Factors associated with fatal outcome of children with enterovirus A71 infection: a case series

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Abstract

Enterovirus A-71 (EV-A71) may be fatal, but the natural history, symptoms, and signs are poorly understood. This study aimed to examine the natural history of fatal EV-A71 infection and to identify the symptoms and signs of early warning of deterioration. This was a clinical observational study of fatal cases of EV-A71 infection treated at five Chinese hospitals between 1 January 2010 and 31 December 2012. We recorded and analysed 91 manifestations of EV-A71 infection in order to identify early prognosis indicators. There were 54 fatal cases. Median age was 21.5 months (Q1−Q3: 12–36). The median duration from onset to death was 78.5 h (range, 6 to 432). The multilayer perceptron analysis showed that ataxia respiratory, ultrahyperpyrexia, excessive tachycardia, refractory shock, absent pharyngeal reflex, irregular respiratory rhythm, hyperventilation, deep coma, pulmonary oedema and/or haemorrhage, excessive hypertension, tachycardia, somnolence, CRT extension, fatigue or sleepiness and age were associated with death. Autopsy findings ($n = 2$) showed neuronal necrosis, softening, perivascular cuffing, colloid and neuronophagia phenomenon in the brainstem. The fatal cases of enterovirus A71 had neurologic involvement, even at the early stage. Direct virus invasion through the neural pathway and subsequent brainstem damage might explain the rapid progression to death.

Introduction

Enterovirus 71 (EV-A71) outbreaks have been raising considerable public health concerns since the late 1990s [1–11]. EV-A71 infection may be asymptomatic of cause diarrhoea, rashes, and hand, foot and mouth disease (HFMD). These symptoms predominated in laboratory-confirmed cases, present in 45% of mild, 80% of severe and 93% of fatal cases [12]. EV-A71 infection is especially known for its role in epidemics of severe neurological diseases in children, sometimes leading to death [13]. Indeed, the mortality rate of severe EV-A71 infection cases was 5.14% [12]. Fatal cases appear about 3 days from the onset of illness [7, 14]. The cause of death tends to be brainstem function failure [15–18]. Studies have reported that most fatal cases were 5 years of age or younger, and all were with similar manifestations, i.e. autonomic nervous system failure and other neurological symptoms before death [1, 2, 7, 14].

Autopsies and animal models indicated that the main lesions appear to be localised to the brainstem [15, 16, 18–25]. Indeed, brainstem encephalitis accounts for 58% of neurological manifestations of EV-A71 infection, followed by aseptic meningitis (36%) [15, 16, 18–26]. Neurological symptoms include myoclonus, ataxia, nystagmus, oculomotor palsies and bulbar palsy, and imaging can be either positive or negative [26]. Receptors for EV-A71 have been identified on human cells; SCARB2 is the main receptor of EV-A71 in nerve tissue, allowing the virus release its RNA into neurones [2–5]. Nevertheless, the reasons for brainstem involvement are unknown [26].

In addition, there is a lack of reliable indicators for the early identification of the cases at higher risk of death. Determining the symptoms and signs that can be associated with a fatal progression of EV-A71 infection could be useful to identify the patients who could benefit from treatments such as intravenous immunoglobulins and milrinone [26]. Therefore, the aim of the present study was to examine the natural history of fatal EV-A71 infection and to identify the symptoms and signs for early warning of deterioration. To do so, a 5-year multicentre clinical observational study was performed.

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Table 1. Signs and symptoms in 54 fatal cases of EV71 infection and time of occurrence (h)

Sign/symptom	Median (range)	Mean ± s.d.
General indicators (four items)		
Fever (n = 52)	0 (0–72)	4.0 ± 13.3
Rash (n = 36)	0 (0–120)	13.2 ± 29.3
Herpangina (n = 8)	3 (0–48)	10.5 ± 17.2
Flu-like symptoms (n = 6)	6 (0–6)	4.0 ± 3.1
Dyskinesia indicators (46 items)		
Emotional movement disorders (six items)		
Startle (n = 25)	24 (0–96)	27.4 ± 26.0
Anxiety (n = 19)	32 (14–72)	38.0 ± 17.9
Dysphoria (n = 18)	53 (6–96)	54.9 ± 23.8
Fright (n = 13)	52 (18–96)	47.9 ± 19.4
Mania (n = 4)	72 (52–96)	73.0 ± 18.0
Gibberish (n = 2)	24	24.0 ± 0.0
Limb/muscle movement disorder (14 items)		
Myoclonic jerks/tremors (n = 21)	36 (0–72)	35.6 ± 22.9
Dysphagia (n = 14)	41 (12–96)	44.4 ± 23.6
Tics (n = 8)	60 (0–27)	54.0 ± 24.8
Limbs weakness (n = 12)	57 (4–90)	53.3 ± 22.4
Neck rigidity (n = 5)	46 (12–72)	45.6 ± 27.4
Inability to rise head (n = 11)	60 (24–96)	56.2 ± 19.8
Hypermyotonia (n = 11)	68 (24–96)	67.3 ± 26.4
Hypomyotonia (n = 8)	72 (4–96)	60.5 ± 33.0
Upper extremity weakness (n = 6)	48 (4–72)	46.7 ± 23.0
Upper extremity weakness (n = 7)	48 (4–72)	50.3 ± 23.1
Galloping tongue (n = 2)	56 (42–70)	56.0 ± 19.8
Thalamus hand/foot (n = 1)	42	42
Body ataxia (n = 0)	–	–
Ptosis (n = 0)	–	–
Abnormal eyeball movement (eight items)		
Nystagmus (n = 16)	42 (18–72)	39.3 ± 17.2
Autokinetic eyeball (n = 13)	42 (24–72)	43.4 ± 15.8
Ataxia eyeball** (n = 11)	48 (24–73)	44.6 ± 20.6
Ocular abduction (n = 10)	39 (12–72)	38.4 ± 19.2
Gaze (n = 9)	60 (6–72)	54.4 ± 20.7
Eyeball turn over (n = 5)	48 (48–72)	57.6 ± 13.2
Eyeball fixed (n = 3)	30 (6–120)	52.0 ± 60.1
Eyeball cohesion (n = 0)	–	–
Involuntary movements (five items)		
Chew (n = 3)	60 (24–72)	52.0 ± 25.0
Molar (n = 3)	72 (42–96)	70.0 ± 27.1
Blink/frown (n = 3)	30 (24–72)	42.0 ± 26.2

