (95%CI); p-value**		**Baseline: healthy**		**Baseline: grade 2a**	
	Univariate	**Multivariate**		**Adjusted**		**Adjusted**
grade 2a	0.15 (-0.13 to 0.44); 0.29	0.12 (-0.19 to 0.43); 0.45	**grade 2a**	0.16 (-0.15 to 0.48); 0.30		
grade 2b	-0.12 (-0.54 to 0.30); 0.58	-0.071 (-0.52 to 0.37); 0.75			**grade 2b**	-0.20 (-0.68 to 0.28); 0.42
grade >2b	0.47 (0.017 to 0.92); 0.042 *	0.42 (-0.051 to 0.89); 0.080			**grade >2b**	0.53 (0.027 to 1.043); 0.041*

Z scores	Gross Motor Z score at 1st assessment					
Method	**Linear regression**		**Propensity weighted ATT**			
Baseline: healthy	**Coefficient (95%CI); p-value**		**Baseline: healthy**		**Baseline: grade 2a**	
	Univariate	**Multivariate**		**Adjusted**		**Adjusted**
grade 2a	-0.22 (-0.46 to 0.027); 0.082	-0.21 (-0.48 to 0.057); 0.12	**grade 2a**	-0.30 (-0.58 to -0.020); 0.037 *		
grade 2b	-0.53 (-0.89 to -0.17); 0.0036 **	-0.57 (-0.95 to -0.20); 0.0027 **			**grade 2b**	-0.35 (-0.87to 0.17); 0.19
grade >2b	-0.38 (-0.79 to 0.029); 0.069.	-0.37 (-0.80 to 0.051); 0.085			**grade >2b**	-0.14 (-0.66 to 0.38); 0.61

Z scores	Gross Motor Z score at 2nd assessment					
Method	**Linear regression**		**Propensity weighted ATT**			
Baseline: healthy	**Coefficient (95%CI); p-value**		**Baseline: healthy**		**Baseline: grade 2a**	
	Univariate	**Multivariate**		**Adjusted**		**Adjusted**
grade 2a	-0.42 (-0.65 to -0.18); 0.00056 ***	-0.34 (-0.59 to -0.088); 0.0083**	**grade 2a**	-0.41 (-0.67 to -0.15); 0.0020 **		
grade 2b	-0.36 (-0.71 to -0.011); 0.043 *	-0.20 (-0.56 to 0.17); 0.29			**grade 2b**	0.0069 (-0.39 to 0.40); 0.97
grade >2b	-0.31 (-0.69 to 0.065); 0.10	-0.28 (-0.67 to -0.11); 0.020*			**grade >2b**	0.16 (-0.26 to 0.58); 0.47

Z scores	Gross Motor Z score difference					
Method	**Linear regression**		**Propensity weighted ATT**			
Baseline: healthy	**Coefficient (95%CI); p-value**		**Baseline: healthy**		**Baseline: grade 2a**	
	Univariate	**Multivariate**		**Adjusted**		**Adjusted**
grade 2a	-0.18 (-0.48 to 0.11); 0.22	-0.097 (-0.41 to 0.21); 0.54	**grade 2a**	-0.15 (-0.45 to 0.15); 0.32		
grade 2b	0.065 (-0.37 to 0.50); 0.77	0.29 (-0.16 to 0.73); 0.21			**grade 2b**	0.31 (-0.23 to 0.84); 0.26
grade >2b	0.097 (-0.37 to 0.56); 0.68	0.15 (-0.32 to 0.62); 0.54			**grade >2b**	0.26 (-0.27 to 0.80); 0.34

4.5.5 Results of linear regression and propensity score analysis

4.5.5.1 Cognitive scores

1st Assessment

Compared to healthy group and following adjustment, grade 2a had lower Z scores up to -0.34 and -0.44 by 95% CI limits linear regression (LG) and propensity weighted score (PWS) respectively. Grade 2b, had lower Z scores up to -0.61 compared to healthy (LR) and -0.6 compared to grade 2a (PWS). Grade >2b had lower Z scores up to -0.60 compared to healthy (LR) and -0.52 compared to grade 2a (PWS).

2nd Assessment

Compared to healthy group and following adjustment, grade 2a had lower Z scores up to -0.43 and -0.51 by LG and PWS respectively. Grade 2b, had lower Z scores up to -0.49 compared to healthy (LR) and -0.54 compared to grade 2a (PWS). Grade >2b had lower Z scores up to -0.71 compared to healthy (LR) and marginally positive coefficients with Z scores up to 0.56 compared to grade 2a (PWS).

Difference in Cognitive scores

Compared to healthy group and following adjustment, grade 2a had negative change in Z scores up to -0.38 by LG and PWS. Grade 2b, had negative change in Z scores up to -0.43 compared to healthy (LR) and marginally positive coefficients with change in Z score up to 0.49 compared to grade 2a (PWS). Grade >2b had negative change in Z scores up to -0.54 compared to healthy (LR) and positive coefficients with change in Z score up to 0.73 compared to grade 2a (PWS).

4.5.5.2 Receptive language scores

1st Assessment

Compared to healthy group and following adjustment, grade 2a had lower Z scores up to -0.39 and -0.37 by LG and PWS respectively. Grade 2b, had significantly lower Z scores up to -0.82 compared to healthy (LR) and -0.75 compared to grade 2a (PWS). Grade >2b had significantly lower Z scores up to -0.92 compared to healthy (LR) and -0.76 compared to grade 2a (PWS), which was not significant.

2nd Assessment

Compared to healthy group and following adjustment, grade 2a had lower Z scores up to -0.51 by LG and PWS. Grade 2b, had lower Z scores up to -0.56 compared to healthy (LR) and -0.58 compared to grade 2a (PWS). Grade >2b had positive coefficient with Z scores up to 0.43 compared to healthy (LR) and 0.93 compared to grade 2a (PWS).

Difference in Receptive language scores

Compared to healthy group and following adjustment, grade 2a had negative change in Z scores up to -0.34 and -0.33 by LG and PWS respectively. Grade 2b, had positive coefficients with change in Z scores up to 0.64 compared to healthy (LR) and 0.54 compared to grade 2a (PWS). Grade >2b had significantly improved change in Z scores up to 1.066 compared to healthy (LR) and 1.30 compared to grade 2a (PWS).

4.5.5.3 Expressive language scores

1st Assessment

Compared to healthy group and following adjustment, grade 2a had lower Z scores up to -0.46 by LG and significantly lower Z scores by -0.49 using PWS.

Grade 2b, had significantly lower Z scores up to -0.71 compared to healthy (LR) and -0.47 compared to grade 2a (PWS), which was not significant. grade >2b had significantly lower Z scores up to -0.78 compared to healthy (LR) and -0.46 compared to grade 2a (PWS), which was not significant.

2nd Assessment

Compared to healthy group and following adjustment, grade 2a had lower Z scores up to -0.46 and -0.48 by LG and PWS respectively. Grade 2b, had lower Z scores up to -0.46 compared to healthy (LR) and -0.40 compared to grade 2a (PWS). Grade >2b had lower Z scores up to -0.40 compared to healthy (LR) and a positive coefficient with Z scores up to 0.84 compared to grade 2a (PWS).

Difference in Expressive language scores

Compared to healthy group and following adjustment, grade 2a had negative change in Z scores up to -0.29 and -0.33 by LG and PWS respectively. Grade 2b, had positive change in Z scores up to 0.52 compared to healthy (LR) and 0.4 compared to grade 2a (PWS). Grade >2b had positive change in Z scores up to 0.77 compared to healthy (LR) and 0.83 compared to grade 2a (PWS).

4.5.5.4 Fine motor scores

1st Assessment

Compared to healthy group and following adjustment, grade 2a had lower Z scores up to -0.44 by LG and significantly lower Z scores by -0.50 using PWS. Grade 2b, had lower Z scores up to -0.50 compared to healthy (LR) and a positive coefficient with higher Z scores up to 0.47 compared to grade 2a (PWS). Grade >2b had significantly lower Z scores up to -0.89 compared to healthy (LR) and -0.79 compared to grade 2a (PWS), which was not significant.

2nd Assessment

Compared to healthy group and following adjustment, grade 2a had negative change in Z scores up to -0.36 and -0.40 by LG and PWS respectively. Grade 2b, had negative change in Z scores up to -0.52 compared to healthy (LR) and -0.45 compared to grade 2a (PWS). Grade >2b had negative change in Z scores up to -0.50 compared to healthy (LR) but positive change in Z scores up to 0.57 compared to grade 2a (PWS).

Difference in Fine Motor scores

Compared to healthy group and following adjustment, grade 2a had a positive change in Z scores up to -0.19 and -0.15 by LG and PWS respectively. grade 2b, had worsened Z scores up to -0.52 compared to healthy (LR) and -0.68 compared to grade 2a (PWS). grade >2b had positive change in Z scores up to 0.89 compared to healthy (LR) and significantly improved Z score up to 1.043 compared to grade 2a (PWS).

4.5.6.5 Gross Motor scores

1st Assessment

Compared to healthy group and following adjustment, grade 2a had lower Z scores up to -0.48 by LG and significantly lower Z scores by -0.58 using PWS. Grade 2b, had significantly lower Z scores up to -0.95 compared to healthy (LR) and -0.87 compared to grade 2a (PWS), but was not significant. Grade >2b had significantly lower Z scores up to -0.80 compared to healthy (LR) and -0.66 compared to grade 2a (PWS), which was not significant.

2nd Assessment

Compared to healthy group and following adjustment, grade 2a had significantly lower Z scores up to -0.59 and -0.67 by LG and PWS respectively. Grade 2b, had

lower Z scores up to -0.56 compared to healthy (LR) but positive coefficient with Z scores up to 0.4 higher compared to grade 2a (PWS). Grade >2b had significantly lower Z scores up to -0.67 compared to healthy (LR) but improved Z scores up to 0.58 compared to grade 2a (PWS), which was not significant.

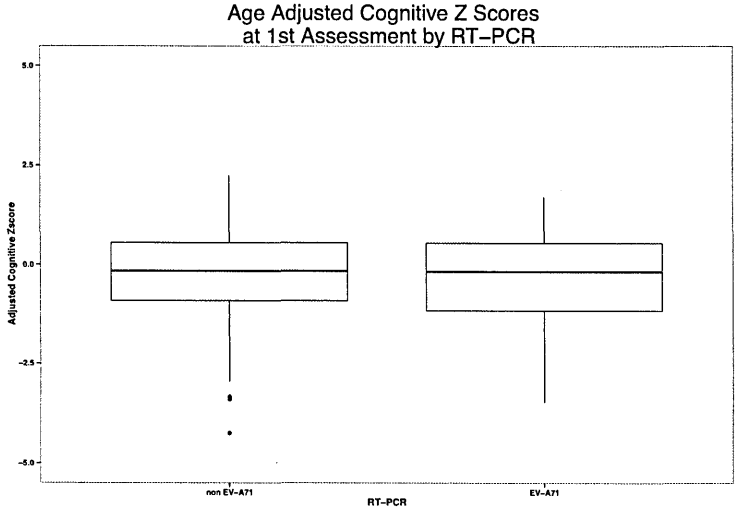
Difference in Gross Motor scores

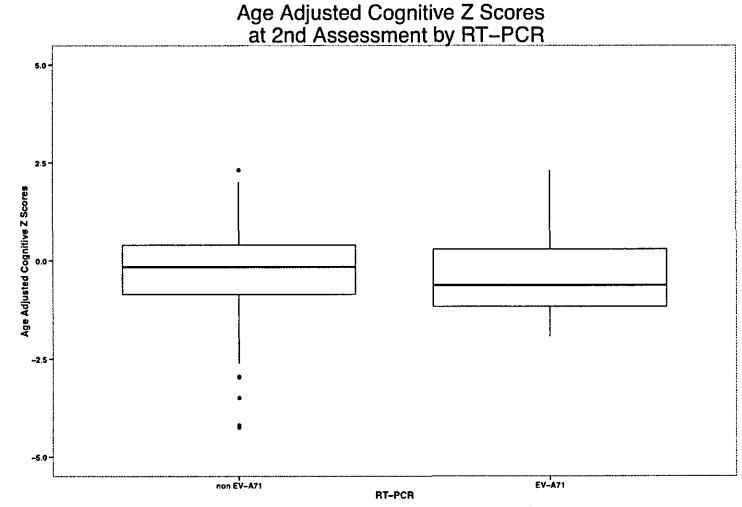
Compared to healthy group and following adjustment, grade 2a had negative change in Z scores up to -0.41 and -0.45 by LG and PWS respectively. Grade 2b, had positive change in Z scores up to 0.73 compared to healthy (LR) and 0.84 compared to grade 2a (PWS). Grade >2b had positive change in Z scores up to 0.62 compared to healthy (LR) and 0.8 compared to grade 2a (PWS).

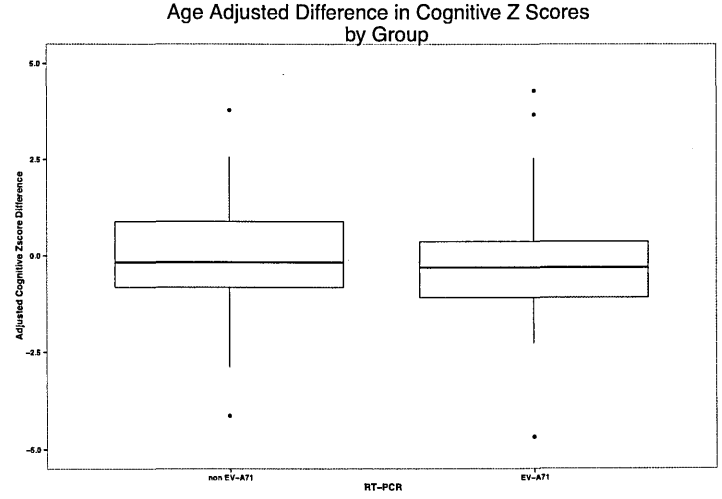
4.6 Bayley III scores by EV-A71

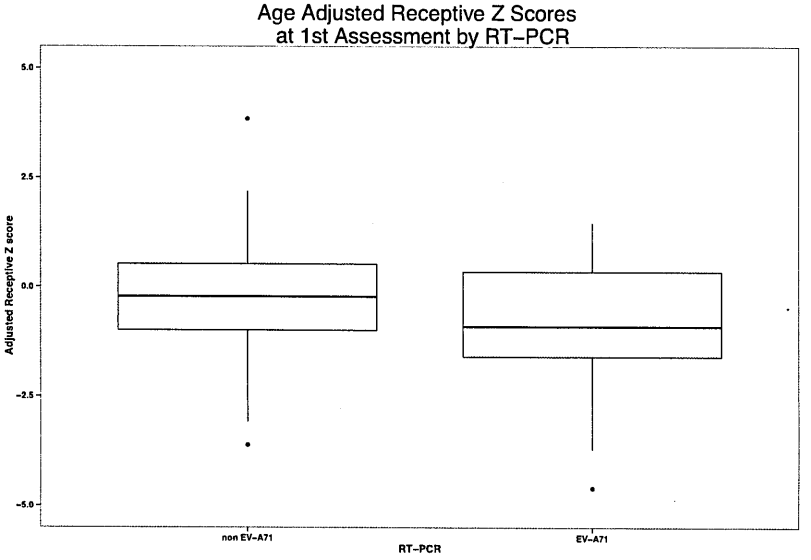
The Bayley III scores were regressed by RT-PCR result to determine influence by EV-A71. (Figure 4 and Table 17). 19% of cases were EV-A71 positive on RT-PCR but the number of severe cases grade 4 was small (7/242, 3%). Unfortunately, one of the two children who did progress could not speak Vietnamese, which limited her language and cognitive scores. She did cooperate at the second assessment and her cognitive, fine motor and gross motor Z scores ranged from -1.1 to -1.9. There were no significant difference in scores between EV-A71 cases and non EV-A71 cases following adjustment for all domains, although there were significant improvements in receptive score and gross motor scores between first and second assessment with coefficients of 0.49 and 0.53 for receptive and gross motor respectively. (Figure 4.5 and Table 4.25)

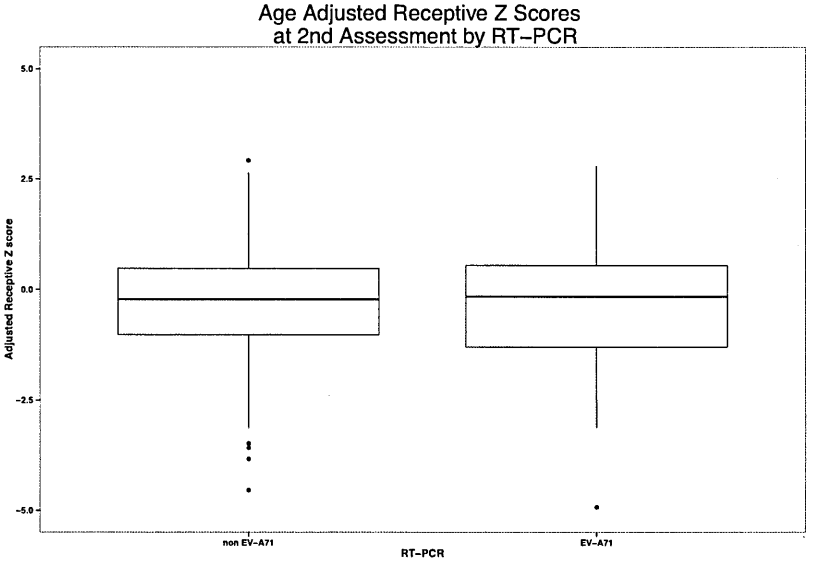
Figure 4.5 Plots of Bayley III Z scores by EV-A71 and non EV-A71. Scores adjusted for age, but not other covariates for each domain and assessment

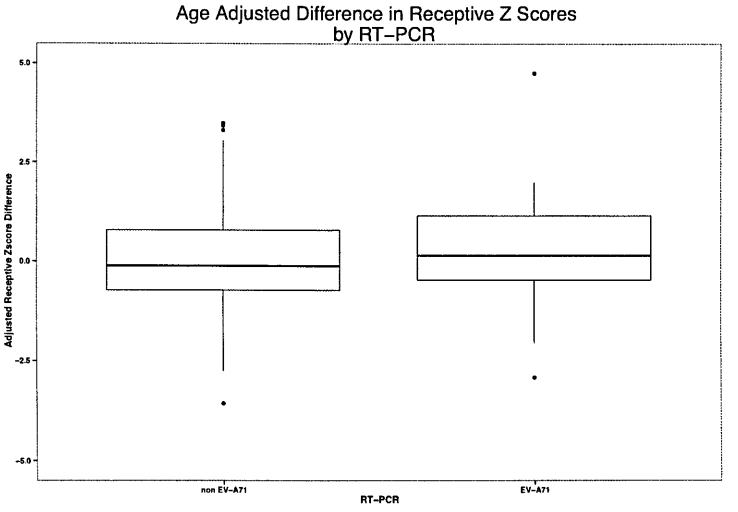


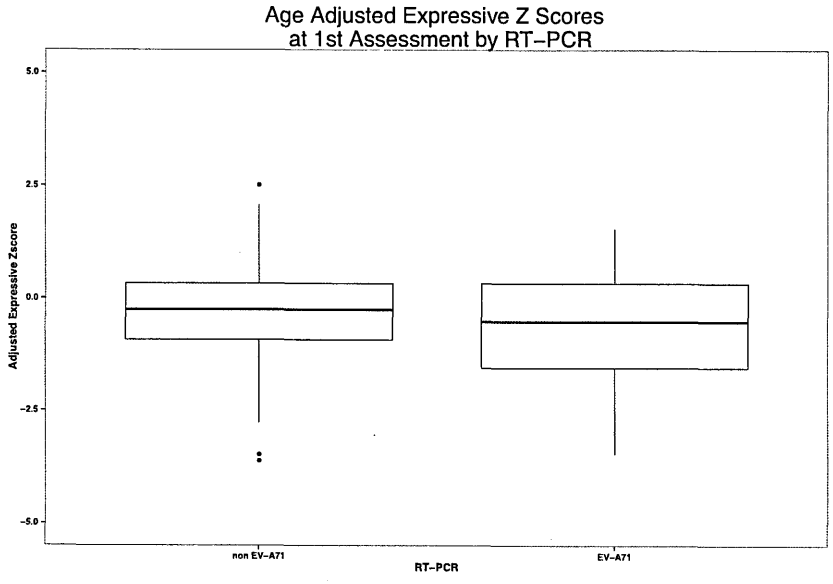


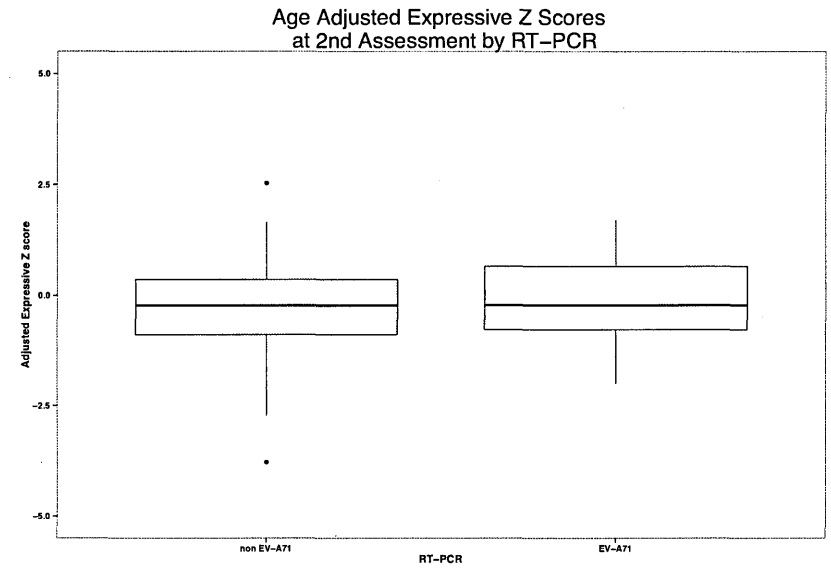


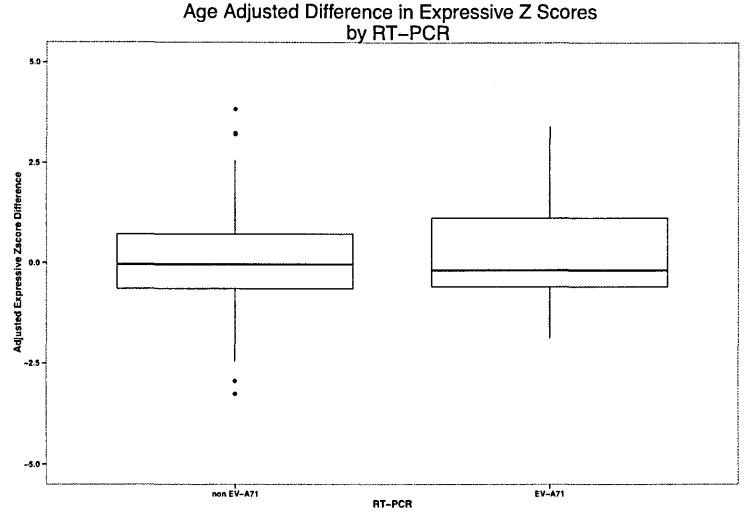

Age Adjusted Receptive Z Scores
at 1st Assessment by RT-PCR
Adjusted Receptive Z score
5.0
2.5
0.0
-2.5
-5.0
non EV-A71
EV-A71
RT-PCR

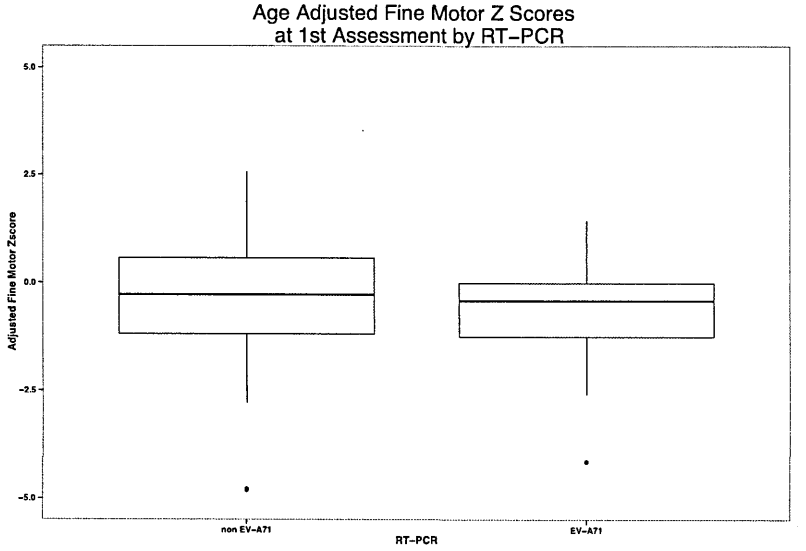

Age Adjusted Receptive Z Scores
at 2nd Assessment by RT-PCR
Adjusted Receptive Z score
5.0
2.5
0.0
-2.5
-5.0
non EV-A71
EV-A71
RT-PCR

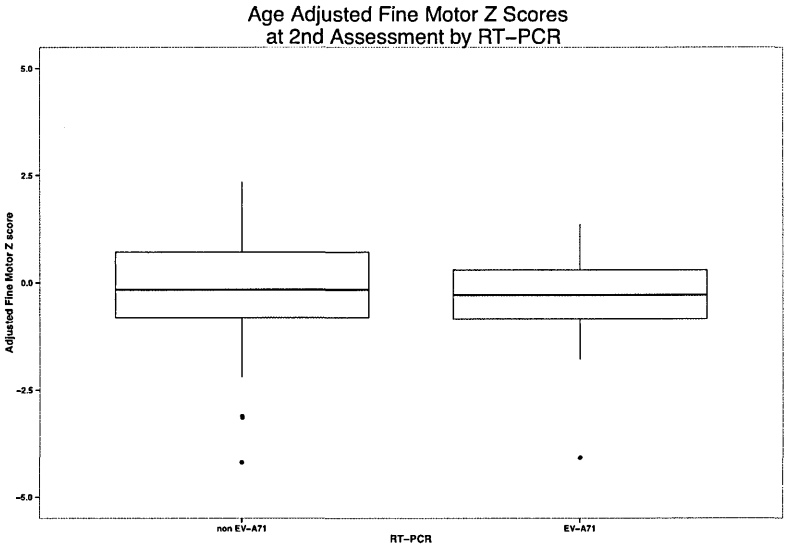

Age Adjusted Difference in Receptive Z Scores
by RT-PCR
Adjusted Receptive Zscore Difference
5.0
2.5
0.0
-2.5
-5.0
non EV-A71
EV-A71
RT-PCR

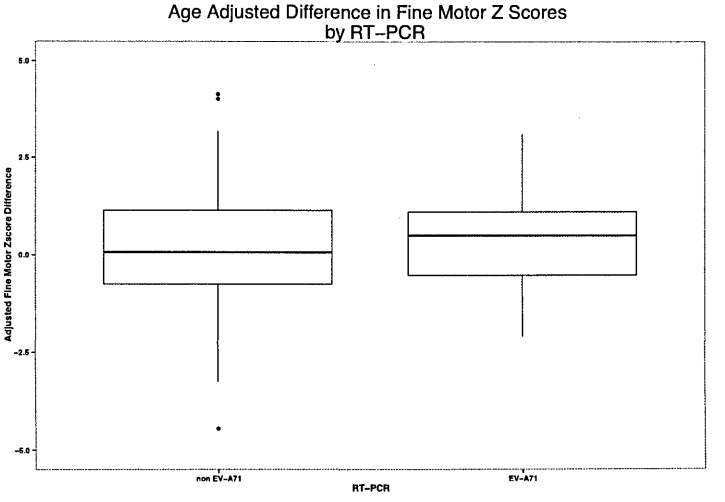

Age Adjusted Expressive Z Scores
at 1st Assessment by RT-PCR
Adjusted Expressive Zscore
5.0
2.5
0.0
-2.5
-5.0
non EV-A71
EV-A71
RT-PCR

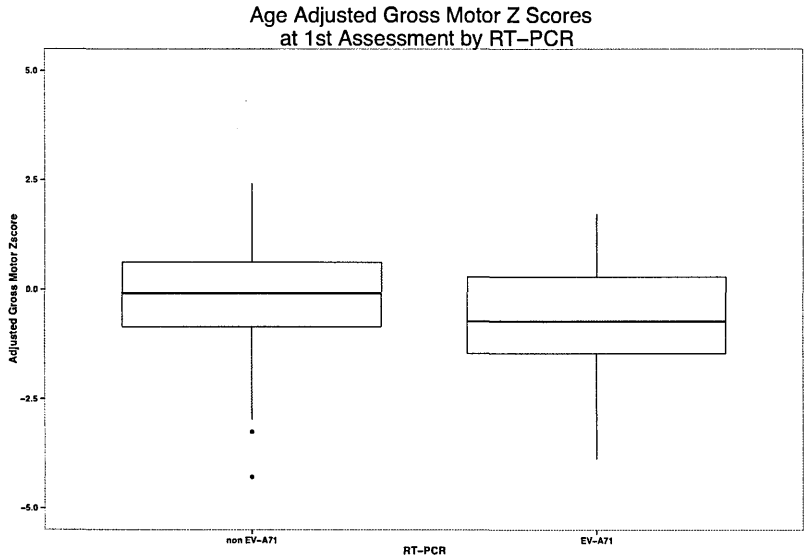

Age Adjusted Expressive Z Scores
at 2nd Assessment by RT-PCR
Adjusted Expressive Z score
5.0
2.5
0.0
-2.5
-5.0
non EV-A71
EV-A71
RT-PCR