(Continued)

Table 1. (*Continued.*)

Sign/symptom	Median (range)	Mean ± s.d.
Suck (n = 3)	48 (24–96)	56.0 ± 36.7
Empty catch/grope (n = 1)	56	56
Abnormal pupil movement (six items)		
Light reflex abnormal (n = 10)	72 (12–96)	64.9 ± 20.8
Myosis (n = 7)	60 (48–408)	176.0 ± 120.3
Mydriasis (n = 4)	77 (6–108)	42.0 ± 45.2
Anisocaria size (n = 6)	27 (10–84)	51.7 ± 29.6
Anisocoria round (n = 4)	27 (6–156)	89.7 ± 39.5
Pupil variable (n = 2)	60 (48–72)	60.0 ± 17.0
Other neural reflex (seven items)		
Pharyngeal reflex absent** (n = 18)	50 (12–120)	61.2 ± 26.8
Corneal reflex absent (n = 6)	108 (6–144)	97.0 ± 52.6
Pathological reflex (n = 7)	48 (12–72)	45.4 ± 22.4
Ankle clonus (n = 8)	72 (36–96)	69.0 ± 26.1
Knee hyperreflexia (n = 6)	45 (6–120)	69.7 ± 40.4
Knee hyporeflexia (n = 4)	78 (18–72)	45.0 ± 22.2
Knee reflex asymmetry (n = 2)	42 (12–72)	42.0 ± 42.4
Non-specific indicators (nine items)		
Vomiting (n = 31)	42 (0–96)	37.2 ± 29.5
Persistent hyperpyrexia (n = 24)	42 (0–144)	48.2 ± 42.4
Hydrostomia (n = 10)	36 (12–84)	36.6 ± 19.7
Headache (n = 1)	36	36
Dizziness (n = 1)	0	0
Palpitation (n = 0)	–	–
Autonomic dysfunction indicators (29 items)		
Poor peripheral perfusion (four items)		
CRT extension (n = 43)	64 (4–144)	56.3 ± 28.2
Clammy skin** (n = 32)	63 (4–144)	59.1 ± 31.3
Pale skin** (n = 27)	70 (4–144)	59.6 ± 33.8
Mottled skin (n = 19)	48 (18–72)	48.8 ± 22.2
Circulatory (seven items)		
Tachycardia (n = 36)	64 (24–96)	58.6 ± 22.0
Ultrahyperpyrexia* (n = 20)	66 (30–144)	75.8 ± 38.5
Excessive tachycardia* (n = 16)	72 (26–88)	61.2 ± 19.8
Ultrahypertension* (n = 15)	72 (30–144)	70.1 ± 31.2
Hypertension** (n = 13)	83 (48–144)	88.0 ± 23.7
Arthythmia (n = 14)	72 (48–120)	83.3 ± 23.0
Bradycardia (n = 4)	65.5 (6–120)	64.3 ± 46.9
Perspiration (three items)		
Hyperhidrosis (n = 15)	48 (24–108)	57.9 ± 23.5
Adiapneustia (n = 2)	72 (24–120)	72.0 ± 67.9

(Continued)

Table 1. (*Continued.*)

Sign/symptom	Median (range)	Mean ± s.d.
Partial body sweating (*n* = 0)	–	–
Respiratory (nine items)		
Tachypnea (*n* = 22)	44 (6–120)	45.4 ± 24.3
Hyperventilation (*n* = 29)	48 (4–120)	54.2 ± 29.7
Irregular respiratory rhythm** (*n* = 28)	55 (20–120)	56.4 ± 23.6
Ataxia respiratory** (*n* = 12)	49 (22–78)	55.1 ± 19.9
Cheyne-stikes breathing (*n* = 3)	72 (72–96)	80.0 ± 13.9
Apneustic breathing (*n* = 5)	29 (24–72)	39.4 ± 20.7
Cluster breathing (*n* = 2)	55 (42–68)	55.0 ± 18.4
Slowed breathing (*n* = 2)	33 (24–42)	33.0 ± 12.7
Paradoxical breathing (*n* = 0)	–	–
Other autonomic dysfunction indicators (four items)		
Frequent vomiting (*n* = 5)	48 (24–64)	46.4 ± 14.3
Variable erythema (*n* = 5)	48 (27–72)	48.2 ± 20.0
Central diabetes insipidus (*n* = 2)	83	83.0 ± 0.0
Anuria (*n* = 1)	120	120
–	–	–
Failure (two items)		
Refractory shock* (*n* = 23)	72 (31–144)	67.1 ± 30.2
Pulmonary oedema and/or haemorrhage** (*n* = 46)	72 (6–144)	66.3 ± 29.1
Death		
Death (*n* = 54)	78.5 (6–432)	100.68 ± 78.88
Disturbance of consciousness indicators (five items)		
Fatigue/sleepiness (*n* = 49)	48 (6–108)	46.0 ± 23.9
Somnolence (*n* = 22)	55 (24–96)	56.3 ± 16.7
Mild coma (*n* = 12)	72 (27–96)	64.8 ± 23.0
Moderate coma (*n* = 13)	72 (27–120)	69.6 ± 25.8
Deep coma* (*n* = 24)	74 (30–144)	81.3 ± 29.9

Note: *Median duration of symptoms/signs occurring within 6 h before death.
**Median duration of symptoms/signs occurring within 12 h before death.

Methods

Study design

This was a clinical observational study of fatal cases of EV-A71 infection. Among the severe cases of EV-A71 infection, we reviewed all HFMD fatal cases records to analyse the risk factors associated with mortality [27–29]. Seven centres designated to treat HFMD in the coastal cities of Guangdong province participated in this study and were trained for the identification of fatal cases. The data of the fatal cases came from five centres; the other two hospitals had no fatal cases.

This study was approved by the Ethics Committee of our institution. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The legal guardians of the children provided a written informed consent to be included in our databank of patients with EV-A71 infection.

Patients

In 2008–2009, all paediatricians working at the Department of Paediatrics of the seven participating hospitals were trained to recognise the symptoms and signs of EV-A71 infection, including the first-, second- and third-line physicians. Sida Yang, the study principal investigator, also performed random inspections to assess accuracy (10% of the entries). Between 1 January 2010 and 31 December 2012, we recorded the symptoms and signs of all EV-A71 infection cases using a standard form. The manifestation, sequence and duration (in hours) of all symptoms and signs were noted.

The inclusion criteria were: (1) <14 years of age; (2) suspected EV-A71 infection (patients with HFMD or patients without rash or herpangina but with neurological symptoms); and (3) confirmed by real-time PCR using a throat swab sample, as previously described (ABI 7300 real-time fluorescence quantitative PCR; C_t value <34.9 was considered positive) [30]. Those who met all the above whole criteria were included.

The exclusion criteria were: (1) tested positive for any pathogens other than EV-A71 (including any other enterovirus or CoxA16); (2) trauma; (3) intoxication; (4) received any vaccine within 4 weeks (to exclude patients with increased susceptibility to EV-A71 because of immune reaction to the vaccine or confounding symptoms from the vaccine); or (5) other central nervous system (CNS) diseases such as cerebral palsy or intracranial space-occupying lesion. Among 5504 confirmed severe cases of EV-A71, 54 fatal cases were identified after application of all above inclusion/exclusion criteria.

Observational indexes

Based on our previous studies [27–29], we identified and defined 91 symptoms and signs of severe cases of EV-A71 infection. The study board included paediatricians and paediatric neurologists, including HFMD specialists from the National Health and Family Planning Commission of the People's Republic of China, experts from the National Clinical Research Centre for Respiratory Disease and from the Institute of Neuroscience of Guangzhou Medical University. These experts reviewed and determined all 91 symptoms and signs. All severe cases were treated according to the national guidelines (in Chinese: http://www.gov.cn/gzdt/2008-05/03/content_960347.htm; or the English version from Hong Kong: http://www.dh.gov.hk/chs/useful/useful_ld/files/ltod20070523.pdf).

The manifestations of fatal cases included general indicators (fever, rash, herpangina and flu-like symptoms), non-specific indicators (vomiting, persistent hyperpyrexia, hydrostomia, headache, dizziness and palpitation), dyskinesia (46 items), disturbance of consciousness (five items) and autonomic dysfunction (29 items) (Supplementary Material 1) [31]. The time from onset of disease was defined as the onset of initial symptom in the patient's medical history.

The autopsy had to be authorised by the legal guardians and was performed at the Forensic Medical Identification Centre of Sun Yat-Sen University.

Statistical analyses

Descriptive statistics were used (frequencies, mean ± S.D., median). Statistical analyses were performed using SPSS 22.0 (IBM, Armonk, NY, USA). Multilayer perceptron analysis [32–34] was used for the stable prediction of the possibility or category of death under the effects of multivariates/multicovariates [35]. Briefly, the 91 indicators observed in this study were divided randomly into two groups (the training and testing groups), and then the possible associations between these indicators with death were assessed. If the percentages in the two groups are similar, the results indicate that the indicators are statistically significant in stably predicting death. This method was used to compensate the limitation of lacking controls for survival.

Results

Characteristics of the patients

Between 1 January 2010 and 31 December 2012, there were 5504 inpatients confirmed with severe EV-A71 infection in five hospitals, including 54 fatal cases (0.98%). Among the fatal cases, there were 31 males and 23 females. There were 26 cases from urban areas and 28 cases from rural areas. Median age was 21.5 months (Q1−Q3: 12–36; mean, 23.0 ± 13.8 months; 47 cases (87.04%) were 3–36 months old. The median duration from onset to death was 3.3 days (range, 0.25–18); 43 cases (79.4%) died within 5 days, and four cases died between 11 and 18 days as they received paediatric advanced life support (PALS) within 5 days after illness onset. Illness onset was defined as the appearance of the first symptom.