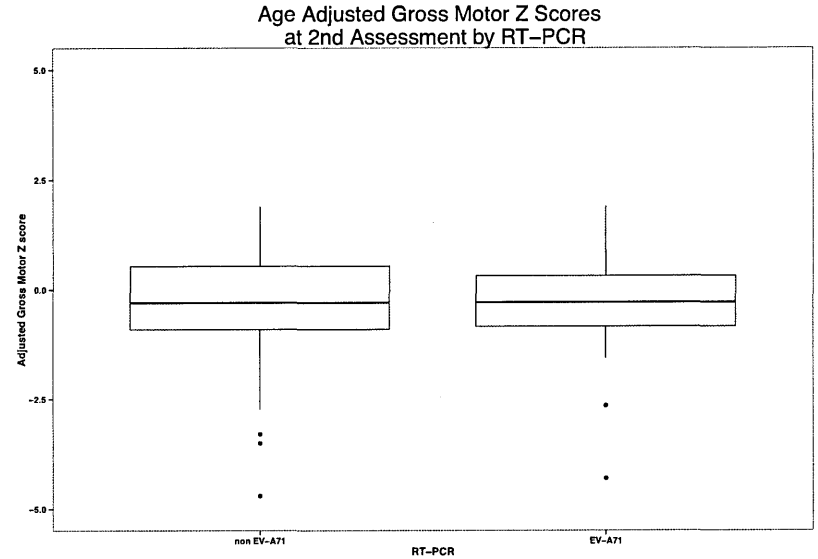

Age Adjusted Difference in Expressive Z Scores
by RT-PCR
Adjusted Expressive Zscore Difference
5.0
2.5
0.0
-2.5
-5.0
non EV-A71
EV-A71
RT-PCR


Age Adjusted Fine Motor Z Scores
at 1st Assessment by RT-PCR
Adjusted Fine Motor Zscore
5.0
2.5
0.0
-2.5
-5.0
non EV-A71
EV-A71
RT-PCR


Age Adjusted Fine Motor Z Scores
at 2nd Assessment by RT-PCR
Adjusted Fine Motor Z score
5.0
2.5
0.0
-2.5
-5.0
non EV-A71
EV-A71
RT-PCR


Age Adjusted Difference in Fine Motor Z Scores
by RT-PCR
Adjusted Fine Motor Zscore Difference
5.0
2.5
0.0
-2.5
-5.0
non EV-A71
EV-A71
RT-PCR


Age Adjusted Gross Motor Z Scores
at 1st Assessment by RT−PCR
Adjusted Gross Motor Zscore
5.0
2.5
0.0
−2.5
−5.0
non EV−A71
EV−A71
RT−PCR


Age Adjusted Gross Motor Z Scores
at 2nd Assessment by RT−PCR
Adjusted Gross Motor Z score
5.0
2.5
0.0
−2.5
−5.0
non EV−A71
EV−A71
RT−PCR

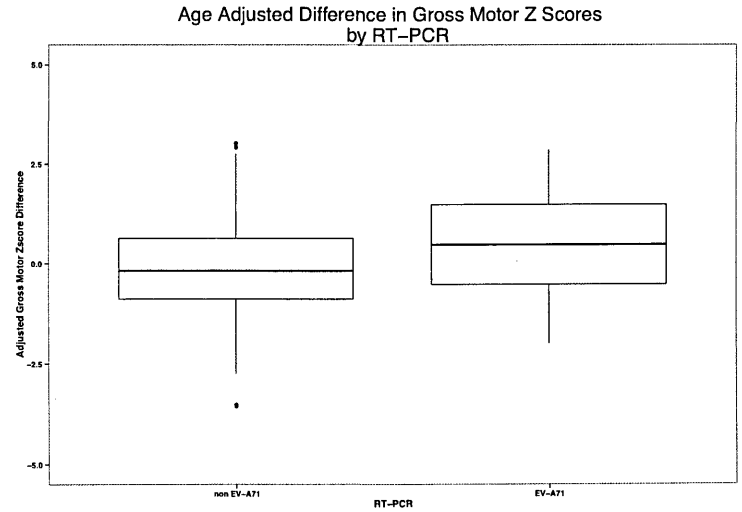

Age Adjusted Difference in Gross Motor Z Scores
by RT−PCR
Adjusted Gross Motor Zscore Difference
5.0
2.5
0.0
−2.5
−5.0
non EV−A71
EV−A71
RT−PCR

Table 4.25 Linear regression of Bayley III Z scores by RT-PCR 197 non EV-A71 vs. 45 EV-A71 (Unstandardised beta coefficients (95%CI) and p-values). Multivariate linear regression and adjustment by age, gender, maternal education and stunting.

	Cognitive Z scores			Receptive Z scores		
	Coefficient (95%CI); p-value			Coefficient (95%CI); p-value		
	1st Assessment	2nd assessment	Difference	1st Assessment	2nd assessment	Difference
EV-A71	-0.15 (-0.55 to 0.25); 0.46	-0.28 (-0.72 to 0.16); 0.21	-0.10 (-0.58 0.38); 0.68	-0.50 (-0.91 to -0.085); 0.018 *	-0.17 (-0.64 to 0.30); 0.48	0.30 (-0.18 to 0.78); 0.216
EV-A71 adjusted	-0.088 (-0.50 to 0.32); 0.67	-0.081 (-0.53 to 0.37); 0.73	0.085 (-0.41 to 0.58); 0.74	-0.27 (-0.67 to 0.13); 0.18	0.15 (-0.32 to 0.61); 0.54	0.49 (0.010 to 0.97); 0.045 *

	Expressive Z scores			Fine Motor Z scores		
	Coefficient (95%CI); p-value			Coefficient (95%CI); p-value		
	1st Assessment	2nd assessment	Difference	1st Assessment	2nd assessment	Difference
EV-A71	-0.28 (-0.66 to 0.096); 0.143	0.062 (-0.32 to 0.44); 0.75	0.24 (-0.19 to 0.67); 0.265	-0.32 (-0.72 to 0.074); 0.11	-0.15 (-0.55 to 0.24); 0.44	0.22 (-0.27 to 0.71); 0.372
EV-A71 adjusted	-0.19 (-0.55 to 0.18); 0.31	0.17 (-0.22 to 0.56); 0.3810	0.27 (-0.16 to 0.70); 0.22	-0.30 (-0.71 to 0.11); 0.15	-0.13 (-0.54 to 0.29); 0.555	0.25 (-0.26 to 0.76); 0.34

	Gross Motor Z scores		
	Coefficient (95%CI); p-value		
	1st Assessment	2nd assessment	Difference
EV-A71	-0.47 (-0.92 to -0.019); 0.041 *	0.091 (-0.32 to 0.51); 0.67	0.57 (0.057 to 1.07); 0.030 *
EV-A71 adjusted	-0.36 (-0.83 to 0.11); 0.13	0.16 (-0.27 to 0.59); 0.46	0.53 (0.0071 to 1.053); 0.047 *

4.7 Summary

Two methods were used to analyse cognitive, language and motor Bayley III scores at two assessments and change in scores between assessments. The first method compared all severities (grade 2a, 2b >2b) to a healthy control group. However, since the missing outcome scores were not missing at random for the healthy group, but were missing at random within the HFMD groups, comparisons between grades of HFMD severity were made using propensity score weighting matching methods and further adjustment for covariates (age, gender, maternal education and stunting). The level of significance was interpreted with caution in view of the small sample sizes. We did not recruit the sample size for which the study was powered to determine differences between groups, hence the focus on the limits of the 95%CI. The results are summarised in Table 4.26.

Table 4.26 summarises the direction of coefficients and limit of 95% confidence intervals of Z score or Z score change.

⇩	Negative coefficient with lower limit of 95% CI up to -0.5 Z score
⇩	Negative coefficient with lower limit of 95% CI between -0.5 and -1 Z score
⇧	Positive coefficient with upper limit of 95% CI up to 0.5 Z score
⇧	Positive coefficient with upper limit of 95% CI between 0.5 and 1 Z score
⇧	Positive coefficient with upper limit of 95% CI between 1 and 1.5 Z score

Cognitive	Multivariate linear regression			Propensity score balanced to grade 2a with adjustment		
Comparative group	Healthy			2a		
	1st	2nd	Difference	1st	2nd	Difference
Grade 2a	⇩	⇩	⇩			
Grade 2b	↓	⇩	⇩	↓	⇧	⇧
Grade >2b	↓	↓	↓	↓	↓	⇧

Receptive	Multivariate linear regression			Propensity score balanced to grade 2a with adjustment		
Comparative group	Healthy			2a		
	1st	2nd	Difference	1st	2nd	Difference
Grade 2a	⇩	↓	⇩			
Grade 2b	↓	↓	⇧	↓	↓	⇧
Grade >2b	↓	⇧	↑	↓	↑	↑

Expressive	Multivariate linear regression			Propensity score balanced to grade 2a with adjustment		
Comparative group	Healthy			2a		
	1st	2nd	Difference	1st	2nd	Difference
2a	⇩	⇩	⇩			
2b	↓	⇩	↑	⇩	⇩	⇧
>2b	↓	⇩	↑	⇩	⇧	↑

Fine motor	Multivariate linear regression			Propensity score balanced to grade 2a with adjustment		
Comparative group	Healthy			2a		
	1st	2nd	Difference	1st	2nd	Difference
2a	⇩	⇩	⇧			
2b	⇩	↓	↓	⇩	⇩	↓
>2b	↓	↓	↑	↓	↑	↑

Gross motor	Multivariate linear regression			Propensity score balanced to grade 2a with adjustment		
Comparative group	Healthy			2a		
	1st	2nd	Difference	1st	2nd	Difference
2a	⇩	↓ **	⇩			
2b	↓ **	↓	↑	↓	⇧	↑
>2b	↓	↓ *	↑	↓	↑	↑

*p<0.05 ** p<0.01

At both assessments, for cognitive, expressive language, fine and gross motor domains, all grades of HFMD had negative Z score coefficients and lower Z score limits compared to healthy group. In addition, the 95% CI limits were lower with increasing severity of HFMD compared to the healthy group. This pattern was not seen in receptive language where grade >2b had a positive coefficient in Z score compared to healthy group.

Compared to changes in Z score over the six months for the healthy group, there was a negative change in Z score for cognitive scale for all grades of HFMD.

There was more variability in the other domains but consistently positive changes in Z scores for grade >2b in receptive language, fine and gross motor domains.

Looking between grades of HFMD, grade 2b and >2b had lower Z scores than grade 2a at the first assessment. This was not consistent at the second assessment with grade 2b having higher Z score limits in cognitive and gross motor domains, and >2b having higher Z score limits in receptive and expressive language, and fine and gross motor. The biggest change in Z scores was seen in grade >2b in all domains.

4.8 Discussion

Amiel-Tison

Modification of the tool was required, as some sections were irrelevant for this population (head circumference, gross motor milestones), as they were also having objective developmental tests, avoiding bias from reported abilities. The sections covered by objective assessment (passive tone, comparison between sides, rigidity, motor activity, primitive reflexes, postural reflexes, gross motor) are relevant for this population. However, the tool is limited to primarily detect cerebral palsy, spasticity and to predict those who may not walk. It would be useful to modify the tool further to look at quality of gait, running, hopping or jumping. The scoring system will have to be modified again after trials on normal children and children with known motor disorders to determine which areas may be more specific for certain milder motor abnormalities and should carry more weight when scoring. There was some variability in scoring, with abnormalities improving between assessments or becoming apparent. More structured reliability assessments between assessors may have been able to distinguish whether the changes were real or due to examiner bias, particularly when children were

uncooperative. Despite these limitations, expansion on the modified criteria did distinguish moderate and mild abnormalities, but these were not significantly different in proportions between grades of HFMD, influenced by the small sample size.

Bayley III

This is the first study to compare neurodevelopmental outcomes of HFMD cases to a healthy comparison group and additionally compare differences between grades by balancing relevant covariates.

Through both methods, the lowest 95%CI limit in Z scores was within minus one Z score, i.e. 1 standard deviation of the comparative group mean. This was also the limit when comparing EV-A71 cases to non EV-A71 cases. In research, large studies following preterm infants have opted to used < 2 SD of the mean of a comparative group to determine impairment.[352] Following this threshold, no group performed significantly worse than the healthy or grade 2a comparative group. Post discharge, the more severe HFMD grades performed worse than the healthy group and this persisted for all domains except receptive language at the second assessment. Interestingly, there were larger positive changes in Z scores between assessments for the grade >2b children, despite negative coefficients of Z scores at the second assessment compared to healthy groups. Between grades of HFMD, the more severe grades had lower Z scores at the first assessment compared to grade 2a but this did not persist at the second assessment. There is a non-significant reduction in Z scores at the first assessment between healthy and all HFMD grades which persists for most domains at six months. However the Z scores between the grades of HFMD appears to narrow and overlap by the six month assessment, with most Z score improvements seen in grade 2b and >2b.

The variability within one Z score may represent variability in performance on the day of assessment rather than actual change in ability, or the improvement may reflect being part of a study interested in neurodevelopment that engages the parents in the assessment. Parents are aware of some of their child's limitations at the first assessment and may actively work on these areas prior to the second assessment.

Results from one assessment may have given biased view of prognosis following severe HFMD. Despite lower Z scores for HFMD compared to healthy, there have been improvements in Z scores for the more severe grades. Encouragingly, all lower limits of 95% CI were within 1 standard deviation of the healthy comparison group, which is deemed to be within normal developmental variability.[409]

5. Brain MRI findings in severe HFMD

5.1 Introduction

There are differing opinions on the clinical evaluation and prognostic value of MRI in HFMD encephalitis. Zeng et al. concluded from examining MRI scans on 42 EV-A71 positive HFMD brainstem encephalitis cases, that MRI changes were relatively specific and could contribute to clinical evaluation and management.[235] Lee et al. were more cautious when describing their findings from 22 EV-A71 HFMD CNS cases, commenting, "clinical features were not always consistent with MRI findings."[242]

As previously reviewed in chapter 1 (Table 1.5), typical MRI findings in EV-A71 HFMD consist of lesions in the dorsal pons, dorsal medulla, midbrain, dentate nuclei, hypothalamus and cervical cord. Prevalence of MRI changes in EV-A71 HFMD range from 46% in cases with myoclonus/ataxia/tremor to 100% in cases with cranial nerve involvement and cardiopulmonary compromise.[126] However, EV-A71 detection can vary from 30% to more than 90% of sampled cases of HFMD, depending on clinical severity. (Table 1.2) Outbreaks in China between 2008-14 have shown fluctuation in detection rates of EV-A71[80] and it is important to identify that other pathogens may have a similar clinical presentation and propensity to severe disease as EV-A71.

Table 1.5 in chapter 1 reviewed MRI findings in the HFMD literature and found stereotypical findings in EV-A71 infected complicated cases with CNS manifestations, but studies did not consistently state when, in the illness or recovery phase, the scans were taken nor do they describe the outcome for the

children who had scans. This prospective study aims to identify whether MRI changes are exclusive to severe HFMD cases associated with EV-A71, and comments on outcome data as measured by standardised tools.

5.2 MRI Scans

The study offered research MRI scans to all enrolled HFMD cases with parental consent when the child was deemed clinically unlikely to progress to more severe disease. The scans were carried out at a private MRI facility 5 km from the hospital. A consultant paediatric neuroradiologist: Dr. Kling Chong at Great Ormond Street Hospital in the United Kingdom, reviewed the scans. Dr. Chong was informed of the age of the child but was not aware of the severity of the child's illness. A proforma (Table 2.5) that included specific regions listed according to the literature was used to document MRI changes.

5.3 Results

5.3.1 Clinical characteristics of sample scanned

A total of 87/242 (36%) HFMD cases had MRI brain scans: 32/147 (22%) Grade 2a, 26/58 (45%) Grade 2b and 29/37 (78%) grade >2b cases. The median day of illness for the scan was day 5, with a range of 4 to 42 days. Tables 5.1- 5.3 compare the characteristics of the children who had MRI compared to those who did not, by HFMD grade. Small sample sizes led to the wide 95% confidence intervals, which limit interpretability. However, the results suggest no significant differences in age, gender, level of maternal education, stunting and viral aetiology between those who had MRI and those who did not, within grades.

There were no significant differences in ages, in the proportion of male sex, level of maternal education, stunting and in the proportion of MRI changes between severity groups (Table 5.4). There was a significant difference in the timing of scan with the more severe cases having scans later after illness onset. All grade 2a and 2b cases and 98% of grade >2b cases recovered by discharge. One 35-month female patient with grade 4 EV71 disease had a persistent 6th and 7th nerve left facial palsy, and a 26-month old male patient with grade 4 disease (EV-A71 PCR-negative) had a tremor at discharge.

Table 5.1 Characteristics of the sample that had MRI out of total cases enrolled grade 2a

Discharge Grade 2a	Had MRI	Total Cases enrolled	Odds Ratio (95%CI); p-value[ϑ]
Number	32	147	
Age at enrolment (median, IQR)	17.6 (13.2-21.8)	15.1 (11.5-20.2)	p-value = 0.13[β]
Gender (male)	22 (69%)	84 (57%)	1.65 (0.78-inf); 0.16
Maternal education			
Primary	6 (19%)	50 (34%)	0.45 (0.17-inf); 0.98
Secondary	22 (69%)	82 (56%)	1.74 (0.82-inf); 0.12
Higher	4 (12%)	15 (10%)	1.26 (0.36-inf); 0.45
Stunted	3 (9%)	22 (15%)	0.59 (0.14-inf); 0.87
Duration in hospital (median, IQR)	6 (5-7)	5 (4-6.5)	p-value = 0.20[β]
Recovery at discharge	32 (100%)	147 (100%)	
PCR			
CV-A10	4 (12%)	16 (11%)	1.17 (0.34-Inf); 0.50
CV-A16	2 (6%)	6 (4%)	1.56 (0.22-Inf); 0.44
EV-71	3 (9%)	13 (9%)	1.07 (0.24-Inf); 0.57
CV-A6	5 (16%)	32 (22%)	0.67 (0.23-Inf); 0.85
EV	10 (31%)	38 (26%)	1.30 (0.58-Inf); 0.34
Negative PCR	8 (25%)	42 (29%)	0.85 (0.36-Inf); 0.72

[ϑ] Fisher's exact test
[β] Kruskal-Wallis chi-squared

Table 5.2 Sample had MRI out of total cases enrolled grade 2b

Discharge Grade 2b	Had MRI	Total Cases enrolled	Odds Ratio (95%CI); p-value [9]
Number	26	58	
Age at enrolment (median, IQR)	16.2 (12.6-21.3)	17.1 (12.6-22.9)	p-value = 0.92 [β]
Gender (male)	15 (58%)	36 (62%)	0.83 (0.34-Inf); 0.73
Maternal education			
Primary	9 (35%)	23 (40%)	0.91 (0.35-inf); 0.66
Secondary	11 (42%)	28 (48%)	0.79 (0.32-inf); 0.77
Higher	6 (23%)	7 (12%)	1.67 (0.51-inf); 0.29
Stunted	2 (8%)	10 (18%)	1.20 (0.53-inf); 0.43
Duration in hospital (median, IQR)	7 (5-8)	6 (5-7)	p-value = 0.68 [β]
Recovery at discharge	26 (100%)	58 (100%)	
PCR			
CV-A10	2 (8%)	10 (17%)	0.40 (0.059-Inf); 0.94
CV-A16	0	1 (2%)	NS
EV-71	3 (12%)	10 (17%)	0.63 (0.14- Inf); 0.84
CV-A6	8 (31%)	12 (21%)	1.90 (0.68- Inf); 0.18
EV	3 (12%)	12 (21%)	0.50 (0.11-Inf); 0.91
Negative PCR	6 (23%)	13 (22%)	1.04 (0.34-Inf); 0.58

Table 5.3 Sample had MRI out of total cases enrolled grade >2b

Discharge Grade >2b	Had MRI	Total Cases enrolled	Odds Ratio (95%CI); p-value [9]
Number	29	37	
Age at enrolment (median, IQR)	21.0 (12.3-29.1)	19.6 (10.5-26.9)	p-value = 0.60 [β]
Gender (male)	18 (62%)	22 (59%)	1.11 (0.43-Inf); 0.52
Maternal education			
Primary	9 (31%)	13 (35%)	0.83 (0.30-inf); 0.73
Secondary	17 (59%)	20 (54%)	1.20 (0.47-inf); 0.45
Higher	3 (10%)	4 (11%)	0.95 (0.17-inf); 0.67
Stunted	2 (7%)	2 (5%)	1.29 (0.13-inf); 0.60
Duration in hospital (median, IQR, range)	6 (5-7) (5-42)	6 (5-7) (4-42)	p-value = 0.84 [β]
Recovery at discharge	27 (93%)	35(95%)	0.77 (0.080- Inf); 0.78
PCR			
CV-A10	1 (3%)	1 (3%)	1.28 (0.032- Inf); 0.69
CV-A16	0	0	
EV-71	17 (59%)	22 (59%)	0.97 (0.38-Inf); 0.63
CV-A6	0	0	
EV	4 (14%)	5 (14%)	1.02 (0.24-Inf); 0.62
Negative PCR	7 (24%)	9 (24%)	0.99 (0.32-Inf); 0.62

Table 5.4 Characteristics of cases that had Brain MRI

Discharge Grade	Grade 2a	Grade 2b	Grade >2b	p-value [ϑ]
Number	32	26	29	
Age at enrolment (median, IQR)	17.6 (13.2-21.8)	16.2 (12.6-21.3)	21.0 (12.3-29.1)	0.51 [β]
Gender (male)	22 (69%)	15 (58%)	18 (62%)	0.64
Maternal education				
Primary	6 (19%)	9 (35%)	9 (31%)	0.41
Secondary	22 (69%)	11 (42%)	17 (59%)	0.14
Higher	4 (12%)	6 (23%)	3 (10%)	0.40
Stunted	3 (9%)	2 (8%)	2 (7%)	1.00
Day illness scan (median, IQR, range)	6 (5-7) (4-16)	7 (6-8) (4-15)	8 (6-8) (5-42)	0.0033** [β]
Acute abnormalities MRI	5 (16%)	6 (23%)	12 (41%)	0.068
Recovery at discharge in those with MRI abnormalities	5 (100%)	6 (100%)	9 (82%)	0.39

[ϑ] Fisher's exact test
[β] Kruskal-Wallis chi-squared

5.3.2 MRI changes

Table 5.5 compares the clinical characteristics and severities between the MRI scans with abnormalities and those with none. There were no significant differences in the age of enrolment, gender, level of maternal education, stunting, median duration of hospitalization, median day of illness of scan and proportion with grade 2a and grade 2b classification between the groups. There were significantly more grade >2b cases in the group with MRI abnormalities. There were no significant differences in the proportion of patients with fever, vomiting, myoclonus, ataxia, cranial nerve abnormalities, rash, ulcer and irritability between groups. There was a trend to MRI abnormalities in the EV-A71 positive cases but this was not significant (p value = 0.098). There were significantly more MRI abnormalities in the CV-A10 positive cases, yet the highest proportion of CV-A10 positive cases were grade 2a (12%).

Table 5.5 Comparison between MRI scans with abnormalities and those without.

	MRI abnormalities	No MRI abnormalities	Odds Ratio (95%CI); p-value [9]
Number of cases	23 (26%)	64 (74%)	
Grade 2a	5 (16%)	27 (84%)	0.38 (0.122-Inf); 0.97
Grade 2b	6 (23%)	20 (77%)	0.78 (0.26- inf); 0.76
Grade >2b	12 (41%)	17 (27%)	2.97 (1.16-Inf) ;0.026*
Age at enrolment in months (median, IQR)	19.7 (15.0-25.9)	17.6 (12.3-22.8)	p-value = 0.18 [β]
Gender (male)	17 (74%)	38 (59%)	1.92 (0.72-Inf); 0.16
Maternal education			
Primary	7 (30%)	17 (27%)	1.21 (0.43-inf); 0.46
Secondary	13 (57%)	37 (58%)	0.48 (0.21-inf); 0.97
Higher	3 (13%)	10 (16%)	0.81 (0.17-inf); 0.73
Stunted	2 (9%)	5 (8%)	1.12 (0.15-inf); 0.60
Day illness scan (median, IQR, range)	7 (6-8) (4-42)	7 (5-8) (4-16)	p-value = 0.50
Clinical features on admission			
Fever	10 (43%)	33 (52%)	0.73; (0.29-Inf); 0.82
Vomiting	0	5 (8%)	0 (0-inf); 1
Rash	9 (39%)	39 (61%)	0.42 (0.18-Inf); 0.98
Ulcer	16 (70%)	54 (86%)	0.43 (0.15-Inf); 0.96,
Myoclonus	4 (17%)	7 (11%)	1.70 (0.42-Inf); 0.32,
Irritability	1 (4%)	7 (11%)	0.37 (0.016-Inf); 0.92
Tremor	1 (5%)	3 (5%)	0.93 (0.034-Inf); 0.72
Cranial N abnormality	1 (5%)	0	
Ataxia	0	1 (2%)	
Nystagmus	1 (4%)	0	
Limb weakness	0	0	

Duration in hospital	6 (2)	6 (2)	p-value = 0.58 [β]
PCR			
CV-A10	5 (22%)	2 (3%)	8.34 (1.39-Inf); 0.013*
CV-A16	1 (4%)	1 (2%)	2.82 (0.07-Inf); 0.46
EV-A71	9 (39%)	14 (22%)	2.27 (0.84-Inf); 0.093
CV-A6	1 (4%)	12 (19%)	0.20 (0.009-Inf); 0.99
EV	1 (4%)	20 (31%)	0.10 (0.0050-Inf); 0.99
Negative PCR	6 (26%)	15 (23%)	1.15 (0.38-Inf); 0.50

[ϑ] Fisher's exact test [β] Kruskal-Wallis chi-squared

Acute abnormalities in T2WI and FLAIR consisted of hyperintense lesions in the pons, dentate nuclei of cerebellum and white matter of the brain (Table 5.6). There was no significant difference in location of lesion between grades of severity. There were significantly more EV-A71 positive PCR cases with MRI changes at grade >2b, reflecting more than 50% of grade >2b cases were EV-A71 positive.

Table 5.6 Acute MRI abnormalities by HFMD grade at discharge and PCR

Discharge Grade	Grade 2a	Grade 2b	Grade >2b	p-value [ϑ]
Number scanned	32	26	29	
Number with abnormalities	5 (16%)	6 (23%)	12 (41%)	0.064
Posterior aspect of pons	0	0	3 (10%)	0.059
Periventricular region	1 (3%)	0	0	1
Dentate nuclei of cerebellum	1 (3%)	5 (19%)	8 (28%)	0.020*
Deep White Matter changes	5 (16%)	4 (15%)	5 (17%)	1
Subcortical white matter	0	0	2 (7%)	0.20
PCR				
CV-A10	3 (9%)	2 (8%)	0	0.27
CV-A16	1 (3%)	0	0	1
EV-71	1 (3%)	0	8 (28%)	0.00075**
CV-A6	0	1 (4%)	0	0.30
EV	0	0	1 (3%)	0.63
Negative PCR	0	3 (12%)	3 (10%)	0.11

[ϑ] Fisher's exact test

Table 5.7 MRI abnormalities by RT-PCR

In total there were 23 cases, which had MRI changes, and 64 with no changes.

	Total Scanned (87)			Grade 2a (32)		Grade 2b (26)		Grade >2b (29)		
	Abnormality	No change	Odds Ratio (95%CI); p-value[9]	Abnormality	No change	Abnormality	No change	Abnormality	No change	p-value[9]
CV-A10 (7)	5 (71%)	2 (29%)	8.34, (1.59-INF); 0.013*	3 (75%)	1 (25%)	2 (100%)	0	0	1 (100%)	0.33
CV-A16 (2)	1 (50%)	1 (50%)	2.82 (0.07-inf);0.46	1 (50%)	1 (50%)	0	0	0	0	1
EV-A71 (23)	9 (39%)	14 (61%)	2.27 (0.84-Inf); 0.093	1 (33%)	2 (66%)	0	3 (100%)	8 (47%)	9 (53%)	0.51
CV-A6 (13)	1 (8%)	12 (92%)	0.20 (0.009-inf); 0.99	0	5 (100%)	1 (13%)	7 (87%)	0	0	1
EV (21)	1 (5%)	20 (95%)	0.10 (0.0047-inf); 0.99	0	10 (100%)	0	7 (100%)	1 (25%)	3 (75%)	0.19
Negative PCR (21)	6 (29%)	15 (71%)	1.15 (0.38-inf); 0.50	0	8 (100%)	3 (50%)	3 (50%)	3 (43%)	4 (57%)	0.057

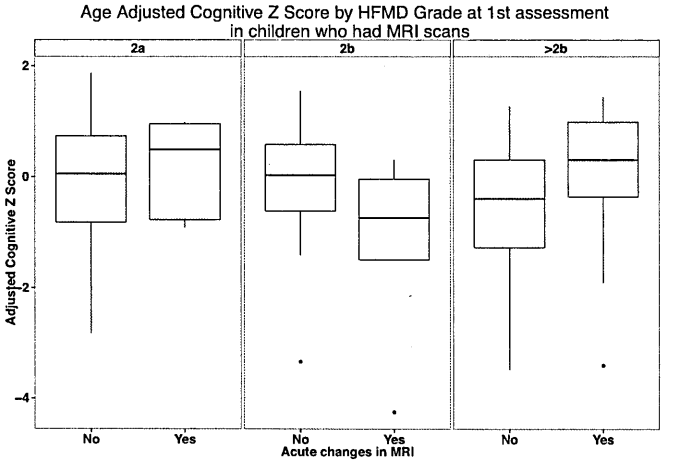
[9] Fisher's exact test

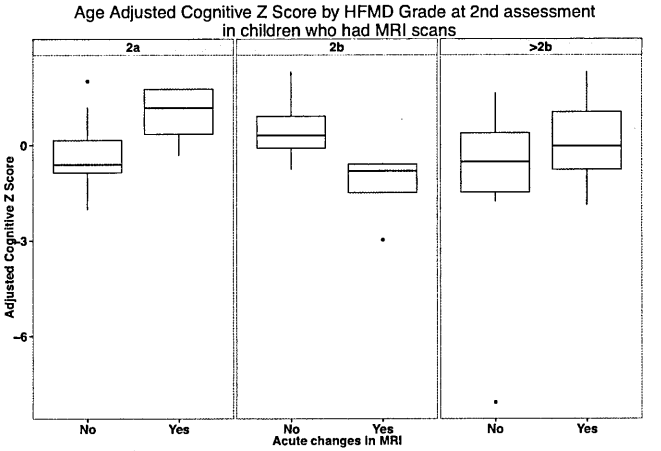
Table 5.7 shows the odds of finding an MRI change in a child with CV-A10 is above 8 times that compared to a child with non CV-A10 virus, and this is significant. No other virus was significant.