Occurrence time of symptoms or signs

Table 1 shows presents the 91 indicators recorded. Except fever, the most common symptom before death was pulmonary oedema and/or haemorrhage ($n = 46$, 85.2%), followed by capillary refill time (CRT) extension ($n = 43$, 79.6%). The signs/symptoms that were present in more than 50% (≥27) of the fatal cases were fever ($n = 52$, 96.3%), pulmonary oedema and/or haemorrhage ($n = 46$, 85.2%), CRT extension ($n = 43$, 79.6%), tachycardia ($n = 36$, 66.7%), rash ($n = 36$, 66.7%), clammy skin ($n = 32$, 59.3%), vomiting ($n = 31$, 57.4%), hyperventilation ($n = 29$, 53.7%), irregular respiratory rhythm ($n = 28$, 51.9%) and pale skin ($n = 27$, 50.0%). The signs/symptoms that were present in
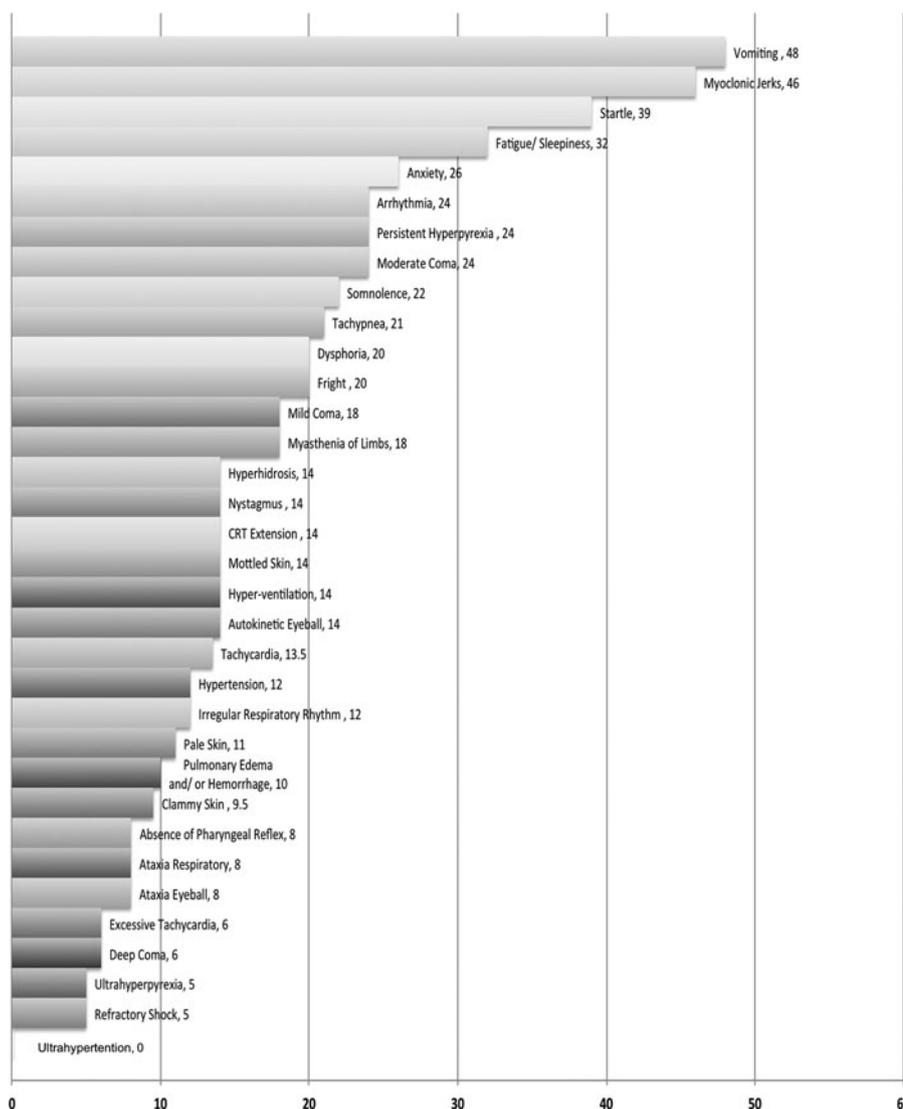

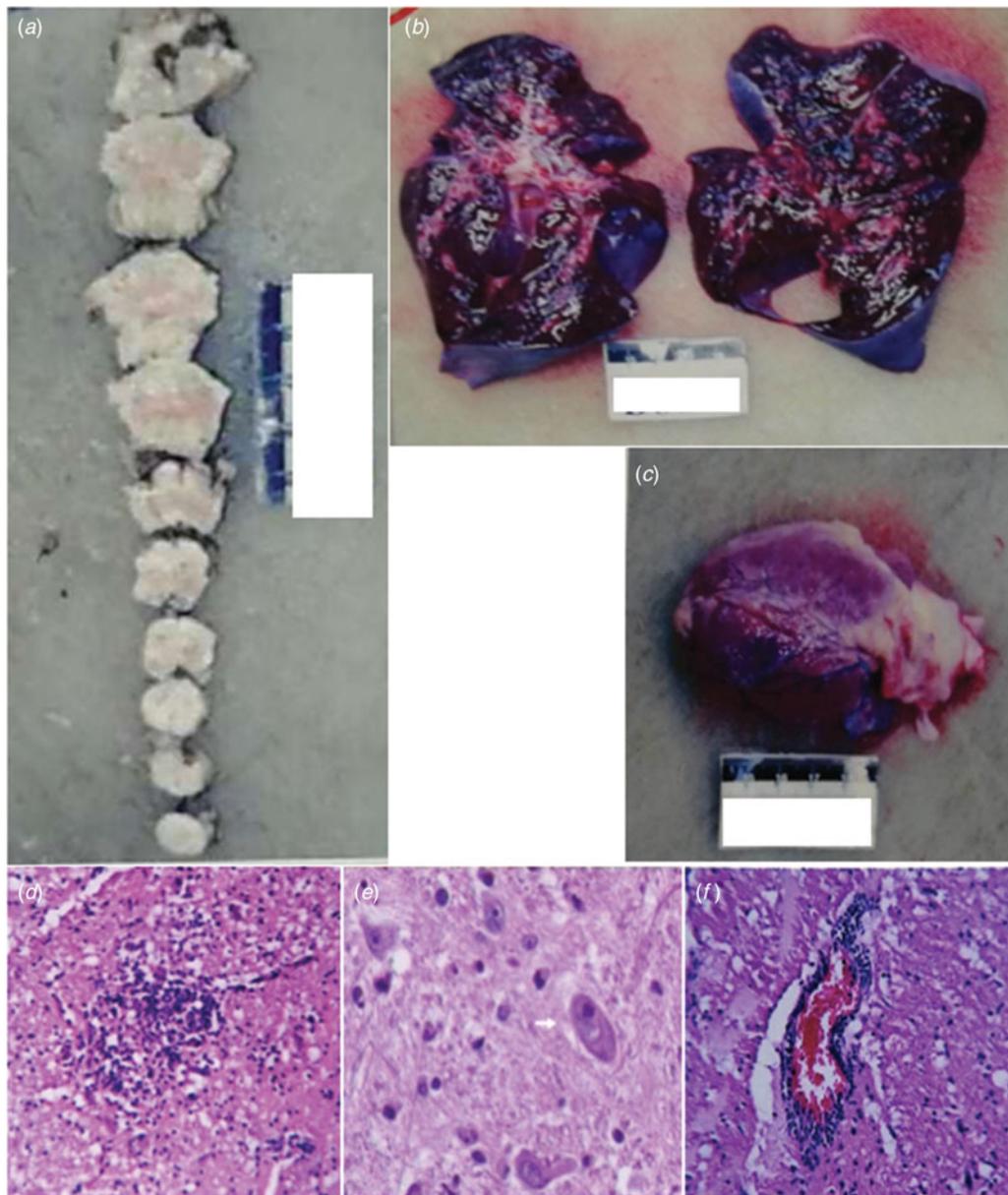Fig. 1. Median duration of symptoms/signs occurring before death in patients with enterovirus A7 infection.