5.3.3 MRI and neurodevelopmental outcomes

The numbers of cases scanned within each grade was small, limiting the strength of this analysis. Nonetheless, looking at scores within each grade, adjusted for age, suggests that acute MRI changes are associated with worse cognitive, fine and gross motor scores at the second neurocognitive assessment for children with grade 2b disease (Figures 5.1-5.5). Surprisingly, after adjusting for age, gender, maternal education and stunting, between the two assessments there were significantly improved cognitive Z scores from the grade 2a with acute MRI changes, but significantly worse Z scores for fine and gross motor domains at the second assessment for those with grade 2b disease. (Table 5.8)

Figure 5.1 Plots of cognitive Z scores by MRI changes identified or not for each HFMD grade (grade>2b includes grade 3 and 4) adjusted for age only





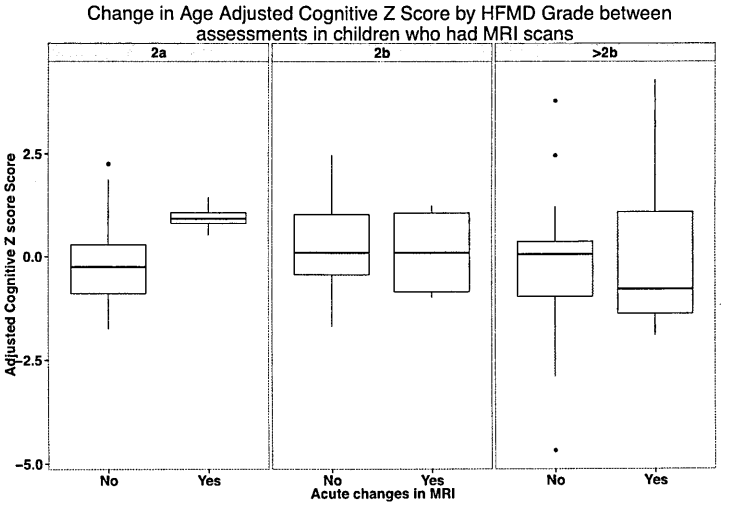
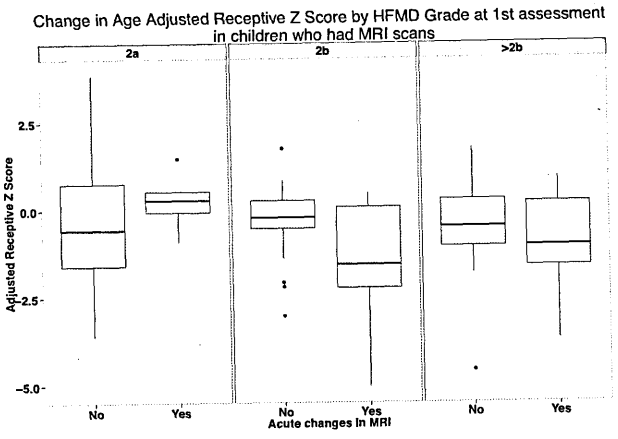
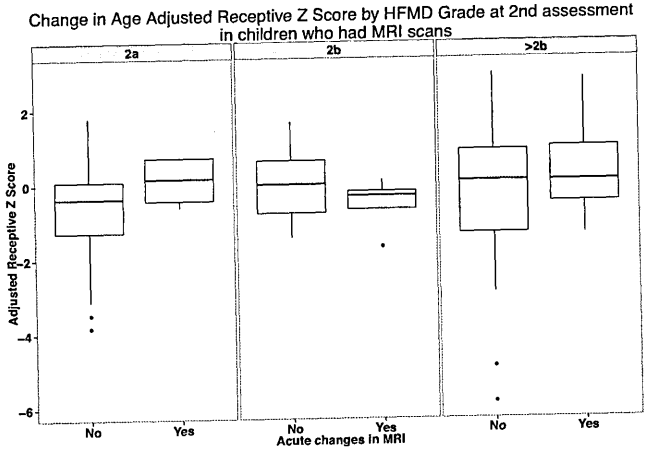


Figure 5.2 Plots of receptive language Z scores by MRI changes identified or not for each HFMD grade (grade>2b includes grade 3 and 4) adjusted for age only





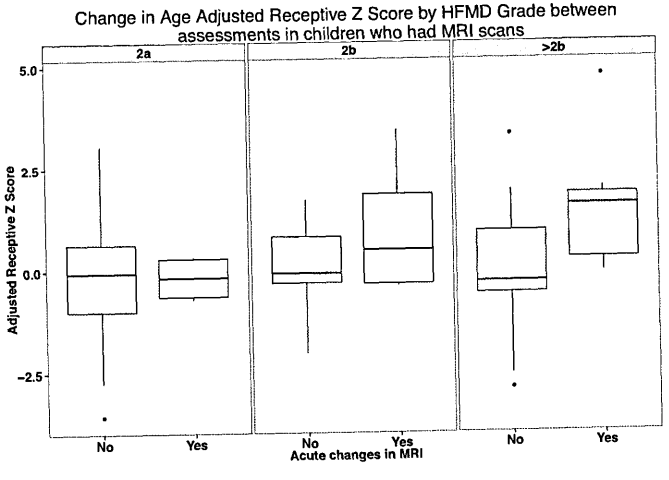
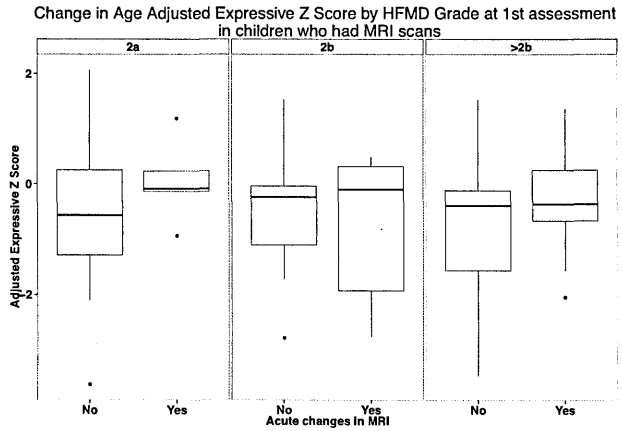
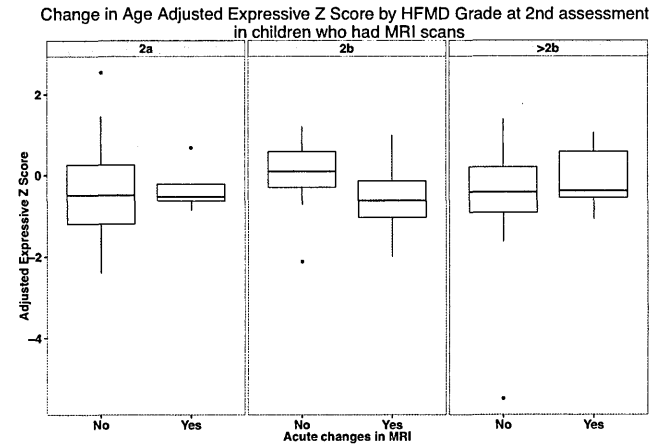


Figure 5.3 Plots of expressive language Z scores by MRI changes identified or not for each HFMD Grade (grade>2b includes grade 3 and 4) adjusted for age only





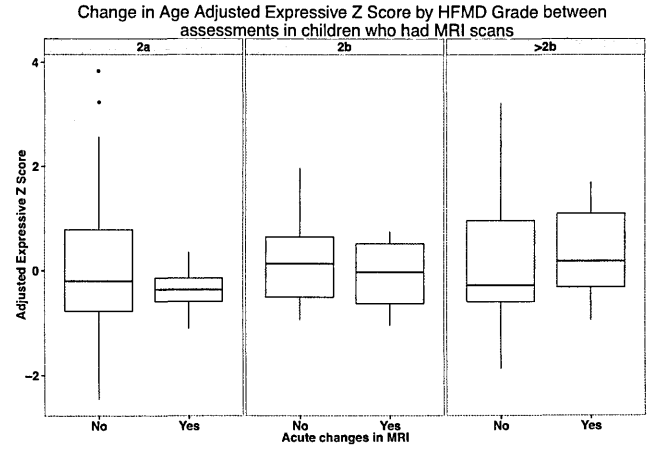
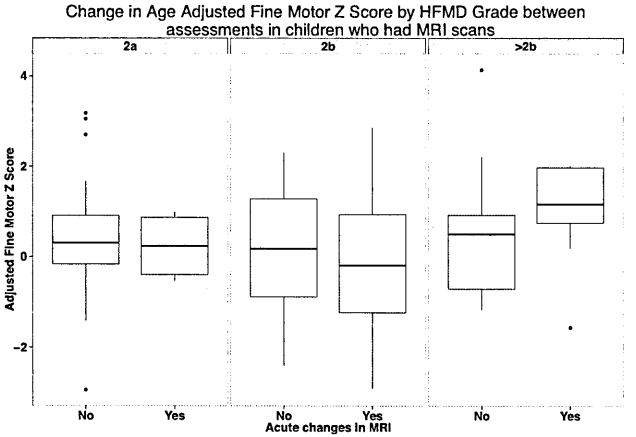
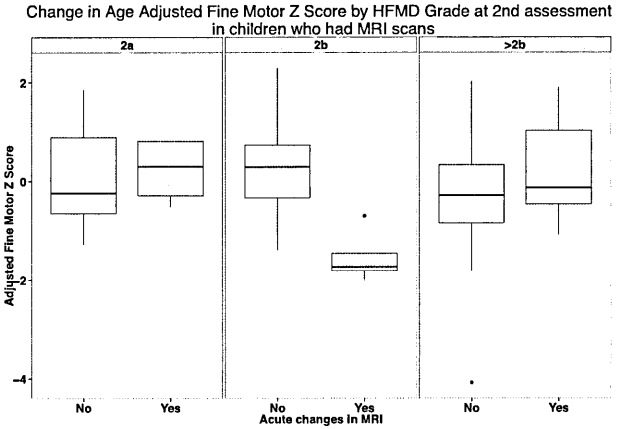


Figure 5.4 Plots of Fine motor Z scores by MRI changes identified or not for each HFMD grade (grade>2b includes grade 3 and 4) adjusted for age only





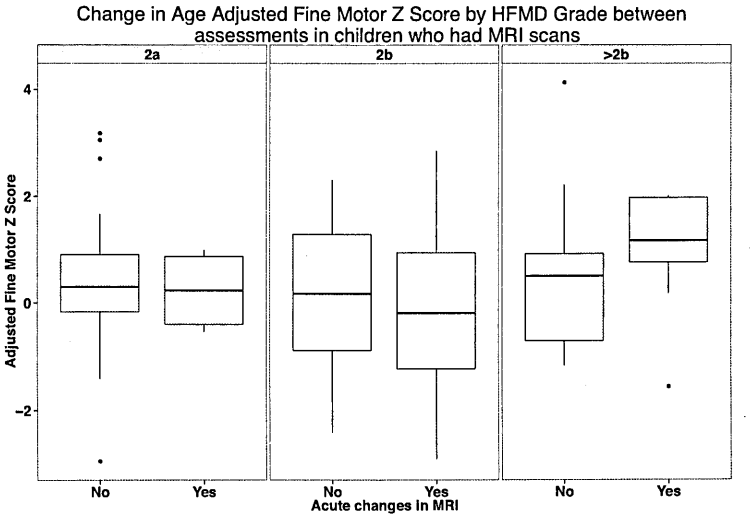
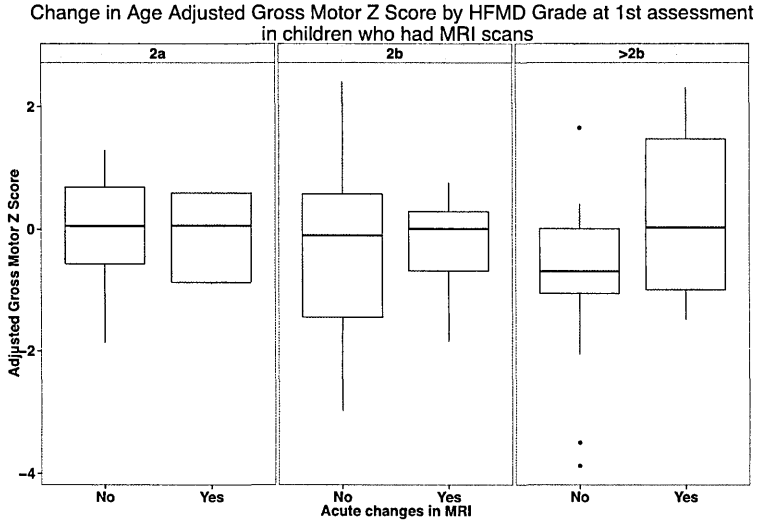
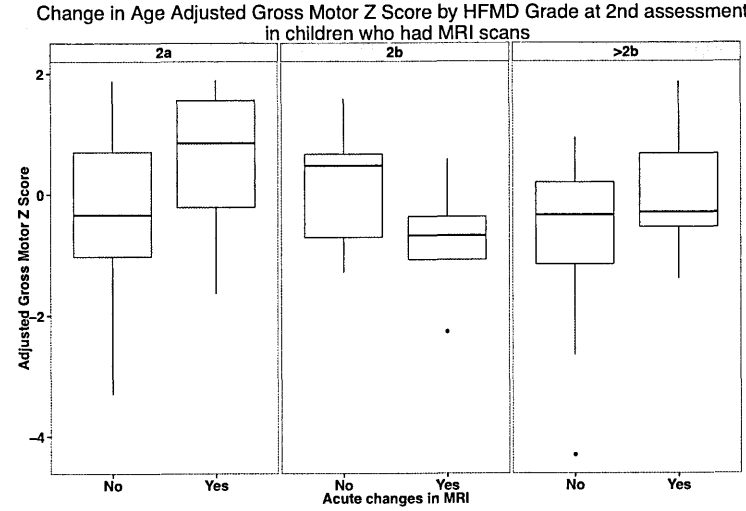


Figure 5.5 Plots of Gross motor Z scores by MRI changes identified or not for each HFMD grade (grade>2b includes grade 3 and 4) adjusted for age only





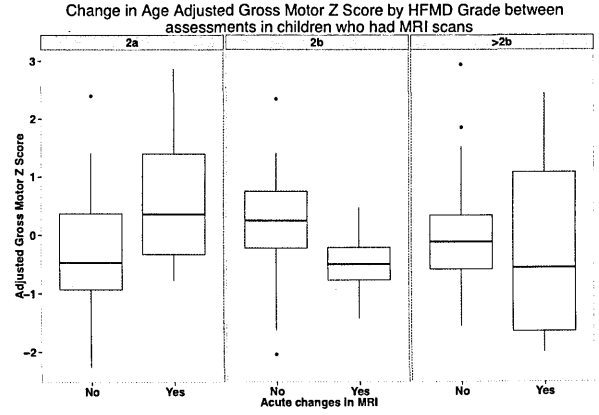


Table 5.8 Adjusted median and range of Bayley III Z score by domain and time tested

Z scores	Cognitive Z score (Coefficient (95%CI); p-value)		
Change vs. No change MRI	1st Assessment	2nd Assessment	Difference in scores
	Multivariate	Multivariate	Multivariate
Grade 2a	0.023 (-1.33 to 1.38); 0.97	1.20 (0.18 to 2.22); 0.023 *	1.27 (0.046 to 2.450); 0.043 *
Grade 2b	-0.91 (-2.32 to 0.50); 0.19	-1.42 (-3.10 to 0.25); 0.086	-4.14e-01 (-3.20 to 2.37); 0.74
Grade >2b	0.77 (-0.49 to 2.03); 0.22	1.09 (-0.50 to 2.70); 0.17	0.32 (-1.28 to 1.92); 0.67

Z scores	Receptive Z score (Coefficient (95%CI); p-value)		
Change vs. No change MRI	1st Assessment	2nd Assessment	Difference in scores
	Multivariate	Multivariate	Multivariate
Grade 2a	0.58 (-0.89 to 2.046); 0.42	0.51 (-0.99 to 2.018); 0.49	0.069 (-1.47 to 1.61); 0.93
Grade 2b	-1.36 (-2.98 to 0.26); 0.093	0.13 (-1.62 to 1.88); 0.87	1.092 (-1.97 to 4.15); 0.44
Grade >2b	-0.39 (-1.69 to 0.91); 0.54	1.29 (-0.063 to 2.65); 0.060	1.68 (0.27 to 3.098); 0.023 *

Z scores	Expressive Z score (Coefficient (95%CI); p-value)		
Change vs. No change MRI	1st Assessment	2nd Assessment	Difference in scores
	Multivariate	Multivariate	Multivariate
Grade 2a	0.41 (-1.086 to 1.90); 0.58	0.17 (-1.26 to 1.60); 0.80	-0.148 (-2.13 to 1.83); 0.88
Grade 2b	-0.51 (-1.68 to 0.66); 0.37	-0.081 (-1.57 to 1.41); 0.90	0.50 (-0.79 to 1.80); 0.40
Grade >2b	0.097 (-0.98 to 1.18); 0.85	0.72 (-0.43 to 1.87); 0.21	0.62 (-0.46 to 1.70); 0.24

Z scores	Fine Motor Z score (Coefficient (95%CI); p-value)		
Change vs. No change MRI	1st Assessment	2nd Assessment	Difference in scores
	Multivariate	Multivariate	Multivariate
Grade 2a	0.46 (-0.75 to 1.67); 0.44	-0.040 (-1.14 to 1.058); 0.94	-0.35 (-2.029 to 1.33); 0.67
Grade 2b	-0.38 (-1.84 to 1.077); 0.59	-2.46 (-4.71 to -0.21); 0.035 *	-1.84 (-5.50 to 1.82); 0.29
Grade >2b	0.41 (-0.91 to 1.74); 0.52	0.87 (-0.43 to 2.16); 0.18	0.45 (-0.81 to 1.72); 0.46

Z scores	Gross Motor Z score (Coefficient (95%CI); p-value)		
Change vs. No change MRI	1st Assessment	2nd Assessment	Difference in scores
	Multivariate	Multivariate	Multivariate
Grade 2a	-0.19 (-1.094 to 0.72); 0.67	0.42 (-1.01 to 1.85); 0.55	0.76 (-0.82 to 2.34); 0.33
Grade 2b	-0.22 (-1.80 to 1.36); 0.77	-1.22 (-2.25 to -0.18); 0.026 *	-0.28 (-2.72 to 2.16); 0.80
Grade >2b	0.82 (-0.33 to 1.96); 0.15	0.43 (-0.62 to 1.49); 0.40	-0.38 (-1.54 to 0.77); 0.49

5.3.4 Case Studies

Four case studies are described with MRI images of changes identified.

Figure 5.6 MRI Case 10.

Male, 34 month old child, grade >2b, EV-A71 PCR positive. Scan at day 7 of illness. Presented at day 3 of illness with rash and ulcers in the mouth. He developed hypertension and was treated with a course of immunoglobulin, intravenous milrinone for 2 days and magnesium sulphate for 3 days. Discharged after 5 days in hospital with complete recovery. (a) Axial FLAIR showed hyperintense signal at the dentates, also visible on (b) sagittal T2WI.

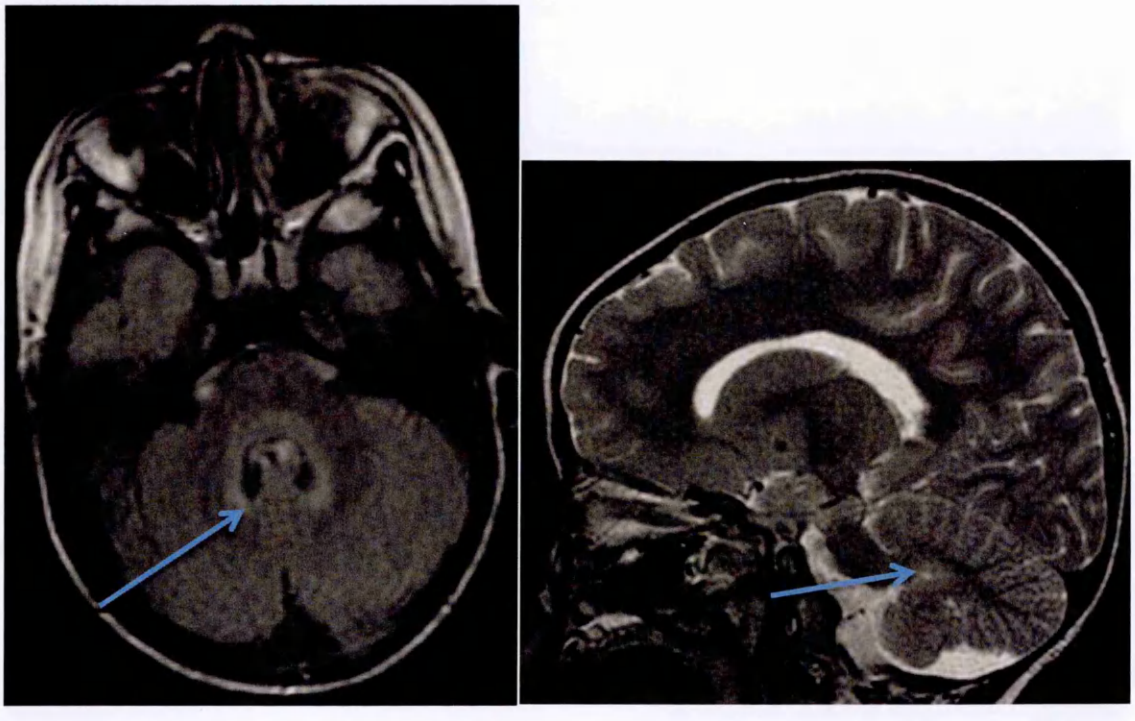


Figure 5.7 MRI Case 18.

Male, 6 month old child, grade >2b, EV-A71 PCR positive. Scan at day 21 of illness. No rash at presentation, day 3 of illness. He developed hypertension and respiratory distress. He was treated with a course of immunoglobulin, ventilated for 6 days and administered intravenous milrinone for 2 days and magnesium sulphate for 3 days. Discharged after 19 days in hospital with complete recovery. Hypointense lesion ponto-medullary junction (a) sagittal and (b) axial T2WI.

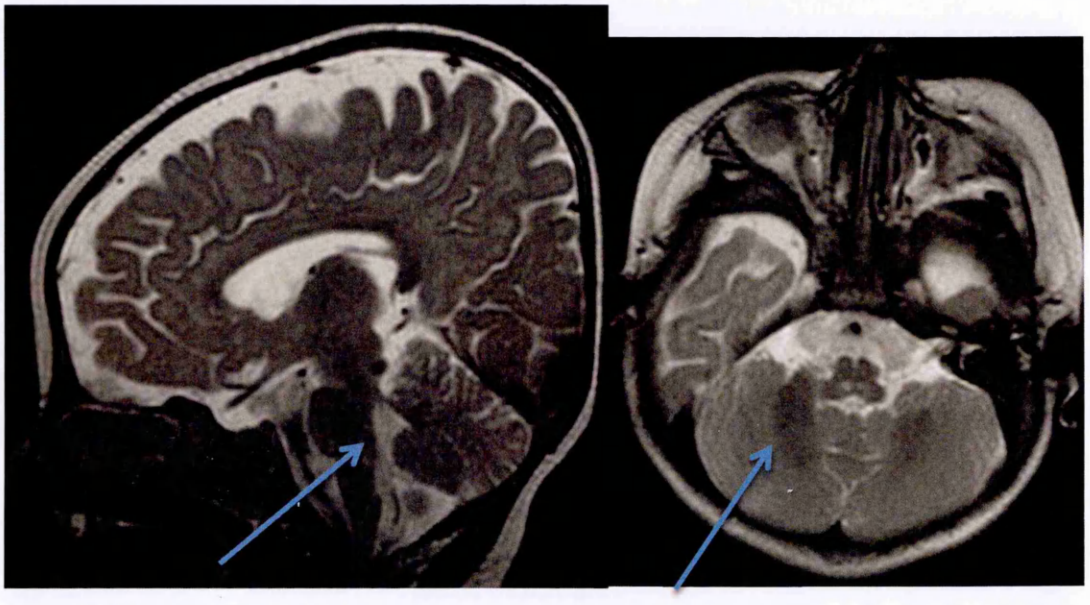


Figure 5.8 MRI Case 43.

Male, 22 month old child, grade >2b, negative PCR. Scan at day 6 of illness. Fever, cough, myoclonus on day 1 of illness. He was treated with a course of immunoglobulin. Discharged after 6 days in hospital with complete recovery. Hyperintense lesion (a) dentates Axial FLAIR and (b) + (c) axial T1WI.

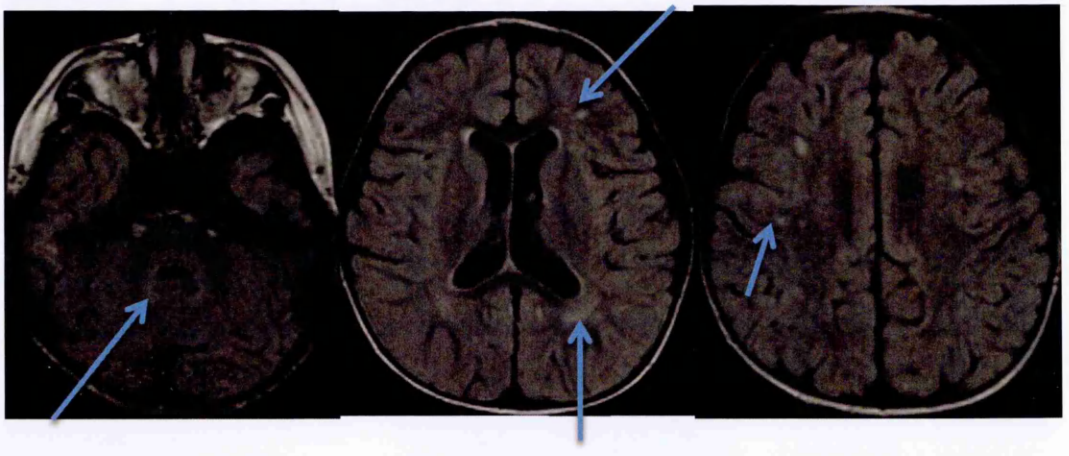


Figure 5.9 MRI Case 79.

Male, 15 month old child, grade >2b, EV-A71 PCR positive. Scan at day 8 of illness. Fever, cough, runny nose, vesicular rash, mouth ulcers and myoclonus on day 3 of illness. He was treated with a course of immunoglobulin. Discharged after 11 days in hospital with complete recovery. Normal appearance of dentate nuclei for age, scattered hyperintense foci on axial FLAIR.