more than 30% ($\geq$16) of the fatal cases were startle ($n = 25$, 46.3%), persistent hyperpyrexia ($n = 24$, 44.4%), deep coma ($n = 24$, 44.4%), refractory shock ($n = 23$, 42.6%), somnolence ($n = 22$, 40.7%), tachypnoea ($n = 22$, 40.7%), myoclonic jerks/tremors ($n = 21$, 38.9%), ultrahyperpyrexia ($n = 20$, 37.0%), anxiety ($n = 19$, 35.2%), mottled skin ($n = 19$, 35.2%), dysphoria ($n = 18$, 33.3%), absence of pharyngeal reflex ($n = 18$, 33.3%) and excessive tachycardia ($n = 16$, 29.6%).

Relation between death and signs/symptoms

Three patients died within 24 h after onset, and 23 symptom or signs had been observed from the list of 91 signs/symptoms. The shortest duration from onset to death was 6 h; this case presented with fever first, clammy limbs followed, then death after from pulmonary haemorrhage. The other two cases presented ataxia respiratory or pulmonary haemorrhage; none of the three were comatose before PALS. Five cases died 1–1.5 days after onset and they showed 58 symptoms or signs. Autonomic dysfunction was found in all five cases, including poor peripheral perfusion and pulmonary oedema and/or haemorrhage. For the patients who died >1.5 days after onset, a wide diversity of manifestations could be observed. These patients deteriorated sharply after autonomic nerve failure, including refractory shock, Neurogenic pulmonary edema (NPE), excessive tachycardia, ultrahypertension and deep coma. When these five symptoms persisted for 6 h (median duration), the patients died (Fig. 1).

Multilayer perceptron analysis

The multilayer perceptron analysis showed that age and 15 symptoms and signs predicted a fatal progression. These symptoms/ signs were ataxia respiratory (100% of training, 100% of testing), ultrahyperpyrexia (92.3%, 85.7%), excessive tachycardia (80.0%, 83.3%), refractory shock (75.7%, 85.0%), pharyngeal reflex absent (71.4%, 75.0%), irregular respiratory rhythm (65.0%, 73.0%), hyperventilation (66.7%, 72.7%), deep coma (60.0%, 66.7%), pulmonary oedema and/or haemorrhage (59.5%, 55.6%), excessive hypertension (50.0%, 42.9%), tachycardia (50.0%, 42.9%), somnolence (43.8%, 50.0%), CRT extension (39.3%, 33.3%), fatigue or sleepiness (34.6%, 39.1%) and age (30.0%, 35.7%) (Table 2).

Autopsy findings

Macroscopic examination showed brainstem oedema (Fig. 2a). The brain and brainstem sections showed no bleeding or hernia. The texture of the two lungs was more solid than normal and there were foam or pale red liquid overflow in the lung sections (Fig. 2b). There were no abnormal appearance of the heart (intracardiac structure and coronary) and no bleeding in the myocardial sections (Fig. 2c).

Microscopic examination of the brain showed that there was no haemorrhage in the brain parenchyma. Necrosis of the brainstem and formation of the softening spots were observed (Fig. 2d). There were microglia infiltration and the phenomenon of neuronophagia (Fig. 2e). Glial nodule was observed (Fig. 2d). Interstitial small vessels were dilated and congested and there were many lymphocytes and mononuclear cells infiltrated around the vessels, showing 'sleeve like' changes (Fig. 2f).

Microscopic examination of the heart, lung and other organs showed that the lungs were with alveolar wall thickening, interstitial fibrous tissue hyperplasia, vascular dilatation and hyperemia,

Table 2. Multilayer perceptron of signs/symptoms associated with death from of non-survivors after enterovirus A71

Sign or symptom	Ataxia respiratory		Ultrahyperpyrexia		Refractory shock		Excessive tachycardia		Pharyngeal reflex absent		Irregular respiratory rhythm		Hyperventilation		Deep coma	
Group	A ($n=9$)	B ($n=3$)	A ($n=13$)	B ($n=7$)	A ($n=16$)	B ($n=7$)	A ($n=10$)	B ($n=6$)	A ($n=14$)	B ($n=4$)	A ($n=21$)	B ($n=7$)	A ($n=18$)	B ($n=11$)	A ($n=15$)	B ($n=9$)
Correct (%)	100	100	92.3	85.7	75.7	85	80	83.3	71.4	75	65	73	66.7	72.7	60	66.7

Sign or symptom	Pulmonary oedema and/or haemorrhage		Ultrahypertension		Tachycardia		Somnolence		CRT extension		Fatigue/sleepiness		Age	
Group	A ($n=37$)	B ($n=9$)	A ($n=8$)	B ($n=7$)	A ($n=25$)	B ($n=11$)	A ($n=16$)	B ($n=6$)	A ($n=28$)	B ($n=15$)	A ($n=26$)	B ($n=23$)	A ($n=40$)	B ($n=14$)
Correct (%)	59.5	55.6	50	42.9	50	42.9	43.8	50	39.3	33.3	34.6	39.1	30	35.7

Group A: Training; Group B: Testing.