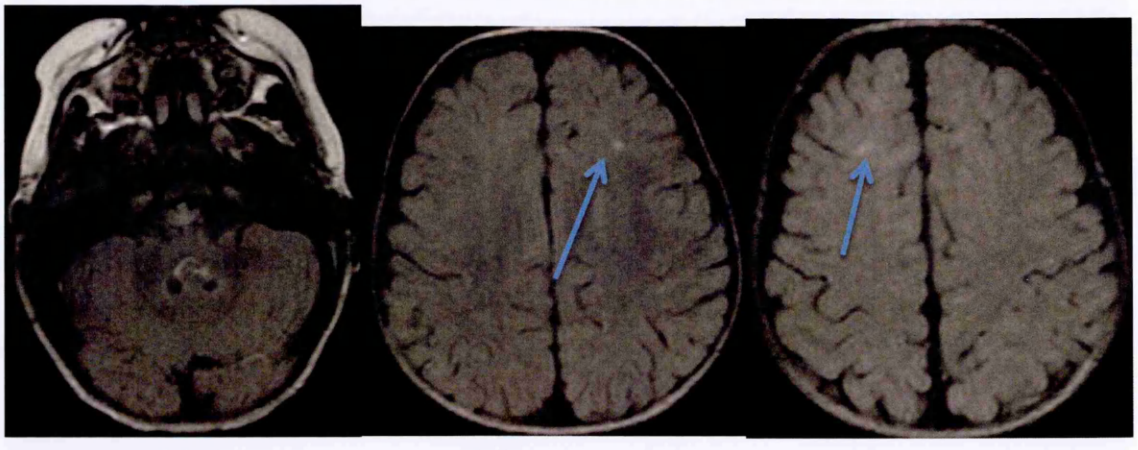
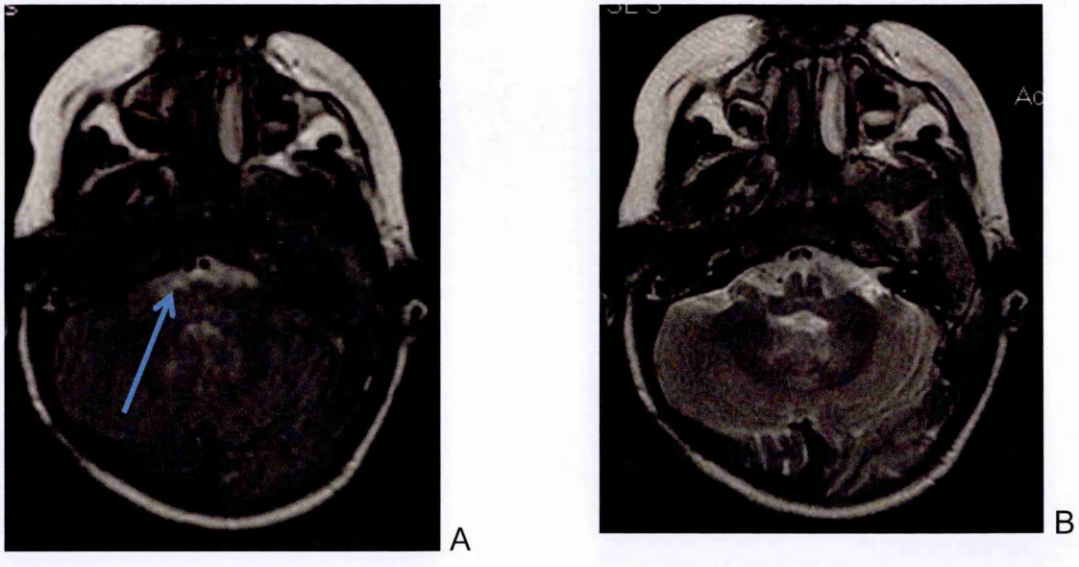


Figure 5.10 MRI Case 01.

Female, 35 month-old child from an ethnic minority group in Dak Nong. She was admitted on day 2 of illness following transfer from a provincial hospital with a GCS of 7/15, scored grade 3 due to right limb weakness and left 6th and 7th cranial nerve palsy (lower motor neuron), EV-A71 RT-PCR positive. Her mother had primary education only. She progressed to grade 4 within one day of admission due to respiratory failure and required ventilation. She was scanned at day 42 of her illness following recovery. MRI abnormalities were noted at the pontomedullary junction on axial FLAIR sequence (A) but not consistent on axial T2WI (B).



5.4 Summary

This is the first study to prospectively evaluate severe HFMD cases including grade 2a cases. I identified MRI changes in 5/32 (16%) Grade 2a cases, 6/26 (23%) Grade 2b cases and 12/29 (41%) >Grade 2b cases. Changes in the posterior aspect of the pons and novel findings of subcortical white matter changes were found in <10% of >grade 2b cases. Changes in the dentate nuclei were present in all severity grades, but was significantly different between HFMD grades, with an association with more severe disease. Deep white matter changes were present in all HFMD grades, and were not significantly different between grades. Of note, the odds of a child with MRI changes having detectable CV-A10 was significantly higher as compared to non-CV-A10 cases, and the highest proportion of CV-A10 cases was in grade 2a.

There were no significant differences in the proportions of children presenting with rash, mouth ulcers and neurological symptoms between the cases with MRI changes and those without.

In one MRI case, who had a scan performed late in the illness (day 21), hypointense lesions were identified on axial FLAIR, which was not seen in scans performed earlier in the illness.

Comparing the scores within each grade, between those with MRI changes and those without, revealed significantly worse scores for those with grade 2b disease for fine motor and gross motor domains at the second assessment, but, interestingly, better cognitive scores at the same time point. This variability may be a chance finding reflecting the small sample size but warrants further

investigation. Outcomes at discharge did not differ between those with MRI changes and those without within each grade.

5.5 Discussion

The literature reports that lesions in the dorsal pons, medulla oblongata and dentate nucleus are the stereotypic areas affected by EV-A71 HFMD-associated brainstem encephalitis. In this study, the dentate nucleus was affected in all severity grades but were significantly more common in those with grade 2b disease or worse. Subcortical white matter lesions on T2WI and FLAIR identified in grade >2b disease are not described in the HFMD literature, but I have identified these occurring in 16%, 15%, 17% of grade 2a, grade 2b, >grade 2b respectively.

The study results suggest the stereotypic findings of EV71 are not necessarily specific for EV-A71 but also occur in CVA10 and more rarely CVA6 infected cases. The significantly higher rates of MRI changes in CVA10 cases must be interpreted with caution in view of the sampling bias, where more EV-A71 cases were scanned overall compared to the other viruses.

MRI abnormalities in grade 2a were predominately within deep white matter. Adjusted Bayley scores identified there was an improvement in cognitive scores between assessments for this group. In more severe grades, there was a higher prevalence of abnormalities of the dentate nuclei of the cerebellum. Despite there being no significant change in Bayley scores between assessments in grade >2b, there were differences between assessments for grade 2b fine and gross motor

scores. Due to small sample sizes it is difficult to explore further. However, the heterogeneity within grade 2b may suggest that this group should be studied in greater detail, as MRI imaging may be of prognostic value. Future work should investigate the effect on scores of grade 2b with specific abnormalities, such as in the dentate nuclei compared to grade 2b with no abnormalities. It would be advisable to have a large enough cohort to determine whether age of onset of disease also makes a difference to prevalence of MRI abnormalities and impact on scores.

In essence we agree with Lee's cautious note in the introduction, that MRI changes are not obviously prognostic or have a clear correlation with clinical disease.

6. Chapter 6 Discussion

6.1 Introduction

Since the 1990s HFMD has emerged as an additional encephalitis threat to children under the age of five years in the Asia Pacific region. The specific encephalitis threat is commonly reported to be mainly associated with EV-A71 infection, with fatality ensuing from neurogenic cardiopulmonary complications. It is a threat that could overwhelm public health, education and rehabilitation services or stay hidden from view as minor impairment results in failure to progress at school or withdrawal from school.

The HFMD literature focuses on virologically positive EV-A71 cases and fails to encompass all cases clinically diagnosed with severe HFMD. This limits the generalisability of the outcome data from the literature, as many viruses are co-circulating during HFMD seasons and/or epidemics. The study aimed to evaluate what outcomes were associated with clinically severe HFMD, irrespective of virological diagnostics, which represents the real situation clinicians are facing during management and follow-up of children in settings where viral diagnostics are not routinely performed.

My study tested the following hypotheses:

1. Children affected by severe HFMD (grade 2a, 2b and >2b as per Vietnamese Ministry of Health classification) under the age of 4 years have lower cognitive, language and motor scores than a comparative group of

healthy children, as measured by internationally recognised adapted tools and measured a week post discharge and at 6 months after discharge.

2. Children with severe HFMD and MRI changes during the acute phase of the illness predict lower outcome scores at 6 months after discharge compared to severe HFMD children with no MRI changes.

I ran this prospective study from the start acknowledging that a suitable outcome measure was lacking. I decided to choose a detailed, standardised assessment test to comprehensively evaluate cognitive, language and motor scores. One major barrier to developing outcome tools is the lack of child development resources, and more specifically of trained staff. This study had to train and develop local professionals in a field that is evolving in Vietnam.

My thesis describes the process of adapting and validating Bayley III for Vietnam, comparing the study raw scores to the original and highlighting the methodological issues in validating a tool for a different culture. I deconstructed the tool to the underlying factors being measured and statistically model relationships between the observed variables or test items and the underlying factors. This procedure is standard for psychometric literature but is poorly understood by allied professionals who routinely use child developmental tools for research.

My thesis tests the above hypotheses through a prospective cohort study, with a healthy comparison group recruited from the district were the majority of HFMD are admitted from at HTD. This health comparison group was used to validate the Bayley III and also create Z scores form which to compare the HFMD cases. Two methods were used to evaluate differences in Z scores. Firstly linear regression

comparing all HFMD grades to healthy group. The limitation of linear regression is when the covariate distributions in each group differed significantly. In my study, there were significantly different proportions of maternal education in the healthy cohort compared to HFMD grade 2a. Propensity weighting balancing of covariates aimed to limit the imbalance in covariates without wasting the participants. This was used to balance the healthy group and grade 2a and then to balance between the different severities of HFMD. Comparison between grade 2a and grade 2b and >2b allowed improved balance in covariates than when balanced with the healthy group, permitting more robust analysis.

Additionally I investigated whether MRI changes were associated with more severe disease, were as stereotypical as previously described and if they were associated with poorer neurodevelopmental outcomes. The literature suggests mainly ascending motor tract lesions in MRI scans, supporting the hypothesis of retrograde axonal transport of EV-A71 into the brainstem.[236] A prospective study is needed to understand whether current findings are due to bias in sampling severe cases and only EV-A71 cases.

6.2 Results: Adaptation and validation of Bayley III for Vietnam

This is the first study to psychometrically evaluate the validity and reliability of a Bayley III adaptation in an LMIC. Despite widespread use of Bayley III in LMICs, few have looked into whether the underlying structure of the test is affected by adaptation. This may avoid the problems faced by Rie et al. when using the previous version of the Bayley (BSID II) in Congo. They identified 24.4% severely delayed children in their healthy control group when using US references. The

authors acknowledge the different environments, parenting styles, and access to toys may have influenced these findings.[410] However, they state they deliberately used a direct translation of a western CDAT without cultural adaptation to compare between groups in the same setting and then compare between past and future studies in other settings.[410] This statement underlies the lack of understanding of the structure and appropriate practice for interpreting results. It is clear that this method does not ensure that the tool is measuring the same underlying ability in all settings, and strict caution should be used when comparing different settings. In Malawi, due to similar high levels of deficits detected by Bayley III, re-standardisation was carried out.[402] However, the pattern of the raw scores with age by domain is very different to that of the original US version, and leaves open to debate whether the Malawian tests are measuring the same underlying factors as the original, and whether the test is reliable and valid. A more recent tool developed by the Intergrowth project[411] had set criteria for what domains the tool tested, the duration of test and ease of application for both high and low income countries. The test was designed with expert consensus, which uses items deemed suitable from a panel of experienced experts. Many of the items in the new tool are from Bayley III, which many of the expert panel have used or are familiar with for research worldwide. Hence their experience is mainly with Bayley items and this influences their contribution when developing new fit-for-purpose tools. Psychometric validation of Bayley III is not routinely done in LMICs, so caution should be used when items are chosen based on expert opinion without psychometric analysis on the perception of the item being universally applicable. Depression or anxiety scales undergo detailed psychometric evaluation when used in different ethnic groups, as is familiar with test developers in the field. Child development tools have been developed by psychologists, but are widely used by paediatricians and allied health

professionals who have only limited understanding of test structure. The implications are highlighted by the difficulty in comparing Bayley III to previous versions. The BSID II measured two constructs: psychomotor and mental index, and the Bayley III measures three. It has been assumed that since both versions have standardised scores, that they should be directly comparable. However there appears to be underestimation of impairment with Bayley III in Australia, compared to BSID II.[412] This difference may be compounded by the fact that in essence, the tests are measuring different constructs, making it difficult to translate one standard score into another. Additionally, there was no underestimation when using the mean and standard deviations (i.e. Z scores) of a control group rather than the standardised scores for Bayley III.

It is unclear how to meaningfully interpret changes in raw score on Bayley III. Makrides et al. ran a multicentre randomised controlled trial of dietary docosahexaenoic acid (DHA) in pregnancy using BSID II as an outcome measure. They reported that "Studies showing differences between nutritional or environment interventions of 4 to 5 points or greater have been catalysts for changes in health policy" and used this difference to calculate sample sizes.[413] Such statements highlights the problems of interpreting findings based on raw scores and the lack of clarity of appreciating the variability of raw scores. Calculating Z scores for group comparison allow meaningful conclusions.

Understanding issues such as gender measurement invariance is important but has never been done for Bayley III. Gender differences should be determined as true difference in ability, or difference due to gender differentiation in response to test item. Makrides et al. ran a trial of high versus low dose DHA in pregnancy using BSID II as the outcome measure and were puzzled by the lack of

responsiveness in boys compared to the girls.[414] Measurement invariance by gender was never mentioned in the study and it was perceived that this was a true gender difference in response to DHA dose. I have no comparable gender information on Bayley III but I have highlighted that gender issues in child development are important and need to be adjusted for in analysis.

This study demonstrated that the Vietnamese adaptation of the Bayley neurocognitive tool is reliable and psychometrically valid. This has implications for interpreting differences between groups in this study and has enormous potential as a clinical and research outcome measure for future studies in Vietnam.

It is accepted that for research a control or healthy comparison group should be used to create Z scores. Different Z score changes are used to correlate with different levels of impairment. This method assumes that the adapted score is measuring the same underlying constructs as the original version. This is the first study in an LMIC setting to test this assumption. The Bayley tool is considered one of the most robust measures in early childhood, with extensive literature discussing its validation and reliability. The publishers of Bayley III describe the tool to measure hypothesised constructs of cognition, language and motor domains. This is the first study to evaluate the same psychometric structure following adaptations and look at gender measurement invariance (whether the same constructs are measured in both genders). The results indicate that the best-fit models for the Vietnamese Bayley score fit the original US version and the distribution of raw scores between countries do not differ significantly (using limited US data from the Bayley III manual). Furthermore, the results show that the test measures the same underlying constructs in both genders, but there are differences in the underlying ability, which is confirmed by the literature in this age

range. This also confirms the need to adjust for gender in further analysis. The methods used are well described in the psychometric literature, but are novel in the application for early childhood development.

The study is limited in that the sample size is not adequate to establish measurement invariance between age groups. However, it is worth noting that the original developers did not do this, although the best hypothesised model was tested in different age groups and found to be similar.

The individuals assessed in this Vietnamese Bayley study were predominately individuals living in urban or semi-urban settings. There are research groups using the Bayley tool in more rural or regional specific groups. It would be of interest to pool data to establish measurement invariance by geographical location and potentially to look at age measurement invariance. It is also possible that the results may differ between rural and urban settings and it would be informative to explore this potential variation in more depth

An understanding of psychometric test evaluation and development is a necessity for allied professionals using these tools for research. This study explains in depth the process for robust psychometric evaluation and provides methodology that can be replicated in both low and high-income countries.

Retrospective studies looking at outcomes following EV-A71 infection have been limited by the lack of locally valid adaptations. Differences in outcomes described in the literature may reflect poor adaptation of the psychometric tool used rather than true differences in scores. This validation study is an essential step prior to investigating the main question of neurocognitive outcome in severe HFMD. In

addition, the adaptation methodology described here has the potential to assist future studies in low and middle-income countries.

6.3 Results: Outcomes following severe HFMD

My study demonstrated that severe HFMD was not associated with lower Z scores compared to a healthy comparison group after adjusting for confounders. The literature in this area typically focused on the most severe cases of HFMD, usually associated with EV-A71 infection, who may have profound disabilities that limit their ability to cooperate with detailed assessments such as the Bayley tool. The number of severe cases in a hospitalised population has previously been poorly documented. These cases are relatively rare and in this study only 1 child (0.004%) had significant neurological complications that affected their ability to participate in the Bayley at the first assessment. At the second assessment their Z score was between -1.1 to -1.9. The added difficulty was that this child was from an ethnic minority group that did not use Vietnamese as their first language meaning it was not possible to reliably assess the language domain.

Previous studies of severe HFMD outcomes focused on EV-A71 during HFMD outbreaks. As reviewed in section 1.5.5 and listed in Table 1.7, none of the published studies used Z scores and only one used a healthy comparison group, preferring to state the proportion of cases which had impairments. Different classification of HFMD severity between retrospective studies makes direct comparison unreliable. Stating proportions only on those with obvious difficulties means more subtle defects may have been missed by crude assessments. None of the studies describe in detail whether the outcome measure had been locally

validated. These retrospective studies fail to estimate the true burden of outcomes following HFMD outbreaks, where virological diagnostic facilities are often limited.

The literature suggests it is the cases with cardiopulmonary complications (grade 4) that have poor outcomes. Although classification differs between settings, this study enrolled 7 (0.03%) grade 4 cases of which 5 (71%) had fully recovered neurologically by discharge. Our data suggest that the majority of grade 4 cases recovered, and that only a small subset of these severe cases have neurological complications. In my study, 2 grade 2b cases had acute neurology evident on admission, which resolved by discharge. There was one child with grade 4 disease who had cranial nerve palsies on admission, which persisted until follow up. In the literature, cases with persistent neurology are usually identified as having infection with EV-A71. These cases are usually grade 4 as in the study described here. In this study population, there were 45 cases EV-A71 infection, 13(29%) of which had grade 2a disease, 10 (22%) had grade 2b disease, 18 (40%) had grade 3 disease, and 4 (9%) had grade 4 disease. Huang et al. identified neurological sequelae in 20% of the EV-A71 cases presenting with cranial nerve involvement.[282] The study population had 1 grade 2b (PCR: CV-A6) and 1 grade 4 case (EV-A71) with cranial nerve involvement and the percentage of EV-A71 cases presenting with cranial nerve involvement was 2% (1/45). This suggests that cranial nerve involvement in this prospective study group was not common in EV-A71 infected cases.

EV-A71 is considered in the literature to be associated with more severe clinical course and poorer outcomes. My prospective study clarifies that EV-A71 is indeed associated with more severe disease and abnormal neurology, but 75% (3/4) of the most severe cases had normal neurology at discharge and did not

significantly differ in neurocognitive outcomes by 6 months when compared to healthy controls. These results suggest it is only a small subset of children with the most severe grade 4 cardiopulmonary complications, who may be at risk of poorer outcomes at discharge and at 6 months. This has important implications for understanding the disease burden. These data suggest that the major disease burden is associated with the costs of acute hospitalization and the difficulty in predicting those most at risk of disease progression (resulting in high rates of hospitalization for observation). This also has vaccination implications, as clearly there are other viruses associated with severe HFMD and hospitalisation. In my study, the virological diagnosis of the 242 cases were 64 (26%) untypable or other viruses, 55 (23%) enteroviruses, (19%) EV-A71, 44 (18%) CVA6, 27 (11%) CVA10 and 7 (3%) CVA16. The majority of the cases were untypable, hence outcome results based on clinical severity is important for clinicians at the front line.

My study is limited by the small numbers of cases with severe disease. This may be due to larger public awareness about HFMD, better public health information on hygiene practices and better acute management of cases. The Vietnamese guidelines can into force following the 2011 outbreak in order to standardise care, and hence differences in management pre 2011 and after in Vietnam and surrounding regions may also limit outcome data between those two time periods.

The socioemotional domain, which was not assessed, is an important functional area in determining social acceptability and social participation. There is a concern in the literature regarding the development of attention deficit hyper-activity disorder (ADHD) symptoms[232] in later childhood and my study was not designed to assess this area. It is possible that infection at specific ages may

result in a greater impact on language development (for example, in those aged less than two years). My study identified some reduced language scores in specific age groups but these changes were not persistent. These results may reflect a lack of cooperation from a degree of anxiety rather than a true poor score. Unfortunately, the study recruited insufficient numbers to compare scores in cases aged under and over two years. This is an important area to address in future studies.

6.4 Results: Brain MRI findings in severe HFMD

This is the first study exploring acute MRI changes in the brain associated with HFMD. The study focused on children with less severe disease who were scanned within two weeks of illness onset. We made the MRI scans optional, as there was risk involved with routine use of sedation at the MRI facility. We did not adjust for this in sample size calculations. We were also limited in that we did not record reasons for declining participation in MRI, which would have been useful when designing future studies with paediatric MRI in this particular patient population. This does introduce bias in the MRI sample selection, but the comparison of the sample to the total study population did not show any significant difference in gender, age, level of maternal education, stunting, duration of hospitalisation, recovery, and virological detection.

My study was the first to include MRI scans of non-severe cases and identified acute changes in 5 (16%), 6 (23%) and 12 (41%) of grade 2a, 2b and >2b cases respectively. The study demonstrated that a variety of viruses were associated with MRI changes, with the novel finding of showing a significant association with CV-A10 detection, which was most prevalent in grade 2a cases. The dentate nucleus abnormality is well documented in the literature but the white matter

abnormalities are a novel finding. The sample that had MRI was representative of the enrolled cases in terms of age, gender and duration in hospital. Despite the small numbers, the grade 2b children who had MRI changes had significantly worse fine and gross motor scores at the second assessment after adjusting for maternal education and stunting. This was not identified in the other groups. This suggests there may be a motor prognostic value from MRI in a specific group of patients and should be researched further. It is also important to establish whether younger age influences MRI abnormalities and hence impacts on outcome. This would require a large cohort of infants participating in sequential scans to identify timing of resolution or on-going persistence.

The neuropathogenesis and neurotropism of the various enteroviruses appears to differ.[415] There is suggestion that particular EV quasispecies contribute to invasion of CNS and to different neuropathology patterns seen with MRI. It may be this phenomena contributing to the novel white matter abnormalities seen on the brain MRI scans. Clearly, a more in depth understanding of the neurotropism of the enteroviruses is an important area to address and these novel findings suggest the neurotropisms to be less stereotypical than previously described.

6.5 Conclusions

I have described the first construct validity adaptation study of the Bayley III tool in a low-income setting. I anticipate that the methodology used in the adaptation process has considerable potential to assist further research exploring the impact of HFMD but also a wide spectrum of other diseases of huge epidemiological importance in Vietnam and other LMIC settings. Secondly, my study identified that

– in a low incidence season with low proportions of EV-A71 detection and very severe cases - with current management strategies in Vietnam, severe HFMD (grade >2a) was not associated with significant neurocognitive sequelae at six months, with differences in Z scores within 2 standard deviations of the mean of the healthy comparison group. While my study was limited by a small sample size, the results have the potential to better inform models of disease burden in Vietnam and other settings where HFMD are endemic. Thirdly, the study found that MRI changes occur at grade 2a, 2b and >2b of HFMD. With small samples, the MRI changes were significantly associated with worse fine and gross motor outcomes at six months for grade 2b cases only. This group have 22% risk of disease progression to >2b[86], and MRI in this group may identify the children more likely to progress. The findings of white matter changes, not previously described associated with HFMD in the literature, raises questions about the neurotropism of the enteroviruses and the need to better understand the neuropathogenesis of these pathogens. This information would aid clinicians and future research on outcomes, by proposing more specific functional areas of development that may be affected, which would need evaluation in children as the developmental complexity progresses with age.

The Vietnamese adaptation of Bayley III demonstrates adequate reliability and validity for research purposes. This was the first evaluation of the construct structure of a Bayley III adaptation, but since the study means scaled scores were not similar to US, local norms are required. Pooling data from contemporaneous studies in one country is an opportunity to develop local norms for clinical use. Sharing of data between publishers and researchers across countries encourages cross-cultural validation of clinical outcome measures, but such practice is not standard.

Bayley III assessment is a recommended tool to determine further specialist support in the clinical setting in high-income countries. However there is limited psychometric evaluation of its invariance over age groups, gender and culture despite the frequency of its use in the literature. My study is an important addition to understanding adaptation of western tools and outlines appropriate methodology to evaluate this.

Bayley III is a complicated tool, requiring high level of assessor skill and training. This is often seen a major limitation for its use in LMIC. However, we found developing the tool in the research setting involves capacity building that is transferable to clinical practice.

6.6 Future Directions

The western literature on long-term outcomes of encephalitis is important to contextualise my study. In 2000, The Encephalitis Society carried out a UK postal survey to 1200 members with 400 respondents, of whom 139 had encephalitis in childhood.[416] All pathologies were included including herpes simplex virus, which is known to have a worse outcome. Mostly parents or carers completed the children's responses. 72% of the children reported that they were unable to 'fully resume their normal life', 44% had a statement of special educational. 53% of those who had contracted the disease as children required significant assistance or regular supervision with personal care tasks and/or had very limited mobility.

The most common difficulties identified in children were concentration and

attention problems followed by tiredness/fatigue; mood swings; and frustration and anger with difficulties with new learning but not with short term memory, planning and problem-solving. Hooper et al. identified problems with organising and planning daily living tasks and regulating emotions persisted over time and were associated with a high level of parental stress and anxiety.[417] One centre in Nottingham, UK had 13 referrals for rehabilitation following childhood encephalitis in 2005 of which 70% were made by the parents, the remainder by health professionals.[418] Even in this high income setting, parents of children who had encephalitis had difficulty navigating the referral pathway, accessing specialist services and receiving adequate support from health professionals.[418]

The paucity of long-term outcome data in encephalitis, suggest that there is long-term negative educational, employment and family impact.[419-421] My study identified differences between groups ranged within 2 standard deviations of the healthy children mean. The impact of even 1 standard deviation below mean on education may be more significant in a system where there are fifty children in a class using limited teaching methods and testing, with scarce specialist educational support. It may also affect poor families' decision to fund education of their child.[422] Behavioural difficulties may be managed with more punitive methods adding to parental stress.[418] Despite this study continuing till 18 months follow up, it is important to evaluate this impact by following-up the HFMD and healthy cohorts with an assessment at school age (age 7 years). This will more accurately determine whether more complex, higher functioning skills have been affected, whether minor cognitive difficulties have a significant impact on schooling in this setting, and to determine socio-emotional and adaptive behaviour difficulties, including self-reported difficulties.

MRI is increasingly used for prognostic information in premature neonates, although its sensitivity and specificity is highest with motor function, and limited with neurocognitive and behavioural function.[423] Interestingly this is also what was identified in this study. Newer quantitative white matter tract measures[424] require specialist skills but may be of benefit in grade 2b HFMD infants. We did not have the facilities to do this in this study, but the study findings suggest knowledge base would increase in pursuing this area further.

Recent work by Seydel et al. suggests transient rise in intracranial pressure in cerebral malaria may account for high fatality rates.[425] MRI during the acute phase identified severe brain swelling and a decrease in brain volume in survivors who initially had brain swelling. Although raised intracranial pressure is not a predominant feature in HFMD, prospective measuring of pressures has not been done. With the study MRI files, the next step could be to look at cerebral hemisphere swelling to help determine whether this area should be explored in future studies.

Carter et al., researching malaria, state rehabilitation must be considered on equal terms to preventative health.[426] Neuropsychological therapies in Vietnam are not included in the public health care system. However, there are effective public funded models of neurorehabilitation that can be delivered in low to middle resourced settings, such as the SARAH network in Brazil.[427] This is a network of neurorehabilitation services working with specialists in a multi-disciplinary team to "progressive care" with the aim to return to school or work. SARAH supports all brain injury including post encephalitis and emphasises research as integral to improving the services for the local population. Such success requires a vision of locally funded neurosciences; (neurology, neuroimaging, neuropsychology,

neurosurgery, neurorehabilitation), building capacity with evidence-based practical interventions suited for low resource settings. My study contributes part of the groundwork of robust CDAT development and local capacity building, working with hospital and community teams that may facilitate a future potential neurosciences network.

References

1. Seddon JH. Research Newsletter No. 2 1961.
2. Duff MF. Hand-foot-and-mouth syndrome in humans: coxackie A10 infections in New Zealand. Br Med J. 1968;2(5606):661-4.
3. Robinson CR, Doane FW, Rhodes AJ. Report of an outbreak of febrile illness with pharyngeal lesions and exanthem: Toronto, summer 1957; isolation of group A Coxsackie virus. Can Med Assoc J. 1958;79(8):615-21.
4. Kravis LP, Hummeler K, Sigel MM, Lecks HI. Herpangina; clinical and laboratory aspects of an outbreak caused by group A Coxsackie viruses. Pediatrics. 1953;11(2):113-9.
5. Alsop J, Flewett TH, Foster JR. "Hand-foot-and-mouth disease" in Birmingham in 1959. Br Med J. 1960;2(5214):1708-11.
6. Adler JL, Mostow SR, Mellin H, Janney JH, Joseph JM. Epidemiologic investigation of hand, foot, and mouth disease. Infection caused by coxsackievirus A 16 in Baltimore, June through September 1968. Am J Dis Child. 1970;120(4):309-14.
7. King AMQ. Virus taxonomy. Ninth report of the International Committee on Taxonomy of Viruses. 9 ed. Waltham (MA), USA: Academic Press; 2011.
8. The Pirbright Institute U. Picornaviridae.com 2016 [Available from: http://www.picornaviridae.com/.
9. Fujimoto T, Iizuka S, Enomoto M, Abe K, Yamashita K, Hanaoka N, et al. Hand, foot, and mouth disease caused by coxsackievirus A6, Japan, 2011. Emerg Infect Dis. 2012;18(2):337-9.
10. Huang WC, Huang LM, Lu CY, Cheng AL, Chang LY. Atypical hand-foot-mouth disease in children: a hospital-based prospective cohort study. Virol J. 2013;10:209.
11. Di B, Zhang Y, Xie H, Li X, Chen C, Ding P, et al. Circulation of Coxsackievirus A6 in hand-foot-mouth disease in Guangzhou, 2010-2012. Virol J. 2014;11:157.
12. Akiyoshi K, Suga T, Mori A. Enteroviruses in patients experiencing multiple episodes of hand, foot, and mouth disease in the same season in Kobe, Japan, 2011. Jpn J Infect Dis. 2012;65(5):459-61.
13. Hu YF, Yang F, Du J, Dong J, Zhang T, Wu ZQ, et al. Complete genome analysis of coxsackievirus A2, A4, A5, and A10 strains isolated from hand, foot, and mouth disease patients in China revealing frequent recombination of human enterovirus A. Journal of clinical microbiology. 2011;49(7):2426-34.
14. Ang LW, Koh BK, Chan KP, Chua LT, James L, Goh KT. Epidemiology and control of hand, foot and mouth disease in Singapore, 2001-2007. Ann Acad Med Singapore. 2009;38(2):106-12.
15. Hughes RO, Roberts C. Hand, foot, and mouth disease associated with Coxsackie A9 virus. Lancet. 1972;2(7780):751-2.
16. Feder HM, Jr., Bennett N, Modlin JF. Atypical hand, foot, and mouth disease: a vesiculobullous eruption caused by Coxsackie virus A6. Lancet Infect Dis. 2014;14(1):83-6.
17. Russo DH, Luchs A, Machado BC, Carmona Rde C, Timenetsky Mdo C. Echovirus 4 associated to hand, foot and mouth disease. Rev Inst Med Trop Sao Paulo. 2006;48(4):197-9.
18. Schmidt NJ, Lennette EH, Ho HH. An apparently new enterovirus isolated from patients with disease of the central nervous system. J Infect Dis. 1974;129(3):304-9.
19. van der Sanden S, Koopmans M, Uslu G, van der Avoort H. Epidemiology of enterovirus 71 in the Netherlands, 1963 to 2008. J Clin Microbiol. 2009;47(9):2826-33.
20. Melnick JL, Schmidt NJ, Mirkovic RR, Chumakov MP, Lavrova IK, Voroshilova MK. Identification of Bulgarian strain 258 of enterovirus 71. Intervirology. 1980;12(6):297-302.
21. Melnick JL. Enterovirus type 71 infections: a varied clinical pattern sometimes mimicking paralytic poliomyelitis. Rev Infect Dis. 1984;6 Suppl 2:S387-90.
22. Lum LC, Wong KT, Lam SK, Chua KB, Goh AY, Lim WL, et al. Fatal enterovirus 71 encephalomyelitis. J Pediatr. 1998;133(6):795-8.

23. Shindarov LM, Chumakov MP, Voroshilova MK, Bojinov S, Vasilenko SM, Iordanov I, et al. Epidemiological, clinical, and pathomorphological characteristics of epidemic poliomyelitis-like disease caused by enterovirus 71. J Hyg Epidemiol Microbiol Immunol. 1979;23(3):284-95.
24. Nagy G, Takatsy S, Kukan E, Mihaly I, Domok I. Virological diagnosis of enterovirus type 71 infections: experiences gained during an epidemic of acute CNS diseases in Hungary in 1978. Arch Virol. 1982;71(3):217-27.
25. Deibel R, Gross LL, Collins DN. Isolation of a new enterovirus (38506). Proc Soc Exp Biol Med. 1975;148(1):203-7.
26. Kennett ML, Birch CJ, Lewis FA, Yung AP, Locarnini SA, Gust ID. Enterovirus type 71 infection in Melbourne. Bull World Health Organ. 1974;51(6):609-15.
27. Blomberg J, Lycke E, Ahlfors K, Johnsson T, Wolontis S, von Zeipel G. Letter: New enterovirus type associated with epidemic of aseptic meningitis and-or hand, foot, and mouth disease. Lancet. 1974;2(7872):112.
28. Hagiwara A, Tagaya I, Yoneyama T. Epidemic of hand, foot and mouth disease associated with enterovirus 71 infection. Intervirology. 1978;9(1):60-3.
29. Ishimaru Y, Nakano S, Yamaoka K, Takami S. Outbreaks of hand, foot, and mouth disease by enterovirus 71. High incidence of complication disorders of central nervous system. Arch Dis Child. 1980;55(8):583-8.
30. Chonmaitree T, Menegus MA, Schervish-Swierkosz EM, Schwalenstocker E. Enterovirus 71 infection: report of an outbreak with two cases of paralysis and a review of the literature. Pediatrics. 1981;67(4):489-93.
31. Samuda GM, Chang WK, Yeung CY, Tang PS. Monoplegia caused by Enterovirus 71: an outbreak in Hong Kong. Pediatr Infect Dis J. 1987;6(2):206-8.
32. Chan LG, Parashar UD, Lye MS, Ong FG, Zaki SR, Alexander JP, et al. Deaths of children during an outbreak of hand, foot, and mouth disease in sarawak, malaysia: clinical and pathological characteristics of the disease. For the Outbreak Study Group. Clin Infect Dis. 2000;31(3):678-83.
33. AbuBakar S, Chee HY, Al-Kobaisi MF, Xiaoshan J, Chua KB, Lam SK. Identification of enterovirus 71 isolates from an outbreak of hand, foot and mouth disease (HFMD) with fatal cases of encephalomyelitis in Malaysia. Virus Res. 1999;61(1):1-9.
34. Lum LC, Wong KT, Lam SK, Chua KB, Goh AY. Neurogenic pulmonary oedema and enterovirus 71 encephalomyelitis. Lancet. 1998;352(9137):1391.
35. Lin TY, Chang LY, Hsia SH, Huang YC, Chiu CH, Hsueh C, et al. The 1998 enterovirus 71 outbreak in Taiwan: pathogenesis and management. Clin Infect Dis. 2002;34 Suppl 2:S52-7.
36. Ho M, Chen ER, Hsu KH, Twu SJ, Chen KT, Tsai SF, et al. An epidemic of enterovirus 71 infection in Taiwan. Taiwan Enterovirus Epidemic Working Group. N Engl J Med. 1999;341(13):929-35.
37. Sanders SA, Herrero LJ, McPhie K, Chow SS, Craig ME, Dwyer DE, et al. Molecular epidemiology of enterovirus 71 over two decades in an Australian urban community. Arch Virol. 2006;151(5):1003-13.
38. McMinn PC. An overview of the evolution of enterovirus 71 and its clinical and public health significance. FEMS Microbiol Rev. 2002;26(1):91-107.
39. Report. IAS. Hand, foot and mouth disease, 2000-2003, Japan. 2004 [Available from: http://idsc.nih.go.jp/iasr/25/295/tpc295.html.
40. Chan KP, Goh KT, Chong CY, Teo ES, Lau G, Ling AE. Epidemic hand, foot and mouth disease caused by human enterovirus 71, Singapore. Emerg Infect Dis. 2003;9(1):78-85.
41. Lin TY, Twu SJ, Ho MS, Chang LY, Lee CY. Enterovirus 71 outbreaks, Taiwan: occurrence and recognition. Emerg Infect Dis. 2003;9(3):291-3.
42. Tseng FC, Huang HC, Chi CY, Lin TL, Liu CC, Jian JW, et al. Epidemiological survey of enterovirus infections occurring in Taiwan between 2000 and 2005: analysis of sentinel physician surveillance data. J Med Virol. 2007;79(12):1850-60.
43. Ministry of Health S. Forum on Hand Foot and Mouth Disease (HFMD) in Asia-Pacific Region: Epidemiological, laboratory, Clinical and Public Health Aspects 2008 [Available from: http://www.aseanplus3fetn.net/doc/hfmd_singapore_redi.pdf.

44. Zhang Y, Tan XJ, Wang HY, Yan DM, Zhu SL, Wang DY, et al. An outbreak of hand, foot, and mouth disease associated with subgenotype C4 of human enterovirus 71 in Shandong, China. J Clin Virol. 2009;44(4):262-7.
45. Zhang Y, Zhu Z, Yang W, Ren J, Tan X, Wang Y, et al. An emerging recombinant human enterovirus 71 responsible for the 2008 outbreak of hand foot and mouth disease in Fuyang city of China. Virol J. 2010;7:94.
46. Tu PV, Thao NT, Perera D, Huu TK, Tien NT, Thuong TC, et al. Epidemiologic and virologic investigation of hand, foot, and mouth disease, southern Vietnam, 2005. Emerg Infect Dis. 2007;13(11):1733-41.
47. Organisation WH. A Guide to Clinical Management and Public Health Response for Hand, Foot and Mouth Disease (HFMD)2011 3rd April 2014. Available from: http://www.wpro.who.int/publications/docs/GuidancefortheclinicalmanagementofHFMD.pdf.
48. Kennett ML, Birch CJ, Lewis FA, Yung AP, Locarnini SA, Gust ID. Enterovirus type 71 infection in Melbourne. Bulletin of the World Health Organization. 1974;51(6):609-15.
49. Deibel R, Gross LL, Collins DN. Isolation of a new enterovirus (38506). Proceedings of the Society for Experimental Biology and Medicine Society for Experimental Biology and Medicine. 1975;148(1):203-7.
50. Melnick JL. Enterovirus type 71 infections: a varied clinical pattern sometimes mimicking paralytic poliomyelitis. Reviews of infectious diseases. 1984;6 Suppl 2:S387-90.
51. Chumakov M, Voroshilova M, Shindarov L, Lavrova I, Gracheva L, Koroleva G, et al. Enterovirus 71 isolated from cases of epidemic poliomyelitis-like disease in Bulgaria. Archives of virology. 1979;60(3-4):329-40.
52. Khan R, Vandelaer J, Yakubu A, Raza AA, Zulu F. Maternal and neonatal tetanus elimination: from protecting women and newborns to protecting all. International journal of women's health. 2015;7:171-80.
53. Nagy G, Takatsy S, Kukan E, Mihaly I, Domok I. Virological diagnosis of enterovirus type 71 infections: experiences gained during an epidemic of acute CNS diseases in Hungary in 1978. Archives of virology. 1982;71(3):217-27.
54. Lu CY, Lee CY, Kao CL, Shao WY, Lee PI, Twu SJ, et al. Incidence and case-fatality rates resulting from the 1998 enterovirus 71 outbreak in Taiwan. Journal of medical virology. 2002;67(2):217-23.
55. Sanders SA, Herrero LJ, McPhie K, Chow SS, Craig ME, Dwyer DE, et al. Molecular epidemiology of enterovirus 71 over two decades in an Australian urban community. Archives of virology. 2006;151(5):1003-13.
56. Gilbert GL, Dickson KE, Waters MJ, Kennett ML, Land SA, Sneddon M. Outbreak of enterovirus 71 infection in Victoria, Australia, with a high incidence of neurologic involvement. Pediatr Infect Dis J. 1988;7(7):484-8.
57. Hayward JC, Gillespie SM, Kaplan KM, Packer R, Pallansch M, Plotkin S, et al. Outbreak of poliomyelitis-like paralysis associated with enterovirus 71. Pediatr Infect Dis J. 1989;8(9):611-6.
58. Alexander JP, Jr., Baden L, Pallansch MA, Anderson LJ. Enterovirus 71 infections and neurologic disease--United States, 1977-1991. The Journal of infectious diseases. 1994;169(4):905-8.
59. Perez-Velez CM, Anderson MS, Robinson CC, McFarland EJ, Nix WA, Pallansch MA, et al. Outbreak of neurologic enterovirus type 71 disease: a diagnostic challenge. Clin Infect Dis. 2007;45(8):950-7.
60. IASR. Hand, foot and mouth disease, 2000-2003, Japan. 2004.
61. AbuBakar S, Chee HY, Al-Kobaisi MF, Xiaoshan J, Chua KB, Lam SK. Identification of enterovirus 71 isolates from an outbreak of hand, foot and mouth disease (HFMD) with fatal cases of encephalomyelitis in Malaysia. Virus research. 1999;61(1):1-9.
62. Singapore MoH. Forum on Hand Foot and Mouth Disease (HFMD) in Asia-Pacific Region: Epidemiological, laboratory, Clinical and Public Health Aspects. 2009.

63. Komatsu H, Shimizu Y, Takeuchi Y, Ishiko H, Takada H. Outbreak of severe neurologic involvement associated with Enterovirus 71 infection. Pediatric neurology. 1999;20(1):17-23.
64. Chen KT, Chang HL, Wang ST, Cheng YT, Yang JY. Epidemiologic features of hand-foot-mouth disease and herpangina caused by enterovirus 71 in Taiwan, 1998-2005. Pediatrics. 2007;120(2):e244-52.
65. Tseng FC, Huang HC, Chi CY, Lin TL, Liu CC, Jian JW, et al. Epidemiological survey of enterovirus infections occurring in Taiwan between 2000 and 2005: analysis of sentinel physician surveillance data. Journal of medical virology. 2007;79(12):1850-60.
66. McMinn PC. An overview of the evolution of enterovirus 71 and its clinical and public health significance. FEMS microbiology reviews. 2002;26(1):91-107.
67. McMinn P, Stratov I, Nagarajan L, Davis S. Neurological manifestations of enterovirus 71 infection in children during an outbreak of hand, foot, and mouth disease in Western Australia. Clin Infect Dis. 2001;32(2):236-42.
68. Fujimoto T, Chikahira M, Yoshida S, Ebira H, Hasegawa A, Totsuka A, et al. Outbreak of central nervous system disease associated with hand, foot, and mouth disease in Japan during the summer of 2000: detection and molecular epidemiology of enterovirus 71. Microbiology and immunology. 2002;46(9):621-7.
69. Nolan MA, Craig ME, Lahra MM, Rawlinson WD, Prager PC, Williams GD, et al. Survival after pulmonary edema due to enterovirus 71 encephalitis. Neurology. 2003;60(10):1651-6.
70. Jee YM, Cheon DS, Kim K, Cho JH, Chung YS, Lee J, et al. Genetic analysis of the VP1 region of human enterovirus 71 strains isolated in Korea during 2000. Archives of virology. 2003;148(9):1735-46.
71. Lum LC, Chua KB, McMinn PC, Goh AY, Muridan R, Sarji SA, et al. Echovirus 7 associated encephalomyelitis. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology. 2002;23(3):153-60.
72. Wong SS, Yip CC, Lau SK, Yuen KY. Human enterovirus 71 and hand, foot and mouth disease. Epidemiology and infection. 2010;138(8):1071-89.
73. Ang LW, Koh BK, Chan KP, Chua LT, James L, Goh KT. Epidemiology and control of hand, foot and mouth disease in Singapore, 2001-2007. Annals of the Academy of Medicine, Singapore. 2009;38(2):106-12.
74. Wang SM, Liu CC. Enterovirus 71: epidemiology, pathogenesis and management. Expert review of anti-infective therapy. 2009;7(6):735-42.
75. AbuBakar S, Sam IC, Yusof J, Lim MK, Misbah S, MatRahim N, et al. Enterovirus 71 outbreak, Brunei. Emerg Infect Dis. 2009;15(1):79-82.
76. WHO. A Guide to Clinical Management and Public Health Response for Hand, Foot and Mouth Disease (HFMD). 2011.
77. Zhang Y, Tan XJ, Wang HY, Yan DM, Zhu SL, Wang DY, et al. An outbreak of hand, foot, and mouth disease associated with subgenotype C4 of human enterovirus 71 in Shandong, China. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology. 2009;44(4):262-7.
78. Yang F, Ren L, Xiong Z, Li J, Xiao Y, Zhao R, et al. Enterovirus 71 outbreak in the People's Republic of China in 2008. Journal of clinical microbiology. 2009;47(7):2351-2.
79. Wang Y, Feng Z, Yang Y, Self S, Gao Y, Longini IM, et al. Hand, foot, and mouth disease in China: patterns of spread and transmissibility. Epidemiology. 2011;22(6):781-92.
80. Liu SL, Pan H, Liu P, Amer S, Chan TC, Zhan J, et al. Comparative epidemiology and virology of fatal and nonfatal cases of hand, foot and mouth disease in mainland China from 2008 to 2014. Reviews in medical virology. 2015;25(2):115-28.
81. Tan X, Huang X, Zhu S, Chen H, Yu Q, Wang H, et al. The persistent circulation of enterovirus 71 in People's Republic of China: causing emerging nationwide epidemics since 2008. PloS one. 2011;6(9):e25662.
82. WHO. Second Meeting on Vaccine Preventable Diseases Laboratory Networks in the Western Pacific Region. 2010.
83. Chen SP, Huang YC, Li WC, Chiu CH, Huang CG, Tsao KC, et al. Comparison of clinical features between coxsackievirus A2 and enterovirus 71 during the enterovirus

outbreak in Taiwan, 2008: a children's hospital experience. J Microbiol Immunol Infect. 2010;43(2):99-104.
84. Ma E, Chan KC, Cheng P, Wong C, Chuang SK. The enterovirus 71 epidemic in 2008--public health implications for Hong Kong. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2010;14(9):e775-80.
85. Ryu WS, Kang B, Hong J, Hwang S, Kim A, Kim J, et al. Enterovirus 71 infection with central nervous system involvement, South Korea. Emerg Infect Dis. 2010;16(11):1764-6.
86. Khanh TH, Sabanathan S, Thanh TT, Thoa le PK, Thuong TC, Hang V, et al. Enterovirus 71-associated hand, foot, and mouth disease, Southern Vietnam, 2011. Emerg Infect Dis. 2012;18(12):2002-5.
87. WHO. Severe complications of hand, foot and mouth disease (HFMD) caused by EV-71 in Cambodia: conclusion of the joint investigation. 2012
88. Horsley E, Just E, Torres C, Huhtinen E, Forssman B, Slade R. Enterovirus 71 outbreak in Northern Sydney, 2013: case series and initial response. J Paediatr Child Health. 2014;50(7):525-30.
89. Chang LY, King CC, Hsu KH, Ning HC, Tsao KC, Li CC, et al. Risk factors of enterovirus 71 infection and associated hand, foot, and mouth disease/herpangina in children during an epidemic in Taiwan. Pediatrics. 2002;109(6):e88.
90. Lee MS, Chiang PS, Luo ST, Huang ML, Liou GY, Tsao KC, et al. Incidence rates of enterovirus 71 infections in young children during a nationwide epidemic in Taiwan, 2008-09. PLoS neglected tropical diseases. 2012;6(2):e1476.
91. (WPRO) WHOWPR. Hand Foot and Mouth Updates 2011 [Available from: http://www.wpro.who.int/emerging_diseases/HFMD/en/index.html
92. Zhao YY, Jin H, Zhang XF, Wang B. Case-fatality of hand, foot and mouth disease associated with EV71: a systematic review and meta-analysis. Epidemiology and infection. 2015:1-9.
93. Xing W, Liao Q, Viboud C, Zhang J, Sun J, Wu JT, et al. Hand, foot, and mouth disease in China, 2008-12: an epidemiological study. The Lancet infectious diseases. 2014;14(4):308-18.
94. Fields BN, Knipe DM, Howley PM. Fields virology. 5th ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2007. 2 v. (xix, 3091, 86 p.) p.
95. Solomon T, Lewthwaite P, Perera D, Cardosa MJ, McMinn P, Ooi MH. Virology, epidemiology, pathogenesis, and control of enterovirus 71. Lancet Infect Dis. 2010;10(11):778-90.
96. Mandell GL, Douglas RG, Bennett JE, Dolin R. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. 6th ed. ed. Philadelphia, Pa.: Elsevier/Churchill Livingstone; 2010. 2 v. (xxxviii, 3661, cxxx p.) p.
97. Koroleva GA, Karmysheva VY, Lukashev AN. Enterovirus 71 pathogenicity in monkeys and cotton rats. Archives of virology. 2014;159(5):1133-8.
98. Yamayoshi S, Yamashita Y, Li J, Hanagata N, Minowa T, Takemura T, et al. Scavenger receptor B2 is a cellular receptor for enterovirus 71. Nat Med. 2009;15(7):798-801.
99. Nishimura Y, Shimojima M, Tano Y, Miyamura T, Wakita T, Shimizu H. Human P-selectin glycoprotein ligand-1 is a functional receptor for enterovirus 71. Nat Med. 2009;15(7):794-7.
100. Yamayoshi S, Iizuka S, Yamashita T, Minagawa H, Mizuta K, Okamoto M, et al. Human SCARB2-dependent infection by coxsackievirus A7, A14, and A16 and enterovirus 71. J Virol. 2012;86(10):5686-96.
101. He Y, Ong KC, Gao Z, Zhao X, Anderson VM, McNutt MA, et al. Tonsillar crypt epithelium is an important extra-central nervous system site for viral replication in EV71 encephalomyelitis. Am J Pathol. 2014;184(3):714-20.
102. Tee KK, Lam TT, Chan YF, Bible JM, Kamarulzaman A, Tong CY, et al. Evolutionary genetics of human enterovirus 71: origin, population dynamics, natural selection, and seasonal periodicity of the VP1 gene. J Virol. 2010;84(7):3339-50.
103. Chan Y, Sam I, Wee K, AbuBakar S. Enterovirus 71 in Malaysia: A decade later. Neurology Asia. 2011;16(1):1-15.

104. Mizuta K, Aoki Y, Suto A, Ootani K, Katsushima N, Itagaki T, et al. Cross-antigenicity among EV71 strains from different genogroups isolated in Yamagata, Japan, between 1990 and 2007. Vaccine. 2009;27(24):3153-8.
105. Huang ML, Chiang PS, Chia MY, Luo ST, Chang LY, Lin TY, et al. Cross-reactive neutralizing antibody responses to enterovirus 71 infections in young children: implications for vaccine development. PLoS neglected tropical diseases. 2013;7(2):e2067.
106. Khetsuriani N, Lamonte-Fowlkes A, Oberst S, Pallansch MA. Enterovirus surveillance--United States, 1970-2005. Morbidity and mortality weekly report Surveillance summaries. 2006;55(8):1-20.
107. Podin Y, Gias EL, Ong F, Leong YW, Yee SF, Yusof MA, et al. Sentinel surveillance for human enterovirus 71 in Sarawak, Malaysia: lessons from the first 7 years. BMC public health. 2006;6:180.
108. Lu CY, Lee CY, Kao CL, Shao WY, Lee PI, Twu SJ, et al. Incidence and case-fatality rates resulting from the 1998 enterovirus 71 outbreak in Taiwan. J Med Virol. 2002;67(2):217-23.
109. Chang LY. Enterovirus 71 in Taiwan. Pediatr Neonatol. 2008;49(4):103-12.
110. Rabenau HF, Richter M, Doerr HW. Hand, foot and mouth disease: seroprevalence of Coxsackie A16 and Enterovirus 71 in Germany. Med Microbiol Immunol. 2010;199(1):45-51.
111. Diedrich S, Weinbrecht A, Schreier E. Seroprevalence and molecular epidemiology of enterovirus 71 in Germany. Arch Virol. 2009;154(7):1139-42.
112. Ooi EE, Phoon MC, Ishak B, Chan SH. Seroepidemiology of human enterovirus 71, Singapore. Emerg Infect Dis. 2002;8(9):995-7.
113. Zhu Z, Zhu S, Guo X, Wang J, Wang D, Yan D, et al. Retrospective seroepidemiology indicated that human enterovirus 71 and coxsackievirus A16 circulated wildly in central and southern China before large-scale outbreaks from 2008. Virol J. 2010;7:300.
114. Luo ST, Chiang PS, Chao AS, Liou GY, Lin R, Lin TY, et al. Enterovirus 71 maternal antibodies in infants, Taiwan. Emerg Infect Dis. 2009;15(4):581-4.
115. Tran CB, Nguyen HT, Phan HT, Tran NV, Wills B, Farrar J, et al. The seroprevalence and seroincidence of enterovirus71 infection in infants and children in Ho Chi Minh City, Viet Nam. PLoS One. 2011;6(7):e21116.
116. Ang LW, Phoon MC, Wu Y, Cutter J, James L, Chow VT. The changing seroepidemiology of enterovirus 71 infection among children and adolescents in Singapore. BMC Infect Dis. 2011;11:270.
117. Wang JR, Tsai HP, Chen PF, Lai YJ, Yan JJ, Kiang D, et al. An outbreak of enterovirus 71 infection in Taiwan, 1998. II. Laboratory diagnosis and genetic analysis. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology. 2000;17(2):91-9.
118. Li CC, Yang MY, Chen RF, Lin TY, Tsao KC, Ning HC, et al. Clinical manifestations and laboratory assessment in an enterovirus 71 outbreak in southern Taiwan. Scand J Infect Dis. 2002;34(2):104-9.
119. Han J, Ma XJ, Wan JF, Liu YH, Han YL, Chen C, et al. Long persistence of EV71 specific nucleotides in respiratory and feces samples of the patients with Hand-Foot-Mouth Disease after recovery. BMC Infect Dis. 2010;10:178.
120. Ooi MH, Solomon T, Podin Y, Mohan A, Akin W, Yusuf MA, et al. Evaluation of different clinical sample types in diagnosis of human enterovirus 71-associated hand-foot-and-mouth disease. J Clin Microbiol. 2007;45(6):1858-66.
121. Kuppahally SS, Fowler MB, Vagelos R, Wang P, Al-Ahmad A, Hsia H, et al. Dyssynchrony Assessment with Tissue Doppler Imaging and Regional Volumetric Analysis by 3D Echocardiography Do Not Predict Long-Term Response to Cardiac Resynchronization Therapy. Cardiology research and practice. 2010;2011:568918.
122. Yerly S, Gervaix A, Simonet V, Caflisch M, Perrin L, Wunderli W. Rapid and sensitive detection of enteroviruses in specimens from patients with aseptic meningitis. Journal of clinical microbiology. 1996;34(1):199-201.
123. Irani DN. Aseptic meningitis and viral myelitis. Neurologic clinics. 2008;26(3):635-55, vii-viii.

124. Chen CS, Yao YC, Lin SC, Lee YP, Wang YF, Wang JR, et al. Retrograde axonal transport: a major transmission route of enterovirus 71 in mice. J Virol. 2007;81(17):8996-9003.
125. Wong KT, Munisamy B, Ong KC, Kojima H, Noriyo N, Chua KB, et al. The distribution of inflammation and virus in human enterovirus 71 encephalomyelitis suggests possible viral spread by neural pathways. J Neuropathol Exp Neurol. 2008;67(2):162-9.
126. Huang CC, Liu CC, Chang YC, Chen CY, Wang ST, Yeh TF. Neurologic complications in children with enterovirus 71 infection. N Engl J Med. 1999;341(13):936-42.
127. Hsueh C, Jung SM, Shih SR, Kuo TT, Shieh WJ, Zaki S, et al. Acute encephalomyelitis during an outbreak of enterovirus type 71 infection in Taiwan: report of an autopsy case with pathologic, immunofluorescence, and molecular studies. Mod Pathol. 2000;13(11):1200-5.
128. Shieh WJ, Jung SM, Hsueh C, Kuo TT, Mounts A, Parashar U, et al. Pathologic studies of fatal cases in outbreak of hand, foot, and mouth disease, Taiwan. Emerg Infect Dis. 2001;7(1):146-8.
129. Lu M, Meng G, He YX, Zheng J, Liao SL, Zhong YF, et al. [Pathology of enterovirus 71 infection: an autopsy study of 5 cases]. Zhonghua Bing Li Xue Za Zhi. 2009;38(2):81-5.
130. Gao L, Lin P, Liu S, Lei B, Chen Q, Yu S, et al. Pathological examinations of an enterovirus 71 infection: an autopsy case. International journal of clinical and experimental pathology. 2014;7(8):5236-41.
131. Zhang YC, Li XW, Zhu XD, Qian SY, Shang YX, Li BR, et al. Clinical characteristics and treatment of severe encephalitis associated with neurogenic pulmonary edema caused by enterovirus 71 in China. World journal of emergency medicine. 2010;1(2):108-13.
132. Yu P, Gao Z, Zong Y, Bao L, Xu L, Deng W, et al. Histopathological features and distribution of EV71 antigens and SCARB2 in human fatal cases and a mouse model of enterovirus 71 infection. Virus research. 2014;189:121-32.
133. Schmahmann JD, Ko R, MacMore J. The human basis pontis: motor syndromes and topographic organization. Brain. 2004;127(Pt 6):1269-91.
134. Mallet L, Polosan M, Jaafari N, Baup N, Welter ML, Fontaine D, et al. Subthalamic nucleus stimulation in severe obsessive-compulsive disorder. N Engl J Med. 2008;359(20):2121-34.
135. Salih F, Breuer E, Harnack D, Hoffmann KT, Ploner CJ. A syndrome of the dentate nucleus mimicking psychogenic ataxia. Journal of the neurological sciences. 2010;290(1-2):183-5.
136. Krebs C, Weinberg, J., Akesson, E. Neuroscience. Harvey RA, editor. Maryland: Lippincott Williams & Wilkins; 2011.
137. Shaikh MG. Hypothalamic dysfunction (hypothalamic syndromes). In: Wass JA, & Stewart, P. M. , editor. Oxford textbook of endocrinology and diabetes: Oxford University Press; 2011.
138. Benarroch EE. Subthalamic nucleus and its connections: Anatomic substrate for the network effects of deep brain stimulation. Neurology. 2008;70(21):1991-5.
139. Castelli L, Perozzo P, Zibetti M, Crivelli B, Morabito U, Lanotte M, et al. Chronic deep brain stimulation of the subthalamic nucleus for Parkinson's disease: effects on cognition, mood, anxiety and personality traits. European neurology. 2006;55(3):136-44.
140. Deuschl G, Mischke G, Schenck E, Schulte-Monting J, Lucking CH. Symptomatic and essential rhythmic palatal myoclonus. Brain. 1990;113 (Pt 6):1645-72.
141. Damier P, Hirsch EC, Agid Y, Graybiel AM. The substantia nigra of the human brain. II. Patterns of loss of dopamine-containing neurons in Parkinson's disease. Brain. 1999;122 (Pt 8):1437-48.
142. Wang D. Reticular formation and spinal cord injury. Spinal cord. 2009;47(3):204-12.
143. Woodruff BK, Wijdicks EF, Marshall WF. Reversible metronidazole-induced lesions of the cerebellar dentate nuclei. N Engl J Med. 2002;346(1):68-9.