Fig. 2. Macroscopic examination of the brainstem (a), lung (b) and heart (c). Gliacyte proliferation and accumulation of tuberculum in the brainstem; vacuolar degeneration of neurons formed reticular necrosis lesion (d). Brainstem neurons showed neuronophagia in fatal cases of enterovirus A71. The pathological studies of brainstem showed neuronophagia, HE × 400 (e). Lymphocytes and small glial cell infiltration around vessels in the brainstem, showing 'sleeve like' change (f).

alveolar cavity pink or pale homogeneous oedema liquid, no inflammatory cell infiltration and a small amount of transparent membrane formation. The heart showed no myocardial necrosis and inflammatory cell infiltration, interstitial mild congestion, oedema, no bleeding and myocardial fibre breakage in a wavy arrangement. There were no obvious pathological changes in the other organs (such as liver, spleen, kidney, adrenal gland, pancreas, thymus, gastrointestinal tract, etc.).

Discussion

EV-A71 infection may be fatal, but the natural history, symptoms and signs are poorly understood. Therefore, this study aimed to examine the natural history of fatal EV-A71 infection and to identify the symptoms and signs for early warning of deterioration.

The results showed that all fatal cases of enterovirus A71 had neurologic involvement, even at the early stage.

Despite the fact that the mechanisms of neurological involvement in EV-A71 infections remain mostly unknown [26], recent advances shed some light on this point. Among the fatal cases, neurological symptoms and signs could be observed at any stage and autonomic dysfunction was the most common before death. Some symptoms observed in the present study were similar to those of other studies [1, 2, 7, 14], but these early reports lack the timing of the manifestations. Compared with the surviving patients, the symptoms of autonomic nerve dysfunction appeared earlier in fatal cases [36]. The neurological involvement of the limbic system, pyramidal system, extrapyramidal and autonomic nerve was similar to that of the macaque model developed by Nagata *et al.* [37, 38]. SCARB2 is essential for neurological

involvement during EV-A71 infection [39–43]. EV-A71 binds to myelin SCARB2, which induces degranulation and neuron damage [40, 41]. Nagata *et al.* [37, 38, 44] showed in a macaque model of EV-A71 infection that the damaged neural area included limbic system, pyramidal system, extrapyramidal and autonomic nerves. By binding to SCARB2 receptors on myelin of these regions, EV-A71 can attack the brainstem within a few hours through reverse axonal transport [45–48]. Autopsy studies showed that the brain, especially the brainstem, was the most severely involved [19, 49–51]. Neurons in areas of inflammation and tissue necrosis have been shown to be positive for EV-A71 by immunohistochemistry [52]. These findings were similar to the autopsy findings of the present study, and could explain, at least in part, the neurological manifestations. Nevertheless, additional studies are necessary to determine whether myelin structures provide a direct neural pathway for EV-A71 invasion since only two patients could be examined post mortem.

In the present study, the patients of 3–36 months of age represented 87.0% of the fatal cases, which was not only similar to a large study in China [53], but also to a number of previous studies [7, 14, 54]. The multilayer perceptron analysis showed that age was one of the valid factors predicting progression to death. This age distribution matches the age of active myelination [55–60]. Nevertheless, whether the age distribution of severe vulnerability is related to active myelination [46, 55–58, 60, 61] will have to be confirmed in a future study.

The rapid progression and the neurological pathway suggested here may be associated with the biology of EV-A71. Indeed, EV-A71 is transmitted by droplets or direct contact [17, 62] and often invades the patients through the mucosa of the oropharynx, throat and eyes [63–65]. Because EV-A71 has muscle- and neurotropism properties [37, 45, 66, 67], the neurological involvement could occur immediately after invasion. Stimulation of invasion by EV-A71 could also lead to startle being the initial symptom [68, 69], as we observed in 46.9% of the patients, In addition to myoclonic jerks/tremors and vomiting. The nerve conduction involved in these onset-symptoms is shown in Table 3. When eye or nasal mucosa invasion triggered visual or smell startle reflex and startle onset [70–72], then facial motor nerve involvement, triggering nerve impulse conducting to the red nucleus, and myoclonic jerks/tremors occurred, while invasion from the pharyngeal mucosa triggered vomiting (see Fig. 3). Binding with SCARB2 in myelin induces EV-A71 uncoating and cause neuronal necrosis in the brainstem [17, 43, 49, 70–77], cervical spine cord, spinal cord ventral horn, cerebellum and interbrain central nervous tissues [18, 20, 45, 51, 77]. As autopsy reports showed [78], the brainstem damage observed in the present study was similar. The damage process of EV-A71 can be seen from the symptoms of nerve involvement. In the case of glossopharyngeal nerve, we observed the damage from the peripheral nerves to the glossopharyngeal nucleus (ambiguus) which locates in the medulla oblongata, i.e. the life-centre (Table 4). The length of the cranial nerve in infants and children is similar, and there was no difference in the occurrence time of neurological symptoms caused by the same nerve structure involvement in our cases (Table 4), suggesting that a cranial nerve pathway could be existing.