144. Khoyratty F, Wilson T. The dentato-rubro-olivary tract: clinical dimension of this anatomical pathway. Case reports in otolaryngology. 2013;2013:934386.
145. Zhang XY, Ai HB, Cui XY. Effects of nucleus ambiguus and dorsal motor nuclei of vagus on gastric H(+) and HCO(3)(-) secretion in rats. World J Gastroenterol. 2006;12(20):3271-4.
146. Walker HK. Cranial Nerves IX and X: The Glossopharyngeal and Vagus Nerves. In: Walker HK, Hall, W.D., Hurst, J.W., editor. Clinical Methods: The History, Physical, and Laboratory Examinations. Boston: Butterworths; 1990.
147. Kao SJ, Yang FL, Hsu YH, Chen HI. Mechanism of fulminant pulmonary edema caused by enterovirus 71. Clin Infect Dis. 2004;38(12):1784-8.
148. Tayal V, Kalra BS. Cytokines and anti-cytokines as therapeutics--an update. European journal of pharmacology. 2008;579(1-3):1-12.
149. Rostene W, Dansereau MA, Godefroy D, Van Steenwinckel J, Reaux-Le Goazigo A, Melik-Parsadaniantz S, et al. Neurochemokines: a menage a trois providing new insights on the functions of chemokines in the central nervous system. J Neurochem. 2011;118(5):680-94.
150. Morganti-Kossman MC, Lenzlinger PM, Hans V, Stahel P, Csuka E, Ammann E, et al. Production of cytokines following brain injury: beneficial and deleterious for the damaged tissue. Mol Psychiatry. 1997;2(2):133-6.
151. McAdams RM, Juul SE. The role of cytokines and inflammatory cells in perinatal brain injury. Neurology research international. 2012;2012:561494.
152. Vitkovic L, Konsman JP, Bockaert J, Dantzer R, Homburger V, Jacque C. Cytokine signals propagate through the brain. Mol Psychiatry. 2000;5(6):604-15.
153. Church LD, Cook GP, McDermott MF. Primer: inflammasomes and interleukin 1beta in inflammatory disorders. Nat Clin Pract Rheumatol. 2008;4(1):34-42.
154. Lin TY, Chang LY, Huang YC, Hsu KH, Chiu CH, Yang KD. Different proinflammatory reactions in fatal and non-fatal enterovirus 71 infections: implications for early recognition and therapy. Acta Paediatr. 2002;91(6):632-5.
155. Kenney MJ, Ganta CK. Autonomic nervous system and immune system interactions. Comprehensive Physiology. 2014;4(3):1177-200.
156. Saindon CS, Blecha F, Musch TI, Morgan DA, Fels RJ, Kenney MJ. Effect of cervical vagotomy on sympathetic nerve responses to peripheral interleukin-1beta. Auton Neurosci. 2001;87(2-3):243-8.
157. Bendall LJ, Bradstock KF. G-CSF: From granulopoietic stimulant to bone marrow stem cell mobilizing agent. Cytokine & growth factor reviews. 2014;25(4):355-67.
158. Yujiri T, Tagami K, Tanimura A, Tanizawa Y. Alteration of adrenergic signals during peripheral blood stem cell mobilization induced by granulocyte colony-stimulating factor. Leukemia research. 2008;32(1):195-7.
159. Katayama Y, Battista M, Kao WM, Hidalgo A, Peired AJ, Thomas SA, et al. Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow. Cell. 2006;124(2):407-21.
160. Zhang ZH, Yu Y, Wei SG, Nakamura Y, Nakamura K, Felder RB. EP(3) receptors mediate PGE(2)-induced hypothalamic paraventricular nucleus excitation and sympathetic activation. Am J Physiol Heart Circ Physiol. 2011;301(4):H1559-69.
161. Goehler LE, Gaykema RP, Nguyen KT, Lee JE, Tilders FJ, Maier SF, et al. Interleukin-1beta in immune cells of the abdominal vagus nerve: a link between the immune and nervous systems? J Neurosci. 1999;19(7):2799-806.
162. Goehler LE, Relton JK, Dripps D, Kiechle R, Tartaglia N, Maier SF, et al. Vagal paraganglia bind biotinylated interleukin-1 receptor antagonist: a possible mechanism for immune-to-brain communication. Brain research bulletin. 1997;43(3):357-64.
163. Banks WA, Erickson MA. The blood-brain barrier and immune function and dysfunction. Neurobiology of disease. 2010;37(1):26-32.
164. Huston JM, Tracey KJ. The pulse of inflammation: heart rate variability, the cholinergic anti-inflammatory pathway and implications for therapy. Journal of internal medicine. 2011;269(1):45-53.
165. Sternberg EM. Neural regulation of innate immunity: a coordinated nonspecific host response to pathogens. Nature reviews Immunology. 2006;6(4):318-28.

166. Tracey KJ. Reflex control of immunity. Nature reviews Immunology. 2009;9(6):418-28.
167. Sedy J, Zicha J, Kunes J, Jendelova P, Sykova E. Mechanisms of neurogenic pulmonary edema development. Physiol Res. 2008;57(4):499-506.
168. Fu YC, Chi CS, Chiu YT, Hsu SL, Hwang B, Jan SL, et al. Cardiac complications of enterovirus rhombencephalitis. Arch Dis Child. 2004;89(4):368-73.
169. Huang YF, Chiu PC, Chen CC, Chen YY, Hsieh KS, Liu YC, et al. Cardiac troponin I: a reliable marker and early myocardial involvement with meningoencephalitis after fatal enterovirus-71 infection. J Infect. 2003;46(4):238-43.
170. Wu JM, Wang JN, Tsai YC, Liu CC, Huang CC, Chen YJ, et al. Cardiopulmonary manifestations of fulminant enterovirus 71 infection. Pediatrics. 2002;109(2):E26-.
171. Lin TY, Hsia SH, Huang YC, Wu CT, Chang LY. Proinflammatory cytokine reactions in enterovirus 71 infections of the central nervous system. Clin Infect Dis. 2003;36(3):269-74.
172. Wang SM, Lei HY, Su LY, Wu JM, Yu CK, Wang JR, et al. Cerebrospinal fluid cytokines in enterovirus 71 brain stem encephalitis and echovirus meningitis infections of varying severity. Clin Microbiol Infect. 2007;13(7):677-82.
173. Erta M, Quintana A, Hidalgo J. Interleukin-6, a major cytokine in the central nervous system. Int J Biol Sci. 2012;8(9):1254-66.
174. Borsody MK, Weiss JM. Alteration of locus coeruleus neuronal activity by interleukin-1 and the involvement of endogenous corticotropin-releasing hormone. Neuroimmunomodulation. 2002;10(2):101-21.
175. Wang SM, Lei HY, Huang KJ, Wu JM, Wang JR, Yu CK, et al. Pathogenesis of enterovirus 71 brainstem encephalitis in pediatric patients: roles of cytokines and cellular immune activation in patients with pulmonary edema. The Journal of infectious diseases. 2003;188(4):564-70.
176. Martin S, Maruta K, Burkart V, Gillis S, Kolb H. IL-1 and IFN-gamma increase vascular permeability. Immunology. 1988;64(2):301-5.
177. Hasko G. Receptor-mediated interaction between the sympathetic nervous system and immune system in inflammation. Neurochem Res. 2001;26(8-9):1039-44.
178. Benarroch EE. Autonomic-mediated immunomodulation and potential clinical relevance. Neurology. 2009;73(3):236-42.
179. Zhang Y, Liu H, Wang L, Yang F, Hu Y, Ren X, et al. Comparative study of the cytokine/chemokine response in children with differing disease severity in enterovirus 71-induced hand, foot, and mouth disease. PloS one. 2013;8(6):e67430.
180. Griffiths MJ, Ooi MH, Wong SC, Mohan A, Podin Y, Perera D, et al. In enterovirus 71 encephalitis with cardio-respiratory compromise, elevated interleukin 1beta, interleukin 1 receptor antagonist, and granulocyte colony-stimulating factor levels are markers of poor prognosis. The Journal of infectious diseases. 2012;206(6):881-92.
181. Offner H, Subramanian S, Parker SM, Afentoulis ME, Vandenbark AA, Hurn PD. Experimental stroke induces massive, rapid activation of the peripheral immune system. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2006;26(5):654-65.
182. Chang LY, Lin TY, Hsu KH, Huang YC, Lin KL, Hsueh C, et al. Clinical features and risk factors of pulmonary oedema after enterovirus-71-related hand, foot, and mouth disease. Lancet. 1999;354(9191):1682-6.
183. Chang LY, Hsia SH, Wu CT, Huang YC, Lin KL, Fang TY, et al. Outcome of enterovirus 71 infections with or without stage-based management: 1998 to 2002. Pediatr Infect Dis J. 2004;23(4):327-32.
184. Nguyen NT, Pham HV, Hoang CQ, Nguyen TM, Nguyen LT, Phan HC, et al. Epidemiological and clinical characteristics of children who died from hand, foot and mouth disease in Vietnam, 2011. BMC Infect Dis. 2014;14:341.
185. Dalakas MC. Mechanisms of action of IVIg and therapeutic considerations in the treatment of acute and chronic demyelinating neuropathies. Neurology. 2002;59(12 Suppl 6):S13-21.
186. Wang SM, Lei HY, Liu CC. Cytokine immunopathogenesis of enterovirus 71 brain stem encephalitis. Clin Dev Immunol. 2012;2012:876241.

187. Yang C, Deng C, Wan J, Zhu L, Leng Q. Neutralizing antibody response in the patients with hand, foot and mouth disease to enterovirus 71 and its clinical implications. Virol J. 2011;8:306.
188. Wang SM, Lei HY, Huang MC, Su LY, Lin HC, Yu CK, et al. Modulation of cytokine production by intravenous immunoglobulin in patients with enterovirus 71-associated brainstem encephalitis. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology. 2006;37(1):47-52.
189. Ooi MH, Wong SC, Podin Y, Akin W, del Sel S, Mohan A, et al. Human enterovirus 71 disease in Sarawak, Malaysia: a prospective clinical, virological, and molecular epidemiological study. Clin Infect Dis. 2007;44(5):646-56.
190. Li ZH, Li CM, Ling P, Shen FH, Chen SH, Liu CC, et al. Ribavirin reduces mortality in enterovirus 71-infected mice by decreasing viral replication. The Journal of infectious diseases. 2008;197(6):854-7.
191. Zhang G, Zhou F, Gu B, Ding C, Feng D, Xie F, et al. In vitro and in vivo evaluation of ribavirin and pleconaril antiviral activity against enterovirus 71 infection. Archives of virology. 2012;157(4):669-79.
192. Rotbart HA. Antiviral therapy for enteroviral infections. Pediatr Infect Dis J. 1999;18(7):632-3.
193. Sawyer MH, Saez-Lliorenz, X., Aviles, C.L., Ryan, M., Romero, J. Oral pleconaril reduces the duration and severity of enteroviral meningitis in children. Program and abstracts of the 1999 Pediatric Academic Societies Annual Meetin; Washington DC: Pediatric Research; 1999.
194. Webster AD. Pleconaril--an advance in the treatment of enteroviral infection in immuno-compromised patients. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology. 2005;32(1):1-6.
195. Abzug MJ, Cloud G, Bradley J, Sanchez PJ, Romero J, Powell D, et al. Double blind placebo-controlled trial of pleconaril in infants with enterovirus meningitis. Pediatr Infect Dis J. 2003;22(4):335-41.
196. Lehtonen LA, Antila S, Pentikainen PJ. Pharmacokinetics and pharmacodynamics of intravenous inotropic agents. Clinical pharmacokinetics. 2004;43(3):187-203.
197. Cuffe MS, Califf RM, Adams KF, Jr., Benza R, Bourge R, Colucci WS, et al. Short-term intravenous milrinone for acute exacerbation of chronic heart failure: a randomized controlled trial. JAMA. 2002;287(12):1541-7.
198. Samiee-Zafarghandy S, Raman SR, van den Anker JN, McHutchison K, Hornik CP, Clark RH, et al. Safety of milrinone use in neonatal intensive care units. Early Hum Dev. 2015;91(1):31-5.
199. Hoffman TM, Wernovsky G, Atz AM, Kulik TJ, Nelson DP, Chang AC, et al. Efficacy and safety of milrinone in preventing low cardiac output syndrome in infants and children after corrective surgery for congenital heart disease. Circulation. 2003;107(7):996-1002.
200. Carcillo JA, Fields AI. Clinical practice parameters for hemodynamic support of pediatric and neonatal patients in septic shock. Crit Care Med. 2002;30(6):1365-78.
201. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. Intensive Care Med. 2013;39(2):165-228.
202. Moore AR, Willoughby DA. The role of cAMP regulation in controlling inflammation. Clinical and experimental immunology. 1995;101(3):387-9.
203. Suleiman MS, Zacharowski K, Angelini GD. Inflammatory response and cardioprotection during open-heart surgery: the importance of anaesthetics. Br J Pharmacol. 2008;153(1):21-33.
204. Taylor KM. SIRS--the systemic inflammatory response syndrome after cardiac operations. The Annals of thoracic surgery. 1996;61(6):1607-8.
205. Hayashida N, Tomoeda H, Oda T, Tayama E, Chihara S, Kawara T, et al. Inhibitory effect of milrinone on cytokine production after cardiopulmonary bypass. The Annals of thoracic surgery. 1999;68(5):1661-7.
206. Gong M, Lin XZ, Lu GT, Zheng LJ. Preoperative inhalation of milrinone attenuates inflammation in patients undergoing cardiac surgery with cardiopulmonary bypass.

Medical principles and practice : international journal of the Kuwait University, Health Science Centre. 2012;21(1):30-5.
207. Wang SM, Lei HY, Huang MC, Wu JM, Chen CT, Wang JN, et al. Therapeutic efficacy of milrinone in the management of enterovirus 71-induced pulmonary edema. Pediatr Pulmonol. 2005;39(3):219-23.
208. Chi CY, Khanh TH, Thoa le PK, Tseng FC, Wang SM, Thinh le Q, et al. Milrinone therapy for enterovirus 71-induced pulmonary edema and/or neurogenic shock in children: a randomized controlled trial. Crit Care Med. 2013;41(7):1754-60.
209. Li YP, Liang ZL, Gao Q, Huang LR, Mao QY, Wen SQ, et al. Safety and immunogenicity of a novel human Enterovirus 71 (EV71) vaccine: a randomized, placebo-controlled, double-blind, Phase I clinical trial. Vaccine. 2012;30(22):3295-303.
210. Zhu FC, Meng FY, Li JX, Li XL, Mao QY, Tao H, et al. Efficacy, safety, and immunology of an inactivated alum-adjuvant enterovirus 71 vaccine in children in China: a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2013;381(9882):2024-32.
211. Zhu FC, Liang ZL, Li XL, Ge HM, Meng FY, Mao QY, et al. Immunogenicity and safety of an enterovirus 71 vaccine in healthy Chinese children and infants: a randomised, double-blind, placebo-controlled phase 2 clinical trial. Lancet. 2013;381(9871):1037-45.
212. Cheng A, Fung CP, Liu CC, Lin YT, Tsai HY, Chang SC, et al. A Phase I, randomized, open-label study to evaluate the safety and immunogenicity of an enterovirus 71 vaccine. Vaccine. 2013;31(20):2471-6.
213. Hwa SH, Lee YA, Brewoo JN, Partidos CD, Osorio JE, Santangelo JD. Preclinical evaluation of the immunogenicity and safety of an inactivated enterovirus 71 candidate vaccine. PLoS neglected tropical diseases. 2013;7(11):e2538.
214. Shimizu H, Nakashima K. Surveillance of hand, foot, and mouth disease for a vaccine. The Lancet Infectious diseases. 2014;14(4):262-3.
215. Ooi MH, Wong SC, Mohan A, Podin Y, Perera D, Clear D, et al. Identification and validation of clinical predictors for the risk of neurological involvement in children with hand, foot, and mouth disease in Sarawak. BMC Infect Dis. 2009;9:3.
216. Ruan F, Yang T, Ma H, Jin Y, Song S, Fontaine RE, et al. Risk factors for hand, foot, and mouth disease and herpangina and the preventive effect of hand-washing. Pediatrics. 2011;127(4):e898-904.
217. Lin H, Sun L, Lin J, He J, Deng A, Kang M, et al. Protective effect of exclusive breastfeeding against hand, foot and mouth disease. BMC Infect Dis. 2014;14:645.
218. Hsia SH, Wu CT, Chang JJ, Lin TY, Chung HT, Lin KL, et al. Predictors of unfavorable outcomes in enterovirus 71-related cardiopulmonary failure in children. Pediatr Infect Dis J. 2005;24(4):331-4.
219. Chong CY, Chan KP, Shah VA, Ng WY, Lau G, Teo TE, et al. Hand, foot and mouth disease in Singapore: a comparison of fatal and non-fatal cases. Acta Paediatr. 2003;92(10):1163-9.
220. Yang T, Xu G, Dong H, Ye M, He T. A case-control study of risk factors for severe hand-foot-mouth disease among children in Ningbo, China, 2010-2011. Eur J Pediatr. 2012;171(9):1359-64.
221. Lu HK, Lin TY, Hsia SH, Chiu CH, Huang YC, Tsao KC, et al. Prognostic implications of myoclonic jerk in children with enterovirus infection. J Microbiol Immunol Infect. 2004;37(2):82-7.
222. Fang Y, Wang S, Zhang L, Guo Z, Huang Z, Tu C, et al. Risk factors of severe hand, foot and mouth disease: a meta-analysis. Scand J Infect Dis. 2014;46(7):515-22.
223. Cheng HY, Huang YC, Yen TY, Hsia SH, Hsieh YC, Li CC, et al. The correlation between the presence of viremia and clinical severity in patients with enterovirus 71 infection: a multi-center cohort study. BMC Infect Dis. 2014;14:417.
224. Vellinga MM, Geurts JJ, Rostrup E, Uitdehaag BM, Polman CH, Barkhof F, et al. Clinical correlations of brain lesion distribution in multiple sclerosis. Journal of magnetic resonance imaging : JMRI. 2009;29(4):768-73.
225. Desai A, Shankar SK, Ravi V, Chandramuki A, Gourie-Devi M. Japanese encephalitis virus antigen in the human brain and its topographic distribution. Acta neuropathologica. 1995;89(4):368-73.

226. Damasio AR, Van Hoesen GW. The limbic system and the localisation of herpes simplex encephalitis. J Neurol Neurosurg Psychiatry. 1985;48(4):297-301.
227. Kuker W, Nagele T, Schmidt F, Heckl S, Herrlinger U. Diffusion-weighted MRI in herpes simplex encephalitis: a report of three cases. Neuroradiology. 2004;46(2):122-5.
228. Misra UK, Kalita J, Phadke RV, Wadwekar V, Boruah DK, Srivastava A, et al. Usefulness of various MRI sequences in the diagnosis of viral encephalitis. Acta tropica. 2010;116(3):206-11.
229. Lian ZY, Huang B, He SR, Liang CH, Guo YX. Diffusion-weighted imaging in the diagnosis of enterovirus 71 encephalitis. Acta radiologica. 2012;53(2):208-13.
230. Huang CC, Liu CC, Chang YC, Chen CY, Wang ST, Yeh TF. Neurologic complications in children with enterovirus 71 infection. The New England journal of medicine. 1999;341(13):936-42.
231. Wang SM, Liu CC, Tseng HW, Wang JR, Huang CC, Chen YJ, et al. Clinical spectrum of enterovirus 71 infection in children in southern Taiwan, with an emphasis on neurological complications. Clin Infect Dis. 1999;29(1):184-90.
232. Gau SS, Chang LY, Huang LM, Fan TY, Wu YY, Lin TY. Attention-deficit/hyperactivity-related symptoms among children with enterovirus 71 infection of the central nervous system. Pediatrics. 2008;122(2):e452-8.
233. Chen CY, Chang YC, Huang CC, Lui CC, Lee KW, Huang SC. Acute flaccid paralysis in infants and young children with enterovirus 71 infection: MR imaging findings and clinical correlates. AJNR American journal of neuroradiology. 2001;22(1):200-5.
234. Jang S, Suh SI, Ha SM, Byeon JH, Eun BL, Lee YH, et al. Enterovirus 71-related encephalomyelitis: usual and unusual magnetic resonance imaging findings. Neuroradiology.
235. Zeng H, Wen F, Gan Y, Huang W. MRI and associated clinical characteristics of EV71-induced brainstem encephalitis in children with hand-foot-mouth disease. Neuroradiology. 2012;54(6):623-30.
236. Shen WC, Tsai C, Chiu H, Chow K. MRI of Enterovirus 71 myelitis with monoplegia. Neuroradiology. 2000;42(2):124-7.
237. Chen F, Li J, Liu T, Wang L, Li Y. MRI characteristics of brainstem encephalitis in hand-foot-mouth disease induced by enterovirus type 71--will different MRI manifestations be helpful for prognosis? European journal of paediatric neurology : EJPN : official journal of the European Paediatric Neurology Society. 2013;17(5):486-91.
238. Liu K, Ma YX, Zhang CB, Chen YP, Ye XJ, Bai GH, et al. [Neurologic complications in children with enterovirus 71-infected hand-foot-mouth disease : clinical features, MRI findings and follow-up study]. Zhonghua Yi Xue Za Zhi. 2012;92(25):1742-6.
239. Li J, Chen F, Liu T, Wang L. MRI findings of neurological complications in hand-foot-mouth disease by enterovirus 71 infection. Int J Neurosci. 2012;122(7):338-44.
240. Lee KY, Lee YJ, Kim TH, Cheon DS, Nam SO. Clinico-radiological spectrum in enterovirus 71 infection involving the central nervous system in children. Journal of clinical neuroscience : official journal of the Neurosurgical Society of Australasia. 2014;21(3):416-20.
241. Gonzalvo A, Fowler A, Cook RJ, Little NS, Wheeler H, McDonald KL, et al. Schwannomatosis, sporadic schwannomatosis, and familial schwannomatosis: a surgical series with long-term follow-up. Clinical article. J Neurosurg. 2011;114(3):756-62.
242. Lee KY, Lee YJ, Kim TH, Cheon DS, Nam SO. Clinico-radiological spectrum in enterovirus 71 infection involving the central nervous system in children. J Clin Neurosci. 2014;21(3):416-20.
243. Chen F, Liu T, Li J, Xing Z, Huang S, Wen G. MRI characteristics and follow-up findings in patients with neurological complications of enterovirus 71-related hand, foot, and mouth disease. International journal of clinical and experimental medicine. 2014;7(9):2696-704.
244. Anderson V, Spencer-Smith M, Wood A. Do children really recover better? Neurobehavioural plasticity after early brain insult. Brain : a journal of neurology. 2011;134(Pt 8):2197-221.

245. Taylor HG, Alden J. Age-related differences in outcomes following childhood brain insults: an introduction and overview. Journal of the International Neuropsychological Society : JINS. 1997;3(6):555-67.
246. Stiles J, Jernigan TL. The basics of brain development. Neuropsychol Rev. 2010;20(4):327-48.
247. Uylings H. Development of the human cortex and the concept of 'critical' or 'sensitive' periods. Language learning. 2006;56:59-90.
248. Gogtay N, Giedd JN, Lusk L, Hayashi KM, Greenstein D, Vaituzis AC, et al. Dynamic mapping of human cortical development during childhood through early adulthood. Proceedings of the National Academy of Sciences of the United States of America. 2004;101(21):8174-9.
249. Mrzljak L, Uylings HB, Van Eden CG, Judas M. Neuronal development in human prefrontal cortex in prenatal and postnatal stages. Progress in brain research. 1990;85:185-222.
250. Huttenlocher PR. Synapse elimination and plasticity in developing human cerebral cortex. American journal of mental deficiency. 1984;88(5):488-96.
251. Thomas MSC, Johnson, M.H. New advances in understanding sensitive periods in brain development. Current Directions in Psychological Science. 2008;17(1):1-5.
252. Anderson V, Spencer-Smith M, Coleman L, Anderson P, Williams J, Greenham M, et al. Children's executive functions: are they poorer after very early brain insult. Neuropsychologia. 2010;48(7):2041-50.
253. Giza C, Prins, M. Is Being Plastic Fantastic? Mechanisms of Altered Plasticity after Developmental Traumatic Brain Injury. Developmental Neuroscience. 2006;28(4-5):364-79.
254. Rothi LJH, J. . Restitution and substitution: Two theories of recovery with application to neurobehavioral treatment. Journal of Clinical Neuropsychology. 1983;5(1):73-81.
255. Fowler A, Stodberg T, Eriksson M, Wickstrom R. Long-term outcomes of acute encephalitis in childhood. Pediatrics. 2010;126(4):e828-35.
256. Anderson V, Anderson P, Grimwood K, Nolan T. Cognitive and executive function 12 years after childhood bacterial meningitis: effect of acute neurologic complications and age of onset. J Pediatr Psychol. 2004;29(2):67-81.
257. Power T, Catroppa C, Coleman L, Ditchfield M, Anderson V. Do lesion site and severity predict deficits in attentional control after preschool traumatic brain injury (TBI)? Brain Inj. 2007;21(3):279-92.
258. Fox SE, Levitt P, Nelson CA, 3rd. How the timing and quality of early experiences influence the development of brain architecture. Child Dev. 2010;81(1):28-40.
259. Joseph R. Fetal Brain Behavior and Cognitive Development. Developmental Review. 2000;20(1):81-98.
260. Blessing WW. Inadequate frameworks for understanding bodily homeostasis. Trends Neurosci. 1997;20(6):235-9.
261. Geva R, Feldman R. A neurobiological model for the effects of early brainstem functioning on the development of behavior and emotion regulation in infants: implications for prenatal and perinatal risk. Journal of child psychology and psychiatry, and allied disciplines. 2008;49(10):1031-41.
262. DeCasper AJ, Lecanuet, J. P., Busnel, M. C., Granier-Deferre, C., & Maugeais, R. . Fetal reactions to recurrent maternal speech. Infant behavior and development. 1994;17(2):159-64.
263. Capute AJ, Shapiro BK, Accardo PJ, Wachtel RC, Ross A, Palmer FB. Motor functions: associated primitive reflex profiles. Dev Med Child Neurol. 1982;24(5):662-9.
264. Pereyra PM, Zhang W, Schmidt M, Becker LE. Development of myelinated and unmyelinated fibers of human vagus nerve during the first year of life. Journal of the neurological sciences. 1992;110(1-2):107-13.
265. Calkins SD, Graziano PA, Keane SP. Cardiac vagal regulation differentiates among children at risk for behavior problems. Biological psychology. 2007;74(2):144-53.
266. Garrard P, Bradshaw D, Jager HR, Thompson AJ, Losseff N, Playford D. Cognitive dysfunction after isolated brain stem insult. An underdiagnosed cause of long term morbidity. J Neurol Neurosurg Psychiatry. 2002;73(2):191-4.