Some animal experiments and autopsy studies reported that EV-A71 was detected in the brainstem tissue [14, 16, 17, 37, 79]. EV-A71 can attack the brainstem within a few hours via motor nerves by reverse axonal transport [45, 46, 75] Animal

Table 3. Nerve conduction involved in the onset-symptoms

| Onset symptoms	n	Occurring time (mean ± s.d.) (h)	Reflection	Afferent nerve	Central integrated neuron	Neural pathway	Nerve conduction bundles	Effectors	Duration of retrograde transport to central neurons (h)
Startle	25	27.4 ± 26	Startle reflex (Short-pathway)	Cranial nerves: II/III/IV/V/VI	Red nucleus	Cranial nerves–red nucleus –amygdala	Cranial nerves: II/III/IV/V/VI, rubrospinal tract, corticospinal tract	Muscles of head, face, limbs and trunk	5–10
Myoclonic jerks/tremors	21	35.6 ± 22.9	Peripheral myoclonus pathway	Head and facial motor nerve fibres	Red nucleus	Cranial nerves–red nucleus (myoclonic triangle: olive nucleus–red nucleus–dentate nucleus)	Cranial nerves, rubrospinal tract, corticospinal tract	Muscles of head, face, limbs and trunk	5–10
Vomiting	31	37.2 ± 29.5	Vomiting reflex	Glossopharyngeal, Vagus nerve	Nucleus Ambiguus	Cranial nerves IX–reticular formation of medulla oblongata (vomiting centre)	Cranial nerves: IX autonomic nerve	Gastrointestinal muscle, diaphragm muscle and abdominal wall muscle	3–11
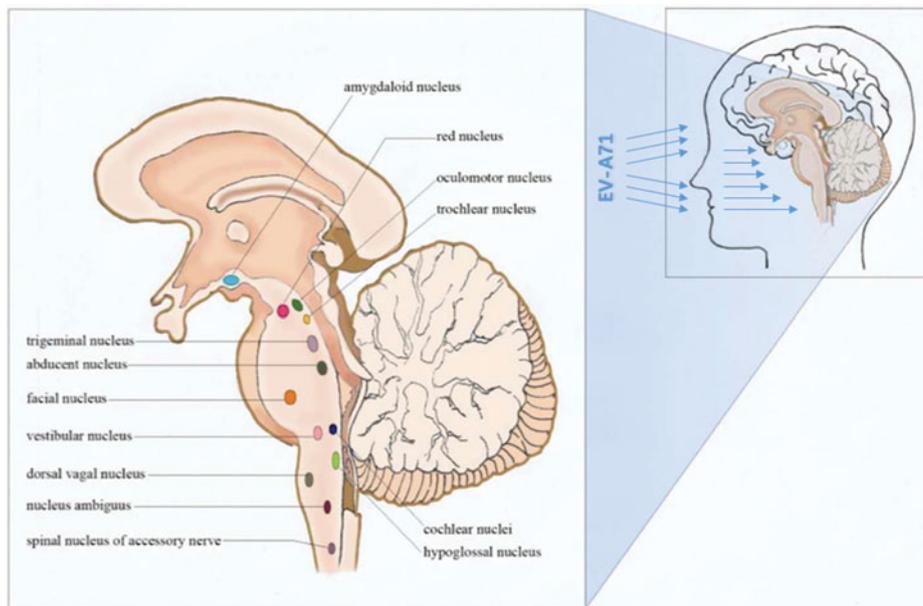
Fig. 3. Invasion entrance of enterovirus A71 (mucosa of the oropharynx, throat, nose and eyes).

experiments showed that EV-A71 entered the brainstem through the cranial nerve [23], which provides a basis for the existence of neural pathways in the disease. The duration from onset to death depends on the velocity of this reverse axonal transportation, the length of the cranial nerves and the characteristics of the virus including its virulence, spreading mode and transmission. Further study is needed to explore whether EV-A71 can hijack and accelerate axonal transport, as other small RNA viruses do [78]. The speed of retrograde axonal transportation is 205 mm/24 h. Because the cranial nerve is shorter and the retrograde axonal transport is faster in infant and young children, the time of transportation of substances through the cranial nerve, including viruses, to the brainstem neurons is shorter (Supplementary Materials 2) Furthermore, the reticular formation of the brainstem creates conditions for the expansion of viral damage, resulting in the loss of a large number of brainstem neurons, especially the medullary neurons, leading to death. It can explain the rapid fatal process of extreme cases from onset to death after only 6 h in the present. Nevertheless, it has to be underlined that these mechanisms are hypotheses based on the literature and were not directly observed in the present study, even if they conveniently fit our observations. The related molecular biological mechanisms need to be further studied.

There are two more noticeable findings in the present study. The occurrence of unconsciousness will progress to death in the fatal cases, regardless of the duration or level of unconsciousness. This phenomenon probably just reflected that EV-A71 invades different areas of the awakening brain centres. In addition, fatigue was the only observed sign when pulmonary oedema and/or haemorrhage occurred in four patients, and this phenomenon might be related to direct attack on the medulla oblongata or spinal cord [80], without attack on the awakening centre.

In summary, direct EV-A71 invasion and subsequent brainstem–neuron damage could be said to be the important causes of death in patients with EV-A71 infection. Combining with the clinical manifestation, the pathological anatomy and previously reported biology of EV-A71, we attempted to describe the neural invasion pathway of EV-A71 (Fig. 4). According to our hypothesis, EV-A71 spreads through droplets or direct contact to infect children. EV-A71 invades the mucosa of oropharynx, throat, eyes and nose (multiplication in the skeletal muscle). EV-A71 reaches the neurons through motor nerves in the muscles of the head, face and oropharynx by reverse axonal transport, and causes the relevant symptoms and signs. EV-A71 will be transmitted through the neural networks to other related CNS areas. Once EV-A71 entered the neural pathway, the distance of attacking medulla