267. Yirmiya R, Goshen I. Immune modulation of learning, memory, neural plasticity and neurogenesis. Brain Behav Immun. 2011;25(2):181-213.
268. McAfoose J, Baune BT. Evidence for a cytokine model of cognitive function. Neuroscience and biobehavioral reviews. 2009;33(3):355-66.
269. Gladstone MJ, Lancaster GA, Jones AP, Maleta K, Mtitimila E, Ashorn P, et al. Can Western developmental screening tools be modified for use in a rural Malawian setting? Archives of disease in childhood. 2008;93(1):23-9.
270. Chang LY, Huang LM, Gau SS, Wu YY, Hsia SH, Fan TY, et al. Neurodevelopment and cognition in children after enterovirus 71 infection. N Engl J Med. 2007;356(12):1226-34.
271. Lee HF, Chi CS, Jan SL, Fu YC, Huang FL, Chen PY, et al. Extracorporeal life support for critical enterovirus 71 rhombencephalomyelitis: long-term neurologic follow-up. Pediatric neurology. 2012;46(4):225-30.
272. Knoester H, Grootenhuis MA, Bos AP. Outcome of paediatric intensive care survivors. Eur J Pediatr. 2007;166(11):1119-28.
273. Hanekamp MN, Mazer P, van der Cammen-van Zijp MH, van Kessel-Feddema BJ, Nijhuis-van der Sanden MW, Knuijt S, et al. Follow-up of newborns treated with extracorporeal membrane oxygenation: a nationwide evaluation at 5 years of age. Critical care. 2006;10(5):R127.
274. Chrysostomou C, Maul T, Callahan PM, Nguyen K, Lichtenstein S, Coate EG, et al. Neurodevelopmental Outcomes after Pediatric Cardiac ECMO Support. Frontiers in pediatrics. 2013;1:47.
275. Sabanathan S, Wills B, Gladstone M. Child development assessment tools in low-income and middle-income countries: how can we use them more appropriately? Arch Dis Child. 2015.
276. Richardson K. Models of cognitive development. East Sussex, UK: Psychology Press; 1998.
277. Anderson VM. Assessing executive functions in children: Biological, psychological, and developmental considerations. Neuropsychological rehabilitation. 1998;8(3):319-49.
278. Wegner LM, Poon, J.K., Macias, M.M. Disorders of Cognition, Attention, Language and Learning. In: Elzouki AY, Harfi, H.A., Nazer, H.M., Stapleton, F.B., Oh, W., Whitley, R.J., editor. Textbook of Clinical Pediatrics. 1. 2nd ed. London: Springer 2001.
279. Grossman L. Normal Child Development. In: Elzouki AY, Harfi, H.A., Nazer, H.M., Stapleton, F.B., Oh, W., Whitley, R.J., editor. Textbook of Clinical Pediatrics. London: Springer; 2001.
280. Child NSCotD. Children's Emotional Development Is Built into the Architecture of Their Brains: Working Paper No. 2. 2004.
281. AAIDD. Definition of Intellectual Disability: American Association on Intellectual and Developmental Disabilities; 2014 [Available from: http://aaidd.org/intellectual-disability/definition - .U-qYvjk3efQ.
282. Huang MC, Wang SM, Hsu YW, Lin HC, Chi CY, Liu CC. Long-term cognitive and motor deficits after enterovirus 71 brainstem encephalitis in children. Pediatrics. 2006;118(6):e1785-8.
283. Lee HF, Chi CS. Enterovirus 71 Infection-Associated Acute Flaccid Paralysis: A Case Series of Long-Term Neurologic Follow-Up. J Child Neurol. 2014.
284. Tsou YA, Cheng YK, Chung HK, Yeh YC, Lin CD, Tsai MH, et al. Upper aerodigestive tract sequelae in severe enterovirus 71 infection: predictors and outcome. Int J Pediatr Otorhinolaryngol. 2008;72(1):41-7.
285. Zhang Q, Macdonald NE, Smith JC, Cai K, Yu H, Li H, et al. Severe enterovirus type 71 nervous system infections in children in the shanghai region of china: clinical manifestations and implications for prevention and care. Pediatr Infect Dis J. 2014;33(5):482-7.
286. Johnson S, Marlow N. Developmental screen or developmental testing? Early human development. 2006;82(3):173-83.
287. The Development Researcher. Country Factsheet in International Development 2012/2013 [Available from: http://developmentresearcher.com/wp-

content/uploads/2014/03/Development-Reseaecher_Country-Factsheet-on-Vietnam_October-2013.pdf.
288. Central Intelligence Agency. The World FactBook [Available from: https://www.cia.gov/library/publications/the-world-factbook/geos/vm.html.
289. Badiani R, Baulch, B., Brandt, L., Dat, V.H.,Giang, N.T., Gibson, J., Giles, J.,Hinsdale, I.,Hung, P., Kozel,V., Lanjouw, P., Marra, M., Ngoc, V.V., Phuong,N.T., Schuler, P., Thang, N.T., Hoang X.,Trung, L.D., Tung, P.D.,Viet Cuong, N.,Vu, L.H., Wells-Dang, A. . 2012 Vietnam poverty assessment : well begun, not yet done - Vietnam's remarkable progress on poverty reduction and the emerging challenges. Washington DC: World Bank, 2013.
290. Institute for State Organization Sciences. Organisational Structure Reform for a Better Governance in Vietnam Hanoi2011 [Available from: http://isos.gov.vn/News_Detail/tabid/179/ArticleId/741/language/en-US/Organisational-Structure-Reform-for-a-Better-Governance-in-Vietnam.aspx.
291. Tien TT, Phuong, H.T., Mathauer, I., Phuong, N.T.K. A Health Financing Review of Vietnam with a focus on Social Health Insurance World Health Organisation (WHO). 2011 [Available from: http://www.who.int/health_financing/documents/oasis_f_11-vietnam.pdf.
292. Central Intelligence Agency. The World FactBook: Physician Density [Available from: https://www.cia.gov/library/publications/the-world-factbook/fields/2226.html.
293. Dao A. Health Insurance in Vietnam: Health Care Reform in a Post-Socialist Context 2012 [Available from: http://www.medanthro.net/research/cagh/insurancestatements/Dao_Vietnam.pdf.
294. Shieh M, Thompson C, Phan VT, Van TT, Tediosi F, Merson L, et al. The policy of free healthcare for children under the age of 6 years in Vietnam: assessment of the uptake for children hospitalised with acute diarrhoea in Ho Chi Minh City. Tropical medicine & international health : TM & IH. 2013;18(12):1444-51.
295. Nguyen H, Wang W. The effects of free government health insurance among small children--evidence from the free care for children under six policy in Vietnam. The International journal of health planning and management. 2013;28(1):3-15.
296. MOH/HPG. Joint Annual Health Review (JAHR) Hanoi: Ministry of Health Vietnam and Health Partnership Group, 2010.
297. Dang V, Do, T., Nguyen, C., Phung, T., Phung, T. . Achievements and challenges in the progress of reaching millennium development goals of Vietnam. Hanoi: Mekong Deelopment Reserach Institute, 2013.
298. World Bank. Education in Vietnam: Development, History, Challenges and Solutions [Available from: http://siteresources.worldbank.org/EDUCATION/Resources/278200-1121703274255/1439264-1153425508901/Education_Vietnam_Development.pdf.
299. MOLISA/UNICEF. Creating a protective environment for children in Vietnam: an assessment if child protection laws and policies, especially children in special circumstances in Vietnam. . Hanoi: MOLISA/UNICEF, 2009.
300. Human Rights Watch. "Children of the Dust": Abuse of hanoi Street Children in Detention. New york2006.
301. United Nations population Fund (UNFA). PEOPLE WITH DISABILITIES IN VIET NAM Key Findings from the 2009 Viet Nam Population and Housing Census. Hanoi: 2011.
302. Mont D, Nguyen, C. Spatial Variation in the Disability-Poverty Correlation: Evidence from Vietnam. London and Hanoi: Leonard Cheshire Disability and Inclusive Development Centre, London and National Economics University, Hanoi 2013.
303. Shin JY, Nhan NV. Predictors of parenting stress among Vietnamese mothers of young children with and without cognitive delay. Journal of intellectual & developmental disability. 2009;34(1):17-26.
304. UNICEF Vuetnam. Inclusive Education in Viet Nam: their Right, our Responsibility [Available from: http://www.unicef.org/vietnam/reallives_20186.html.
305. Le HM. Opening the Gates for Children with Disabilities: An Introduction to inclusive Education in Vietnam. Washington, D.C.: Aspen institute, 2013.

306. Vasiljev I. The Disabled and Their Organizations – The Emergence of New Paradigms [Available from: http://www.reachingoutvietnam.com/public_html/Media/disabled_organisations.html.
307. Velentgas P, Dreyer, NA., Wu, AW. Outcome Definition and Measurement. In: Velentgas P, Dreyer, NA., Nourjah, P. et al., editor. Developing a Protocol for Observational Comparative Effectiveness Research: A User's Guide. Rockville: Agency for Healthcare Research and Quality; 2013.
308. Stahlmann N, Hartel C, Knopp A, Gehring B, Kiecksee H, Thyen U. Predictive value of neurodevelopmental assessment versus evaluation of general movements for motor outcome in preterm infants with birth weights <1500 g. Neuropediatrics. 2007;38(2):91-9.
309. El-Dib M, Massaro AN, Glass P, Aly H. Neurodevelopmental assessment of the newborn: An opportunity for prediction of outcome. Brain Dev. 2011;33(2):95-105.
310. Fowler A, Stodberg T, Eriksson M, Wickstrom R. Childhood encephalitis in Sweden: etiology, clinical presentation and outcome. European journal of paediatric neurology : EJPN : official journal of the European Paediatric Neurology Society. 2008;12(6):484-90.
311. Goelman H, Ford, L., Pighini, M., Dahinten, S., Harris, S., Synnes, A., Tse, L., Ball, J., and Hayes, V. . What we learned about identification and screening. In: Goelman H, Pivik, J., Guhn, M., editor. The CHILD project: New approaches for reserach in child development: Rules rituals and realities. New York: ManMillian; 2011.
312. Adeyemi TO. The Effective use of Standard Scores for Research in Educational Management. Research Journal of Mathematics and Statistics 2011;3(3):91-6.
313. Phillips GW, Finn CE. The Lake Wobegon Effect: A Skeleton in the Testing Closet? Educational Measurement: Issues and Practice. 1998;7(2):10-1.
314. Benetti AR, Jacobsen J, Lehnhoff B, Momsen NC, Okhrimenko DV, Telling MT, et al. How mobile are protons in the structure of dental glass ionomer cements? Scientific reports. 2015;5:8972.
315. Pena ED. Lost in translation: methodological considerations in cross-cultural research. Child Dev. 2007;78(4):1255-64.
316. Hambleton RK. Issues, designs, and technical guidelines for adapting tests into multiple languages and cultures. In: Hambleton RK, Merenda, P. F. , & Spielberger, C. D. , editor. Adapting educational and psychological tests for cross-cultural assessment Mahwah, NJ: Lawrence Erlbaum Associates; 2005. p. 3-38.
317. Streiner DL, Norman, G.R. Health Measurement Scales: A practical guide to their development and use. Oxford: Oxford University Press; 2008.
318. Fernald L, Kariger P, Engle P, Raikes A. Examining Early Child Development in Low-Income Countries: A Toolkit for the Assessment of Children in the First Five Years of Life. World Bank, Washington DC, 2009.
319. Cicchetti DV. Guidelines, criteria, and rules of thumb for evaluating normed and standardized assessment instruments in psychology. Psychological assessment 1994;6(4):284-90.
320. Prado EL, Hartini S, Rahmawati A, Ismayani E, Hidayati A, Hikmah N, et al. Test selection, adaptation, and evaluation: a systematic approach to assess nutritional influences on child development in developing countries. Br J Educ Psychol. 2010;80(Pt 1):31-53.
321. Glascoe FP, Byrne KE, Ashford LG, Johnson KL, Chang B, Strickland B. Accuracy of the Denver-II in developmental screening. Pediatrics. 1992;89(6 Pt 2):1221-5.
322. Commonwealth Fund. Part II: Guides to Facilitate Your Choice and Use of Screening Instruments [Available from: http://www.commonwealthfund.org/publications/resources/2007/dec/part-ii--guides-to-facilitate-your-choice-and-use-of-screening-instruments.
323. Jones HB, N. . The Berkeley growth study. Child development 1941;12:167–73.
324. Simard MN, Lambert J, Lachance C, Audibert F, Gosselin J. Interexaminer reliability of Amiel-Tison neurological assessments. Pediatric neurology. 2009;41(5):347-52.

325. Paro-Panjan D, Neubauer D, Kodric J, Bratanic B. Amiel-Tison Neurological Assessment at term age: clinical application, correlation with other methods, and outcome at 12 to 15 months. Dev Med Child Neurol. 2005;47(1):19-26.
326. Deschenes G, Gosselin J, Couture M, Lachance C. Interobserver reliability of the Amiel-Tison neurological assessment at term. Pediatric neurology. 2004;30(3):190-4.
327. Barrett MA, Trapp M, Lohstroh W, Seydel T, Ollivier J, Ballauff M, et al. Alzheimer's peptide amyloid-beta, fragment 22-40, perturbs lipid dynamics. Soft matter. 2016;12(5):1444-51.
328. Fehlmann F, Seydel P, Misirlic M, Staudacher D. [Better day by day - for a lifetime]. Krankenpflege Soins infirmiers. 2015;108(10):24-5.
329. Garrigan E, Belkin NS, Seydel F, Han Z, Carter J, McDuffie M, et al. Csf2 and Ptgs2 Epigenetic Dysregulation in Diabetes-prone Bicongenic B6.NODC11bxC1tb Mice. Genetics & epigenetics. 2015;7:5-17.
330. Hochman SE, Madaline TF, Wassmer SC, Mbale E, Choi N, Seydel KB, et al. Fatal Pediatric Cerebral Malaria Is Associated with Intravascular Monocytes and Platelets That Are Increased with HIV Coinfection. mBio. 2015;6(5):e01390-15.
331. van der Meulen B, Smrkovsky, M. Factor Analyses of Bayley's Infant Behavior Record: A Dutch Replication and Extension. British Journal of Developmental Psychology 1985;3:345-52.
332. Walldorf JA, Cohee LM, Coalson JE, Bauleni A, Nkanaunena K, Kapito-Tembo A, et al. School-Age Children Are a Reservoir of Malaria Infection in Malawi. PloS one. 2015;10(7):e0134061.
333. Marlow N, Wolke D, Bracewell MA, Samara M. Neurologic and developmental disability at six years of age after extremely preterm birth. N Engl J Med. 2005;352(1):9-19.
334. Burankova T, Hempelmann R, Fossog V, Ollivier J, Seydel T, Embs JP. Proton Diffusivity in the Protic Ionic Liquid Triethylammonium Triflate Probed by Quasielastic Neutron Scattering. The journal of physical chemistry B. 2015;119(33):10643-51.
335. Seydelmann N, Wanner C, Stork S, Ertl G, Weidemann F. Fabry disease and the heart. Best practice & research Clinical endocrinology & metabolism. 2015;29(2):195-204.
,336. Bayley N. Bayley Scales of Infant and Toddler Development 3rd Edition Technical Manual. 2006.
337. Gosselin J, Gahagan S, Amiel-Tison C. The Amiel-Tison Neurological Assessment at Term: conceptual and methodological continuity in the course of follow-up. Mental retardation and developmental disabilities research reviews. 2005;11(1):34-51.
338. Gosselin J, Amiel-Tison, C. Neurological assessment from birth to six years [Évaluation neurologique de la naissance à 6 ans] [, French]
. 2nd ed. Montreal: Éditions du CHU Sainte-Justine; 2007.
339. Harmon HM, Taylor HG, Minich N, Wilson-Costello D, Hack M. Early school outcomes for extremely preterm infants with transient neurological abnormalities. Dev Med Child Neurol. 2015.
340. Zeng H, Lu J, Zheng H, Yi L, Guo X, Liu L, et al. The Epidemiological Study of Coxsackievirus A6 revealing Hand, Foot and Mouth Disease Epidemic patterns in Guangdong, China. Sci Rep. 2015;5:10550.
341. Commission IT. International test commission guidelines for translating and adapting tests.
Retrieved from http://www.intestcom.org2010 [Available from: http://www.intestcom.org.
342. Gonzalez de Dios J, Moya M. [Perinatal asphyxia, hypoxic-ischemic encephalopathy and neurological sequelae in full-term newborns. II. Description and interrelation]. Rev Neurol. 1996;24(132):969-76.
343. Murray DM, Bala P, O'Connor CM, Ryan CA, Connolly S, Boylan GB. The predictive value of early neurological examination in neonatal hypoxic-ischaemic encephalopathy and neurodevelopmental outcome at 24 months. Dev Med Child Neurol. 2010;52(2):e55-9.

344. Leroux BG, N'Guyen The Tich S, Branger B, Gascoin G, Rouger V, Berlie I, et al. Neurological assessment of preterm infants for predicting neuromotor status at 2 years: results from the LIFT cohort. BMJ open. 2013;3(2).
345. Thanh TT, Anh NT, Tham NT, Van HM, Sabanathan S, Qui PT, et al. Validation and utilization of an internally controlled multiplex Real-time RT-PCR assay for simultaneous detection of enteroviruses and enterovirus A71 associated with hand foot and mouth disease. Virol J. 2015;12:85.
346. Tan le V, Tuyen NT, Thanh TT, Ngan TT, Van HM, Sabanathan S, et al. A generic assay for whole-genome amplification and deep sequencing of enterovirus A71. J Virol Methods. 2015;215-216:30-6.
347. Molyneux ME, Taylor TE, Wirima JJ, Borgstein A. Clinical features and prognostic indicators in paediatric cerebral malaria: a study of 131 comatose Malawian children. The Quarterly journal of medicine. 1989;71(265):441-59.
348. Molyneux EM, Walsh AL, Malenga G, Rogerson S, Molyneux ME. Salmonella meningitis in children in Blantyre, Malawi, 1996-1999. Ann Trop Paediatr. 2000;20(1):41-4.
349. Mallewa M, Fooks AR, Banda D, Chikungwa P, Mankhambo L, Molyneux E, et al. Rabies encephalitis in malaria-endemic area, Malawi, Africa. Emerg Infect Dis. 2007;13(1):136-9.
350. Solomon T, Thao TT, Lewthwaite P, Ooi MH, Kneen R, Dung NM, et al. A cohort study to assess the new WHO Japanese encephalitis surveillance standards. Bulletin of the World Health Organization. 2008;86(3):178-86.
351. Fernandez E, Perez R, Hernandez A, Tejada P, Arteta M, Ramos JT. Factors and Mechanisms for Pharmacokinetic Differences between Pediatric Population and Adults. Pharmaceutics. 2011;3(1):53-72.
352. Johnson S, Wolke D, Hennessy E, Marlow N. Educational outcomes in extremely preterm children: neuropsychological correlates and predictors of attainment. Developmental neuropsychology. 2011;36(1):74-95.
353. Hillemeier MM, Morgan PL, Farkas G, Maczuga SA. Perinatal and socioeconomic risk factors for variable and persistent cognitive delay at 24 and 48 months of age in a national sample. Maternal and child health journal. 2011;15(7):1001-10.
354. Walker SP, Wachs TD, Grantham-McGregor S, Black MM, Nelson CA, Huffman SL, et al. Inequality in early childhood: risk and protective factors for early child development. Lancet. 2011;378(9799):1325-38.
355. Shen WC, Chiu HH, Chow KC, Tsai CH. MR imaging findings of enteroviral encephaloymelitis: an outbreak in Taiwan. AJNR Am J Neuroradiol. 1999;20(10):1889-95.
356. R Core Team R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna2013.
357. Wolak ME, Fairbairn, D.J., Paulsen, Y.R. Guidelines for Estimating Repeatability. Methods in Ecology and Evolution. 2012;3(1):129-37.
358. Walker SP, Wachs TD, Gardner JM, Lozoff B, Wasserman GA, Pollitt E, et al. Child development: risk factors for adverse outcomes in developing countries. Lancet. 2007;369(9556):145-57.
359. WHO. WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development. Geneva: 2006.
360. Rubin DB, Thomas, N. . Combining propensity score matching with additional adjustments for prognostic covariates. . Journal of the American Statistical Association. 2000;95:573-85.
361. Stuart EA. Matching methods for causal inference: A review and a look forward. Statistical science : a review journal of the Institute of Mathematical Statistics. 2010;25(1):1-21.
362. Winkelmayer WC, Kurth T. Propensity scores: help or hype? Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2004;19(7):1671-3.

363. Glynn RJ, Schneeweiss S, Sturmer T. Indications for propensity scores and review of their use in pharmacoepidemiology. Basic & clinical pharmacology & toxicology. 2006;98(3):253-9.
364. Martens EP, Pestman WR, de Boer A, Belitser SV, Klungel OH. Systematic differences in treatment effect estimates between propensity score methods and logistic regression. International journal of epidemiology. 2008;37(5):1142-7.
365. He J, van de Vijver, F. . Bias and Equivalence in Cross-Cultural Research. Online Readings in Psychology and Culture. 2012;2(2).
366. Malda M, van de Vijver, A. J. R., Srinivasan, K., Transler, C., Sukumar, P., & Rao, K. . Adapting a cognitive test for a different culture: An illustration of qualitative procedures. . Psychology Science Quarterly,
. 2008;50(4):451-68.
367. Van de Vijver F, Hambleton, R. K. Translating Tests: Some Practical Guidelines. European Psychologist. 1996;1(2):88-99.
368. Tran J. The Acquisition of Vietnamese Classifiers. Unpublished PhD Thesis at .... 2011.(accessed 17 Jan2014). University of Hawai'i at Manoa; 2011.
369. Viet Nam Multiple Indicator Cluster Survey Ha Noi, Viet Nam: General Statistical Office (GSO), 2011.
370. The 2009 Vietnam Population and Housing Census: Major Findings. Hanoi, Vietnam: Ministry of Planning and Investment: Geberal Statistics Office, 2009.
371. Timmerman ME. Factor analysis. Groningen: Heymans Institute for Psychology, 2005.
372. Hox JJ, Bechger, T.M. An Introduction to Structural Equation Modeling. Family Science Review. 1998;11:354-73.
373. Hirschfeld G, von Brachel, R. Improving Multiple-Group confirmatory factor analysis in R – A tutorial in measurement invariance with continuous and ordinal indicators. Practical Assessment, Research
& Evaluation. 2014;19(7).
374. Mundfrom DJ, Shaw, D.G., Ke, T.L. . Minimum Sample Size Recommendations for Conducting Factor Analyses,. International Journal of Testing. 2005;5(2):159-68.
375. Clench-Aas J, Nes RB, Dalgard OS, Aaro LE. Dimensionality and measurement invariance in the Satisfaction with Life Scale in Norway. Qual Life Res. 2011;20(8):1307-17.
376. Hooper D, Coughlan, J., Mullen, M.:, 6(1), 53-60. Structural Equation Modelling: Guidelines for Determining Model Fit. . Electronic Journal of
Business Research Methods. 2008;6(1):53-60.
377. Revelle W. psych: Procedures for Personality and Psychological Research. 1.5.1. ed. Illinois: Northwestern University, Evanston.; 2015.
378. Rosseel Y. lavaan: An R Package for Structural Equation Modeling. Journal of Statistical Software
. 2012;48(2):1-36.
379. Milfont TL, Fischer, R. . Testing measurement invariance across groups: Applications in cross-cultural research. International Journal of psychological research. 2010;3(1):111-30.
380. Raines-Eudy R. Using Structural Equation Modeling to Test for Differential Reliability and Validity: An Empirical Demonstration. Structural Equation Modeling: A Multidisciplinary Journal. 2000;7(1):124-41.
381. Hirschfeld G, & von Brachel, R. . Multiple-Group confirmatory factor analysis in R–A tutorial in measurement invariance with continuous and ordinal indicators. Practical Assessment, Research & Evaluation. 2014;19(7):2.
382. Wu AD, Li, Z., & Zumbo, B. D. . Decoding the meaning of factorial invariance and updating the practice of multi-group confirmatory factor analysis: A demonstration with TIMSS data. Practical Assessment, Research and Evaluation. 2007;12(3):1-26.
383. Chen FF. What happens if we compare chopsticks with forks? The impact of making inappropriate comparisons in cross-cultural research. . Journal of personality and social psychology. 2008;95(5):1005.
384. Ory DT, & Mokhtarian, P. L. . The impact of non-normality, sample size and estimation technique on goodness-of-fit measures in structural equation modeling:

evidence from ten empirical models of travel behavior. Quality & Quantity. 2010;44(3):427-45.
385. Wicherts JM, Dolan CV, Hessen DJ. Stereotype threat and group differences in test performance: a question of measurement invariance. Journal of personality and social psychology. 2005;89(5):696-716.
386. Mokkink LB, Terwee CB, Patrick DL, Alonso J, Stratford PW, Knol DL, et al. The COSMIN checklist for assessing the methodological quality of studies on measurement properties of health status measurement instruments: an international Delphi study. Qual Life Res. 2010;19(4):539-49.
387. Grantham-McGregor S, Cheung YB, Cueto S, Glewwe P, Richter L, Strupp B. Developmental potential in the first 5 years for children in developing countries. Lancet. 2007;369(9555):60-70.
388. Berkman DS, Lescano AG, Gilman RH, Lopez SL, Black MM. Effects of stunting, diarrhoeal disease, and parasitic infection during infancy on cognition in late childhood: a follow-up study. Lancet. 2002;359(9306):564-71.
389. Mendez MA, Adair LS. Severity and timing of stunting in the first two years of life affect performance on cognitive tests in late childhood. J Nutr. 1999;129(8):1555-62.
390. Walker SP, Chang SM, Powell CA, Grantham-McGregor SM. Effects of early childhood psychosocial stimulation and nutritional supplementation on cognition and education in growth-stunted Jamaican children: prospective cohort study. Lancet. 2005;366(9499):1804-7.
391. Chang SM, Walker SP, Grantham-McGregor S, Powell CA. Early childhood stunting and later behaviour and school achievement. Journal of child psychology and psychiatry, and allied disciplines. 2002;43(6):775-83.
392. Galler JR, Ramsey F. A follow-up study of the influence of early malnutrition on development: behavior at home and at school. J Am Acad Child Adolesc Psychiatry. 1989;28(2):254-61.
393. Semba RD, de Pee S, Sun K, Sari M, Akhter N, Bloem MW. Effect of parental formal education on risk of child stunting in Indonesia and Bangladesh: a cross-sectional study. Lancet. 2008;371(9609):322-8.
394. Sabates R. Paper commissioned for the EFA Global Monitoring Report 2013/4, Teaching and learning: Achieving quality for all. Can maternal education hinder, sustain or enhance the benefits of early life interventions? Evidence from the Young Lives Longitudinal Study. Young Lives, 2014.
395. Nguyen HT, Eriksson B, Petzold M, Bondjers G, Tran TK, Nguyen LT, et al. Factors associated with physical growth of children during the first two years of life in rural and urban areas of Vietnam. BMC Pediatr. 2013;13:149.
396. Burr R. The complexity of morality: Being a 'good child'in Vietnam? Journal of Moral Education. 2014;43(2):156-68.
397. Siegel LS. Infant tests as predictors of cognitive and language development at two years. Child Development. 1981;52(2):545-57.
398. Carlson AG, Rowe, E. Curby, T.W. Disentangling Fine Motor Skills' Relations to Academic Achievement: The Relative Contributions of Visual-Spatial Integration and Visual-Motor Coordination. The Journal of Genetic Psychology. 2013;174(5):514-33.
399. Hedges LV, Nowell A. Sex differences in mental test scores, variability, and numbers of high-scoring individuals. Science. 1995;269(5220):41-5.
400. Hyde JS, & Linn, M. C. Gender differences in verbal ability: A meta-analysis. Psychological bulletin. 1988;104(53-69).
401. Ardila A, Rosselli M, Matute E, Inozemtseva O. Gender differences in cognitive development. Developmental Psychology. 2011;47(4):984-90.
402. Cromwell EA, Dube Q, Cole SR, Chirambo C, Dow AE, Heyderman RS, et al. Validity of US norms for the Bayley Scales of Infant Development-III in Malawian children. European journal of paediatric neurology : EJPN : official journal of the European Paediatric Neurology Society. 2014;18(2):223-30.
403. Von Hippel PT. Regression with missing Ys: An improved strategy for analyzing multiply imputed data. Sociological Methodology. 2007;37(1):83-117.
404. Rosenbaum P, Rubin, D. . The central role of the propensity score in observational studies for causal effects. Biometrika. 1983;70:41-55.

405. Stuart EA, Rubin, D. B. . Matching methods for causal inference: Designing observational studies. Harvard University Department of Statistics mimeo. 2004.
406. Ridgeway G, McCaffrey, D. F., Morral, A. R., Burgette, L. F., Griffin, B. A. (2014). . Toolkit for weighting and analysis of nonequivalent groups 2014 [Available from: http://www.rand.org/pubs/tools/TL136z1.html.
407. Olmos A, & Govindasamy, P. A practical guide for using propensity score weighting in R. Practical Assessment, Research & Evaluation. 2015;20(13):2.
408. Belitser SV, Martens EP, Pestman WR, Groenwold RH, de Boer A, Klungel OH. Measuring balance and model selection in propensity score methods. Pharmacoepidemiol Drug Saf. 2011;20(11):1115-29.
409. Woods SP, Childers M, Ellis RJ, Guaman S, Grant I, Heaton RK. A battery approach for measuring neuropsychological change. Archives of clinical neuropsychology : the official journal of the National Academy of Neuropsychologists. 2006;21(1):83-9.
410. Van Rie A, Mupuala A, Dow A. Impact of the HIV/AIDS epidemic on the neurodevelopment of preschool-aged children in Kinshasa, Democratic Republic of the Congo. Pediatrics. 2008;122(1):e123-8.
411. Fernandes M, Stein A, Newton CR, Cheikh-Ismail L, Kihara M, Wulff K, et al. The INTERGROWTH-21st Project Neurodevelopment Package: a novel method for the multi-dimensional assessment of neurodevelopment in pre-school age children. PloS one. 2014;9(11):e113360.
412. Anderson PJ, De Luca CR, Hutchinson E, Roberts G, Doyle LW. Underestimation of developmental delay by the new Bayley-III Scale. Archives of pediatrics & adolescent medicine. 2010;164(4):352-6.
413. Makrides M, Gibson RA, McPhee AJ, Yelland L, Quinlivan J, Ryan P. Effect of DHA supplementation during pregnancy on maternal depression and neurodevelopment of young children: a randomized controlled trial. JAMA. 2010;304(15):1675-83.
414. Makrides M, Gibson RA, McPhee AJ, Collins CT, Davis PG, Doyle LW, et al. Neurodevelopmental outcomes of preterm infants fed high-dose docosahexaenoic acid: a randomized controlled trial. JAMA. 2009;301(2):175-82.
415. Rhoades RE, Tabor-Godwin JM, Tsueng G, Feuer R. Enterovirus infections of the central nervous system. Virology. 2011;411(2):288-305.
416. Dowell E, Easton, A., & Solomon, T. . The consequences of encephalitis Malton, UK: Encephalitis Society; 2000 [Available from: http://www.encephalitis.info/images/iPdf/Research2/CONSEQUENCES1.pdf.
417. Hooper L, Williams WH, Sarah EW, Chua KC. Caregiver distress, coping and parenting styles in cases of childhood encephalitis. Neuropsychological rehabilitation. 2007;17(4-5):621-37.
418. Starza-Smith A, Talbot E, Grant C. Encephalitis in children: a clinical neuropsychology perspective. Neuropsychological rehabilitation. 2007;17(4-5):506-27.
419. Schmolck H, Maritz E, Kletzin I, Korinthenberg R. Neurologic, neuropsychologic, and electroencephalographic findings after European tick-borne encephalitis in children. J Child Neurol. 2005;20(6):500-8.
420. Campbell JM, Linc LG, Mutersbaugh K. Viral encephalitis: a challenging diagnosis in an ICU. Critical care nurse. 1998;18(3):58-65.
421. McGrath N, Anderson NE, Croxson MC, Powell KF. Herpes simplex encephalitis treated with acyclovir: diagnosis and long term outcome. J Neurol Neurosurg Psychiatry. 1997;63(3):321-6.
422. Holding PA, Taylor HG, Kazungu SD, Mkala T, Gona J, Mwamuye B, et al. Assessing cognitive outcomes in a rural African population: development of a neuropsychological battery in Kilifi District, Kenya. J Int Neuropsychol Soc. 2004;10(2):246-60.
423. Van't Hooft J, van der Lee JH, Opmeer BC, Aarnoudse-Moens CS, Leenders AG, Mol BW, et al. Predicting developmental outcomes in premature infants by term equivalent MRI: systematic review and meta-analysis. Systematic reviews. 2015;4:71.
424. Massaro AN, Evangelou I, Fatemi A, Vezina G, McCarter R, Glass P, et al. White matter tract integrity and developmental outcome in newborn infants with hypoxic-ischemic encephalopathy treated with hypothermia. Dev Med Child Neurol. 2015;57(5):441-8.