Table 4. The comparison of occurring-time of symptoms with glossopharyngeal involvement (h)

| Symptoms	Onset symptom: vomiting (n = 31)	Pharyngeal reflex absent* (n = 18)	Excessive tachycardia* (n = 16)	Refractory shock** (n = 23)	NPE** (n = 46)	P*	P**
Mean ± s.d.	37.2 ± 29.5	61.2 ± 26.8	61.2 ± 19.8	67.1 ± 30.2	66.3 ± 29.1	1.000	0.921
Involved tissues	Pharynx	Pons – medulla oblongata	Pons – Medulla oblongata	Medulla oblongata	Reticular formation of medulla oblongata		
Neural localisation	Glossopharyngeal nerve ending	The upper part of the glossopharyngeal nerve nucleus ambiguus	The upper part of the glossopharyngeal nerve nucleus ambiguus	The lower part of the glossopharyngeal nerve nucleus ambiguus	The lower part of the glossopharyngeal nerve nucleus ambiguus		
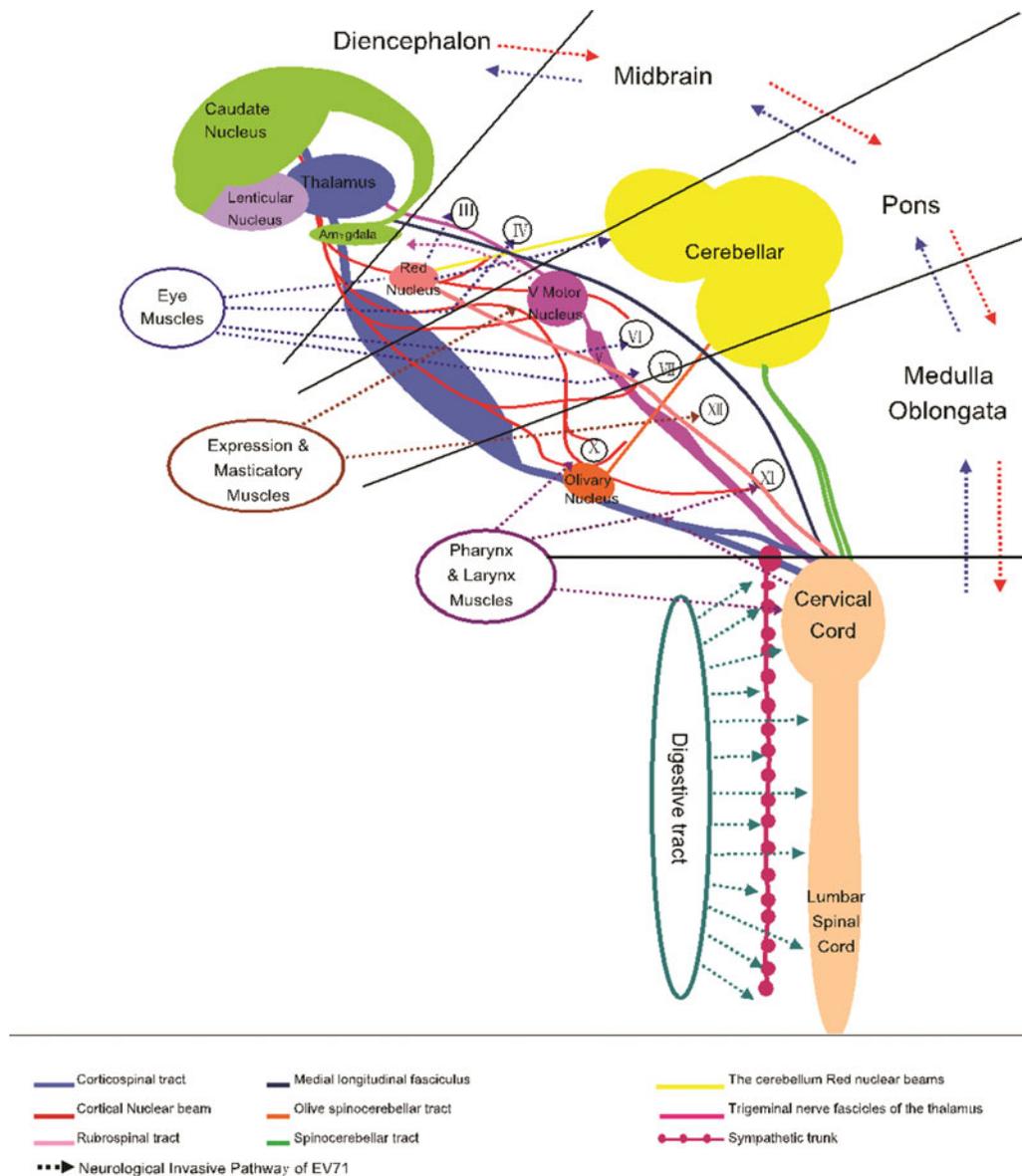
Fig. 4. Neurological invasion pathway of enterovirus A71.

could be calculated in millimetres and the duration from onset to death could be calculated in minutes. The present study strongly suggests that it is important for clinicians to identify neurological manifestations of EV-A71. In practice, identification of severe cases will help clinicians provide appropriate assessment and intervention.

The World Health Organization suggests that the clinical monitoring indicators for HFMD should include basic vital signs, myoclonic jerks, profuse sweating, central venous pressure, arterial blood gases and echocardiography [79]. In the present study, abnormalities could be observed in <50% of the cases and these patients could progress rapidly to death. In these circumstances, the cranial nerve symptoms and signs, the level of consciousness and autonomic nerve function like poor peripheral circulation should be assessed and reassessed, in order to more fully understand the degree of disease progression, then get early warning of critical state.

This study has some limitations. Firstly, the observed cases were only from coastal cities in Guangdong Province, and the sample size was small, precluding any reliable calculation of sensitivity and specificity. Therefore, the generalisability of our study might be challengeable. Secondly, we only interpreted the observation and analysis of the clinical facts. The present facts supported that neurological symptoms were valid for the early assessment of the risk of death, but the actual validity will have to be tested in a future study that will include controls. Thirdly, due to ethical reasons, autopsy could only confirm the main cause of death, and no exploratory and comprehensive autopsy could be performed. Thus, brainstem lesion could not be explained as an independent reason for death. In addition, due to failure to obtain the legal guardians' consent, the autopsy cases were limited, and EV-A71 virus isolation or PCR was not performed due to Chinese regulations.

Conclusion

The fatal cases of enterovirus A71 had neurologic involvement, even at the early stage. Direct virus invasion through neural pathway and subsequent brainstem damage might explain the rapid progress to death.

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