425. Seydel KB, Kampondeni SD, Valim C, Potchen MJ, Milner DA, Muwalo FW, et al. Brain swelling and death in children with cerebral malaria. N Engl J Med. 2015;372(12):1126-37.
426. Carter JA, Ross AJ, Neville BG, Obiero E, Katana K, Mung'ala-Odera V, et al. Developmental impairments following severe falciparum malaria in children. Tropical medicine & international health : TM & IH. 2005;10(1):3-10.
427. Braga LW. The SARAH Network of Rehabilitation Hospitals: International Brain Injury Association; 2012 [Available from: http://www.internationalbrain.org/articles/the-sarah-network-of-rehabilitation-hospitals/.

Enrolment	ENROL
Participant number 08RS-[_0_|_3_]-[_|_|_]	Initials [_|_|_|_|_|_]

7. Appendix 1: Case record form HFMD cases

General information
1) Date of enrolment: [_|_]/[_|_]/[_|_] (dd/mm/yy)
2) Patient's Full name:* [_____]
3) Hospital number:* [_____]
4) Ward of enrolment: O Ward C O PICU
5) Gender: O male O female
6) Date of birth: [_|_]/[_|_]/[_|_|_|_] *dd mm yyyy*
7) Address: a. Home number/street:* [_____] Province/ Centrally governed city: _____ Provincial City/ District: _____ Town/ Ward/Commune: _____ Hamlet: _____ b. Contact no.1 Name* [_____] c. Relationship to child * [_____] d. Phone Number 1*: [_____] e. Contact no.2 Name* [_____] f. Relationship to child* [_____] g. Phone Number 2*: [_____]

**Information is not recorded into the database*

Completed by (initials): _____ *Date completed:* _____

History at ENROLMENT	HIST
Participant number 08RS-[_0_|_3_]-[_|_|_]	Initials [_|_|_|_|_|_]

History at ENROLMENT

Date of onset of illness [_|_]/[_|_]/[_|_] (dd/mm/yy)
Date of admission to this hospital [_|_]/[_|_]/[_|_] (dd/mm/yy)
Transfer from another hospital O Yes O No
If yes:
Which hospital [_____]
Date admitted to the other hospital: [_|_]/[_|_]/[_|_] (dd/mm/yy) O Unknown/No documentation
Grade of HFMD when child admitted to previous hospital
O Grade 1 O Grade 2a O Grade 2b(1) O Grade 2b(2) O Grade 3 O Grade 4 O unknown

What grade of HFMD does the child have today?
O Grade 1 O Grade 2a O Grade 2b(1) O Grade 2b(2) O Grade 3 O Grade 4
What grade of HFMD did the child have on the day s/he was first admitted to hospital?
O Same as above because child was admitted today O Grade 1
O Grade 2a O Grade 2b(1) O Grade 2b(2) O Grade 3 O Grade 4 O Unknown

Did the receive any of the following, during this illness episode?
a. Cardiac arrest O Yes O No O Unknown
b. Mechanical Ventilation O Yes O No O Unknown
c. Hemofiltration O Yes O No O Unknown
d. Intravenous Immunoglobulin O Yes O No O Unknown
e. Milrinone O Yes O No O Unknown
f. IV Magnesium O Yes O No O Unknown
g. Phenobarbital O Yes O No O Unknown
h. Corticosteroids / Methylprednisolone O Yes O No O Unknown
i. Antibiotics O Yes O No O Unknown

Completed by (initials): _____ *Date completed:* _____

Contact History at ENROLMENT	**CONHIST**
Participant number 08RS-[_0_|_3_]-[_|_|_]	Initials [_|_|_|_|_|_]

Contact History at ENROLMENT

Does the patient attend:

O Daycare facility O Kindergarten O School O Stay at Home

If attends, Daycare/Kindergarten record address

Street:* [_____]

Province/ Centrally governed city: _____

Provincial City/ Town/ District: _____

Town/ Ward/Commune: _____

Hamlet: _____

How many adults normally live in your house (including parent/guardian)?

[_|_]

How many children under the age of 15 (including other people's children) normally live in your house? [_|_]

In the 2 weeks before onset of illness, was there

someone in the child's household with HFMD: O Yes O No O Unkown

someone at the child's daycare/kindergarten/school with HFMD: O Yes O No O Unkown

did the child have any contact with someone with HFMD: O Yes O No O Unkown

Completed by (initials): _____ *Date completed:* _____

Symptoms and Signs at ENROLMENT	EXAM
Participant number 08RS-[_0_|_3_]-[_|_|_]	Initials [_|_|_|_|_|_]

Symptoms and Signs at ENROLMENT

Headache : O Yes O No O Unknown
Fever: O Yes O No O Unknown
Cough: O Yes O No O Unknown
Runny nose: O Yes O No O Unknown
Vomiting: O Yes O No O Unknown
Diarrhea: O Yes O No O Unknown
Drowsiness: O Yes O No O Unknown
Irritability: O Yes O No O Unknown
Myoclonus: O Yes O No O Unknown
Sweating O Yes O No O Unknown
Lethargy O Yes O No O Unknown
Temperature: [_|_].[_] °C
Pulse [_|_|_]/min
Blood pressure [_|_|_]/[_|_|_] mmHg
Respiratory rate [_|_]/min
Weight [_|_].[_] Kg
Height [_|_|_] Cm
Head Circumference [_|_] Cm

Respiratory pattern O Normal OTachypnea O Irregular O wheeze
Ostridor O Cheyne-stokes O Apnea
Conjunctivitis: O Yes O No
Rash O Yes O No
If Yes,Document location of rash and how many spots/vesicles visible at ENROLMENT
Hands and Feet O 0 O 1-5 O >5
Knees and Elbows O 0 O 1-5 O >5
Buttocks O 0 O 1-5 O >5
Descibe rash: O mostly vesicular O mostly macular O both
Ulcer in the mouth O Yes O No
If Yes, Location of ulcers: ☐ Tongue ☐ Palate ☐ Buccal ☐ Lips ☐ Tonsil

If today is not admission day, record any of the following which occurred since admission

Admission GCS documented O Yes O No Date of GCS: [_|_]/[_|_]/[_|_] (dd/mm/yy)
If Yes, state components of GCS: E: [_] / 4 V: [_] / 5 M: [_] / 6
Nystagmus O Yes O No O Unknown
If yes: Date first documented: [_|_]/[_|_]/[_|_] (dd/mm/yy)
Tremor O Yes O No O Unknown
If yes: Date first documented: [_|_]/[_|_]/[_|_] (dd/mm/yy)
Ataxia O Yes O No O Unknown
If yes: Date first documented: [_|_]/[_|_]/[_|_] (dd/mm/yy)
Limb weakness O Yes O No O Unknown
If yes: Date first documented: [_|_]/[_|_]/[_|_] (dd/mm/yy)
If Yes, specify: Upper limb O Right O Left
Lower limb O Right O Left

LAB RESULTS AT ADMISSION	LAB
Participant number 08RS-[_0_|_3_]-[_|_|_]	Initials [_|_|_|_|_|_]

LAB RESULTS AT ADMISSION – First results available in hospital chart	
Date sample taken:	[_|_]/[_|_]/[_|_] (dd/mm/yy)
White blood cell count	[_|_].[_] * 10^9/ml
Neutrophil %	[_|_].[_] %
Lymphocyte %	[_|_].[_] %
Blood glucose	[_|_|_] mg/dL or [_|_].[_]mmol/L
CRP	[_|_].[_]mg/L

Completed by (initials): _____ *Date completed:* _____

Admission Neurological Examination **NEU**

Participant number
08RS-[_0_|_3_]-[_|_|_]

Initials
[_|_|_|_|_|_]

NEUROLOGICAL EXAM

1. Date of exam [_|_]/[_|_]/[_|_] (dd/mm/yy)
2. Has the child had Phenobarbital within the last 24 hours? ○ Yes: oral ○ Yes: intravenous ○ No ○ Not known

Blantyre Coma Score

3. Best motor response	Localises painful stimulus (Rub knuckles on patient's sternum)	2	○
	Withdraws limb from pain (Firm pressure on thumbnail bed with horizontal pencil)	1	○
	Nonspecific or absent response	0	○
4. Verbal response	Appropriate cry	2	○
	Innappropriate cry	1	○
	None	0	○
5. Eye movements	Directed (e.g follows other's face)	1	○
	Not directed	0	○

Neurological Examination at ENROLMENT

6. Nystagmus	○ Yes ○ No ○ Unknown (sleeping or not rousable to assess)
7. Tremor	○ Yes ○ No ○ Unknown (sleeping or not rousable to assess)
8. Ataxia	○ Yes ○ No ○ Unknown (child can not stand)
9. Limb weakness	○ Yes ○ No ○ Unknown (sleeping or not rousable to assess) a. If Yes, specify: Upper limb ○ Right ○ Left Lower limb ○ Right ○ Left
10. Cranial nerve palsies (check and circle all that apply)	☐ Left (circle number) 3 / 4 / 5 / 6 / 7 ☐ Right (circle number) 3 / 4 / 5 / 6 / 7 ☐ Other, specify: ☐ None ☐ Unable to assess

If abnormal at number 7: ○ Upper motor neuron ○ Lower motor neuron ○ Unknown

Completed by (initials): _____ *Date completed:* _____

Daily Review	DAILY
Participant number 08RS-[_0_|_3_]- [_|_|_]	Initials [_|_|_|_|_|_|_]

DAILY REVIEW: DAY 2-6

1. Date and time of exam [_|_]/[_|_]/[_|_] (dd/mm/yy) Time: [_|_]:[_|_]
2. Has the child had Phenobarbital within the last 24 hours? ○ Yes: oral ○ Yes: intravenous ○ No

Blantyre Coma Score

3. Best motor response	Localises painful stimulus (Rub knuckles on patient's sternum)	2	○
	Withdraws limb from pain (Firm pressure on thumbnail bed with horizontal pencil)	1	○
	Nonspecific or absent response	0	○
4. Verbal response	Appropriate cry	2	○
	Innappropriate cry	1	○
	None	0	○
5. Eye movements	Directed (e.g follows other's face)	1	○
	Not directed	0	○

6. Nystagmus	○ Yes ○ No ○ Unknown (sleeping or not rousable to assess)
7. Tremor	○ Yes ○ No ○ Unknown (sleeping or not rousable to assess)
8. Ataxia	○ Yes ○ No ○ Unknown (child can not stand)
9. Limb weakness	○ Yes ○ No ○ Unknown (sleeping or not rousable to assess) a. If Yes, specify: Upper limb ○ Right ○ Left Lower limb ○ Right ○ Left
10. Cranial nerve palsies (check and circle all that apply)	☐ Left (circle number) 3 / 4 / 5 / 6 / 7 ☐ Right (circle number) 3 / 4 / 5 / 6 / 7 ☐ Other, specify: ☐ None ☐ Unable to assess
If abnormal at number 7: ○ Upper motor neuron ○ Lower motor neuron ○ Unknown	

11. Irritability:	○ Yes	○ No	○ Unknown
12. Vomiting	○ Yes	○ No	○ Unknown
13. Lethargy	○ Yes	○ No	○ Unknown
14. Fussiness	○ Yes	○ No	○ Unknown
15. Myoclonus:	○ Yes	○ No	○ Unknown
16. Sweating	○ Yes	○ No	○ Unknown
17. Temperature:	[_|_].[_] °C		

Daily Review	**DAILY**
Participant number 08RS-[_0_|_3_]- [_|_|_]	Initials [_|_|_|_|_|_]

18. Maximum Pulse [_|_|_]/min
19. Minimum Pulse [_|_|_]/min
20. Maximum Blood pressure [_|_|_]/[_|_|_] mmHg
21. Minimum Blood pressure [_|_|_]/[_|_|_] mmHg
22. Respiratory rate [_|_]/min
23. Respiratory pattern O Normal OTachypnea O Irregular O wheeze Ostridor O Cheyne-stokes O Apnea
24. **What grade of HFMD does the child have today?**
    O Grade 1 O Grade 2a O Grade 2b(1) O Grade 2b(2) O Grade 3 O Grade 4

Completed by (initials): _____ *Date completed:* _____

Medication	**MED**
Participant number 08RS-[_0_|_3_]-[_|_|_]	Initials [_|_|_|_|_|_]

Medication/Intervention: Complete from hospital file at DISCHARGE **(if patient withdraws, record data up until the day of withdrawal)**

MEDICATIONS		If Yes: Date of first dose:	Date of last dose:	Total number of days that the drug was given
1. Midazolam	O Yes O No	[_|_]/[_|_]/[_|_]	[_|_]/[_|_]/[_|_]	[_|_|_]
2. Phenobarbitone oral	O Yes O No	[_|_]/[_|_]/[_|_]	[_|_]/[_|_]/[_|_]	[_|_|_]
3. Phenobarbitone IV	O Yes O No	[_|_]/[_|_]/[_|_]	[_|_]/[_|_]/[_|_]	[_|_|_]
4. Milrinone	O Yes O No	[_|_]/[_|_]/[_|_]	[_|_]/[_|_]/[_|_]	[_|_|_]
5. Inotropics (dopamine/dobutamine / epinephrin)	O Yes O No	[_|_]/[_|_]/[_|_]	[_|_]/[_|_]/[_|_]	[_|_|_]
6. Methylprednisolone	O Yes O No	[_|_]/[_|_]/[_|_]	[_|_]/[_|_]/[_|_]	[_|_|_]
7. Magnesium	O Yes O No	[_|_]/[_|_]/[_|_]	[_|_]/[_|_]/[_|_]	[_|_|_]
8. Nasal cannulla	O Yes O No	[_|_]/[_|_]/[_|_]	[_|_]/[_|_]/[_|_]	[_|_|_]
9. Tracheostomy	O Yes O No	[_|_]/[_|_]/[_|_]	[_|_]/[_|_]/[_|_] ☐ still in place at discharge	[_|_|_] (until discharge)
10. CPAP	O Yes O No	[_|_]/[_|_]/[_|_]	[_|_]/[_|_]/[_|_]	[_|_|_]
11. Intubation	O Yes O No	[_|_]/[_|_]/[_|_]	[_|_]/[_|_]/[_|_]	[_|_|_]
12. CVVH	O Yes O No	[_|_]/[_|_]/[_|_]	[_|_]/[_|_]/[_|_]	[_|_|_]
13. IVIg	O Yes O No	1st dose date: [_|_]/[_|_]/[_|_]		
		2nd dose date: [_|_]/[_|_]/[_|_] ☐ No 2nd dose		

Medication	**MED**
Participant number 08RS-[_0_|_3_]-[_|_|_]	Initials [_|_|_|_|_|_]

If patient required chest compressions (CPR) for a cardiac arrest, select how many episodes (at least 1 hour apart) where CPR was used.

14. Resucutation/CPR	O Yes O No	O 1 episode >15 minutes no cardiac output oYes oNo	O 2 episode >15 minutes no cardiac output oYes oNo	O >2nd episodes >15 minutes no cardiac output oYes oNo

Completed by (initials): _____ Date completed: _____

DEVELOPMENTAL HISTORY

a. Child lives with: O mother O father Oboth O grandparents Orelatives O other If lives with grandparents/relatives for how long of last 12 months:O <2 months O 2-6 months O >6 months

INFORMATION ABOUT PREGNANCY AND BIRTH

2. Birth order: O 1st, O 2nd, O3rd O>3rd child
3. The child is : O a singleton Otwin Otriplet Omore than triplet
4. Delivery at birth O Vaginal O Caesarean section
5. Birth weight: [_].[_] kgs O Unknown
6. Breastfeeding: Child was exclusively breastfed until 6 weeks (no formula) OYes ONo
   If Yes, was child exclusively breastfed until 3 months (no formula)? OYes ONo

CHILD'S HISTORY

Developmental history (gross motor)

7. Age Sit O =<6m months O between 6-9months O >9months O unknown O Not yet
8. Age walks O =<12 months O between 12-18 months O >18months Ounknown O Not yet

Fine motor

9. Age pick up small objects O =<9 months O between 9-12 months O >12months ONot yet O unknown

Language

10. Age says mama/dada appropriately for mom and dad O =<9 months O between 9-12 months O >12months O unknown ONot yet
11. 2-3 word utterance O =<24 months O between 24-36 months O >36months Ounknown O Not yet

Ask the question and record the one most suitable option. If it is not clear then list the options to the mother

12. What is mother's occupation?
    - O Stay at home/homemaker
    - O Agriculture, forestry and fishing
    - O Mining
    - O Manufacture
    - O Electricity, gas and water supply
    - O Construction
    - O Trade and repair of motor vehicles

	O Hotels and restaurants O Transport, storage and communication O Financial intermediation O Science and technology O Real estate O Public administration and defence O Training and education O Health and social work O Culture and sport O Party union and associations O Personal and public service O Employment in private households O Other employment O Student O Unemployed
13. What is father's occupation?	O Stay at home/homemaker O Agriculture, forestry and fishing O Mining O Manufacture O Electricity, gas and water supply O Construction O Trade and repair of motor vehicles O Hotels and restaurants O Transport, storage and communication O Financial intermediation O Science and technology O Real estate O Public administration and defence O Training and education O Health and social work O Culture and sport O Party union and associations O Personal and public service O Employment in private households O Other employment O Student O Unemployed
14. What is the highest level of mother's education attained?	O Never been to school O Attended some primary school O Completed primary school (5th gr) O Completed lower secondary school (9th gr) O Completed higher secondary school

(12th gr)

O Completed university/college degree

O Completed postgraduate degree

15. What is the highest level of father's education attained?

O Never been to school
O Attended some primary school
O Completed primary school (5th gr)
O Completed lower secondary school (9th gr)
O Completed higher secondary school (12th gr)
O Completed university/college degree
O Completed postgraduate degree

Refers to accomodation in HCMC

16. Do you own or rent your accomodation? O Own O Rent O House owned by relatives

The house/household refers to their accommodation in HCMC, not their hometown.
If the child usually lives with grandparents and the parents live in HCMC away from their child, document details about the parents' accommodation in HCMC.

Ask the question and list the options to the parent. Record the parent's answer.

17. How many levels in your house? O 1 O >1

18. What is the floor of your house made from? (This refers to the surface you walk on, not what is underneath the surface)

O Cement
O Earth/sand
O Ceramic tiles
O Cane/palm/bamboo
O None of above

19. What is the roof of your home made from?

O Metal
O Calamine/Cement fibre
O Ceramic tiles
O Cement
O Thatch/palm leaf
O Other, specify

[_____]

20. What kind of toilet do members of your household usually use?

O Own flush toilet
O Shared flush toilet
O Traditional pit toilet
O Ventilation improved pit toilet
O No facility/bush/field

		O None of above
21. How many rooms are there in your house that are used for living and/or sleeping (excluding bathroom and kitchen)? [_|_] *(Count all the indoor rooms in your house not including bathrooms and kitchens. If a room has a kitchen or toilet/shower in it but is also used regularly for sleeping or other activities besides cooking and eating, it should be included.)*		
Does your house have:		
22. Electricity?	O Yes	O No
23. Internet access?	O Yes	O No
24. A television?	O Yes	O No
25. A refrigerator?	O Yes	O No
26. An air-conditioner?	O Yes	O No
How many of the following are owned by people in your household:		
27. Bicycle?	[_]	
28. Motorbike?	[_]	
29. Car?	[_]	
30. What is the main type of fuel you use for cooking?		O Wood O Gas O Electricity O Coal O Kerosene O Straw O None of above
31. Where do you get your drinking water (main source)?		O Private tap O Public standpipe O Bottled water O Well in own residence O Public well O Rain water O Spring O River/lake/pond O None of the above
32. Do you usually treat the water prior to drinking?		O Boil O Bleach/chlorine added O Filter O Solar disinfection O No treatment O None of the above

Completed by (initials): _____ *Date completed:* _____

DISCHARGE SUMMARY OF HOSPITAL STAY (Complete from hospital file)

1. Date of discharge: [_|_]/[_|_]/[_|_] (dd/mm/yy)
   (or date of study withdrawl [_|_]/[_|_]/[_|_] (dd/mm/yy))

2. Discharge Diagnosis HFMD: O Yes O No

3. If No, specify discharge diagnosis:
   - ☐ skin infection
   - ☐ upper respiratory tract infection
   - ☐ sepsis
   - ☐ pneumonia
   - ☐ meningitis
   - ☐ other viral infection
   - ☐ other

4. Highest Grade HFMD during admission: O Grade 1 O Grade 2a O Grade 2b(1) O Grade 2b(2) O Grade 3 O Grade 4 O Not Applicable

5. Date of onset of Highest Grade HFMD: [_|_]/[_|_]/[_|_] (dd/mm/yy)

6. Ventilator associated pneumonia O Yes O No

Definition of sepsis: ALL OF THESE

- rectal temperature >38.5°C or <35°C
- signs and symptoms of infection
- tachycardia (may be absent in hypothermic patients)

PLUS >=1 of following:

- organ dysfunction,
- altered mental status
- hypoxemia (arterial PaO2/FIO2 <300)
- increased serum lactate level (> upper limit of lab normal)

7. Treated for sepsis during admission O Yes O No

8. Result of hospital throat swab PCR: a. O EV positive O EV negative O Not done

If EV positive: b. O EV-71 positive O EV-71 negative

10. Outcome at discharge:
    - O Full recovery without complication: no obvious neurological problem, normal age appropriate development, feeds and breathes without medical intervention.
    - O Incomplete recovery: see list below
    - O Transferred to another hospital

○ Taken home without approval ○ Death ○ Discharged to die
11. If incomplete recovery choose as many of the relevant options listed. ☐ Seizures (requiring medication to continue after discharge) ☐ Hypertonicity (stiffness/abnormal posturing) ☐ Limb Paralysis ☐ Persistent cranial nerve palsy ☐ Diaphragmatic weakness ☐ Discharged with tracheostomy ☐ Discharged with nasogastric tube ☐ Behavioural/Personality changes (more talkative, aggressive or inappropriate)

Completed by (initials): _____ *Date completed:* _____

Discharge Summary	**FU**
Participant number 08RS-[_0_|_3_]-[_|_|_]	Initials [_|_|_|_|_|_]

1. Date of assessment [_|_]/[_|_]/[_|_] (dd/mm/yy)

2. Attends with O mother O father Oboth O grandparents Orelatives Oother

3. In the two weeks before admission to HTD with HFMD, was the child living in HCMC:
O Yes O No

4. Address child comes to review from: O same as front page O different address:

Home number/street:* [_____]

Province/ Centrally governed city: _____

Provincial City/ District: _____

Town/ Ward/Commune: _____

Hamlet: _____

5. Verify phone numbers on contact page – correct if necessary*

Study nurse documents the following

6. tracheostomy in place O Yes O No

7. nasogastric feeding O Yes O No

8. Was the child re-admitted between discharge and review: OYes ONo

9. What was the diagnosis: O respiratory illness Odiarrhoeao OfeverO other please specify_____

10. Patient has

☐ Bayley/Movement ABC by psychologist

☐ Neurological examination by Dr Saras

☐ Review by PICU doctor

8. Appendix 2: leaflets for healthy participants

Mỗi trẻ được sinh ra đều có những khả năng thiên bẩm. Hãy cho trẻ cơ hội thực hiện khả năng đó !

Lưu ý các phụ huynh có con dưới 4 tuổi. Chúng tôi đang tìm kiếm những tình nguyện viên cho con mình tham gia vào việc đánh giá dựa trên trò chơi, nhằm tìm hiểu thêm về sự phát triển của trẻ em Việt Nam.

Con của bạn sẽ được khám 3 lần trong 18 tháng, với các chuyên gia về phát triển trẻ em.

Xin liên hệ với chúng tôi để có thêm thông tin, hoặc đăng ký thời gian hẹn:

Xin gửi email cho tôi – bác sĩ Saras:

✉swhitehorn@oucru.org

☎0906741371

Cô Kim Anh: ✉kimanh161@gmail.com

☎0973360382

Cô Trà: ✉psythanhtra@yahoo.com

☎ 0909275429

NGHIÊN CỨU VỀ SỰ PHÁT TRIỂN CỦA TRẺ EM

Hình vẽ *của bé Minh Khuê, 5 tuổi*

Nghiên cứu về sự phát triển của trẻ em

Việc đánh giá sử dụng đồ chơi, lắp ghép hình và sách tranh.

Trẻ dưới 42 tháng

Đây là một nghiên cứu lý thú để xem cách trẻ phát triển kỹ năng vận động, ngôn ngữ và hiểu biết. Chúng tôi dùng một công cụ của Mỹ đã được quốc tế công nhận, gọi là Bayley – Thang đánh giá sự Phát triển của Trẻ Nhũ nhi và Trẻ Nhỏ phiên bản lần 3 (Bayley III).

Đây là một cơ hội tuyệt vời để tìm hiểu xem con bạn đang phát triển như thế nào, và bạn có thể hỗ trợ cho con bạn ra sao, trong suốt quá trình học hỏi của con.

Trẻ trên 42 tháng

Movement ABC là một công cụ đánh giá kỹ năng vận động tinh, vận động thô, và sự phối hợp vận động của trẻ.

Trắc nghiệm này chưa được sử dụng rộng rãi tại Việt Nam, và chỉ có thể thực hiện bởi các chuyên gia đã được huấn luyện.

Thang Bayley đánh giá sự phát triển của trẻ nhũ nhi và trẻ nhỏ - phiên bản lần 3

Đây là một công cụ đánh giá dựa trên trò chơi. Trẻ sẽ được yêu cầu xây tháp, vẽ hình dạng, hoàn thành hình lắp ghép và những hoạt động vui thích khác.

Movement ABC - phiên bản lần 2

Việc đánh giá này dùng hình lắp ghép, bóng và những bài tập để kiểm tra kỹ năng vận động.

Cuối buổi đánh giá, bạn sẽ được thông báo về cách con bạn đã thực hiện, và những gì bạn có thể làm để hỗ trợ sự phát triển của trẻ.

Ai đánh giá ?

Nhóm gồm các chuyên viên tâm lý và giáo viên giáo dục đặc biệt, được hướng dẫn bởi BS Phạm Ngọc Thanh, Bệnh viện Nhi Đồng 1.

CÁC BÁC SĨ KHÔNG MẶC ÁO CHOÀNG TRẮNG

Việc đánh giá sẽ kéo dài trong bao lâu ?

Thời gian đánh giá thay đổi theo tuổi của trẻ, từ 30 phút đến tối đa là 2 giờ. Bạn sẽ được báo thời gian dự tính theo tuổi của con bạn.

Mỗi trẻ sẽ được đánh giá 3 lần trong 18 tháng để theo dõi sự phát triển của trẻ.

Ai có thể tham gia?

Chúng tôi mong muốn công cụ đánh giá này được dùng cho mọi trẻ, nhưng bước đầu, chúng tôi cần thông tin về trẻ khỏe mạnh dưới 4 tuổi. Chúng tôi cần những trẻ sinh đủ tháng, chưa bao giờ nằm viện vì một bệnh nặng hoặc có bệnh mạn tính. Chúng tôi sẽ không đánh giá những trẻ có khiếm khuyết học tập.

Lợi ích của việc tham gia ?

Đây là một cơ hội, để bạn dành thời gian thảo luận, về sự phát triển của con bạn với các chuyên gia trong lĩnh vực này.

Địa điểm đánh giá ?

Tại một phòng trong trường mầm non, phụ huynh cần có mặt trong suốt buổi đánh giá.