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Viral Encephalitis with Focus on Human Enteroviruses

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1. Introduction

The field of infectious diseases is an exciting field with new agents emerging continuously. The emergence of new diseases is partly contributed by our growing population, expansion of residential areas into previously uninhabited areas and increase in global travel. Most of the emerging new infections are due to RNA viruses and nearly half have been described to cause encephalitis or significant neurological symptoms [1].

This review will focus on encephalitis caused by enteroviruses. Encephalitis is basically inflammation of the brain. Several viruses can result in encephalitis and some viruses, such as Enterovirus 71 (EV71), have been documented to cause epidemics.

2. Virology of enterovirus

Enteroviruses are single-stranded, positive-sense RNA viruses belonging to the *Picornaviridae* family. They are associated with various human and animal diseases, and are traditionally classified into 4 groups, namely, Coxsackie A viruses, Coxsackie B viruses, echoviruses and polioviruses, depending on the clinical presentation. Recently, however, enteroviruses have been named numerically e.g. EV70 and EV71 in recognition of the similarities among the 4 groups noted on genomic studies [2].

RNA viruses are known for their high spontaneous mutation rate, which is attributed to the absence of proof reading in viral RNA polymerases. This invariably leads to the emergence of new enteroviruses and consequently, new clinical presentations. Enterovirus 71 was first described in California USA in 1969 [3] and will arguably be the next most important enterovirus after the eradication of poliovirus.

The enterovirus is a small, non-enveloped spherical particle around 30nm in diameter. The viral genomic RNA is encapsidated within the capsid shell comprising the VP1-4 capsid proteins. The capsid proteins are arranged into a symmetrical icosahedral lattice. These capsid proteins recognize receptors on host cells and demonstrate antigenicity. The viral genome is translated into a polyprotein around 250kDa which then undergoes cleavage via the viral proteases. The viral non-structural proteins 2A-C and 3A-D are essential for the replication of the virus within infected cells [4].

Phylogenetic studies of enterovirus 71 have identified 3 genotypes and numerous subtypes. The 3 genotypes are A, B and C, whereas the subtypes are classified numerically. Increased neurovirulence have been attributed to certain subtypes, such as genotype C1 [5]. Still, the exact pathogenesis for the variation in disease presentation is unknown.

3. Epidemiology

Herpes simplex virus holds the dubious honor of being the commonest cause of acute focal encephalitis, and is thus the presumptive diagnosis in patients with viral encephalitis. However, prospective studies have shown that about 9% have a different etiology and may be due to enteroviruses [6, 7]. Enteroviruses have a worldwide distribution, but recent outbreaks of EV71 have been centered in Asia, particularly East and Southeast Asia [8-16]. Enterovirus 71 is not the only enterovirus that involves the central nervous system (CNS). In a Canadian survey of enteroviral infections of the CNS from 1973 to 1981, coxsackie-virus A9, B1, B2, B3 and B5, echoviruses type 6, 7, 9, 11, 30, poliovirus type 2 were isolated as well [17]. The incidence of encephalitis specifically in enterovirus infections is reported to be at 3% [18], with the majority presenting meningitis.

Clinically evident infection occurs mainly in children with few cases reported in adults [19]. There is a male preponderance [19]. In children, the infection usually presents as hand, foot and mouth disease (HFMD). Yet from the late 1990s onwards, increasingly severe cases caused by enterovirus have been documented, particularly involving EV71. In adults, there have been a few case reports occurring after immunosuppressive therapy such as rituximab [20]. Rituximab is a chimeric anti-CD20 monoclonal antibody that can cause profound B-cell lymphopenia and antibody deficiency. There are 3 main different clinical neurological complications of EV71 infection; 1.flaccid paralysis and encephalitis [3, 21], 2.HFMD and meningoencephalitis [22-24] and 3.HFMD or herpangina and rhombencephalitis with neurogenic pulmonary edema [16, 25-27].

The incidence of CNS complications in enterovirus infection has been reported to range from 2-10% [28]. Even so, according to a prospective study of 773 children [5] and retrospective study of 423 patients [19], it can go as high as to 19-42%, respectively. Of the 773 children, EV71 was isolated in 277 (41%) and out of the 277 children, a further 28 had coinfections with a second virus (other enteroviruses, adenovirus and unidentified virus) [5]. Coxsackie A virus was isolated in 85 patients and out of these, 4 had coinfections as well. Other enteroviruses, adenoviruses or unidentified viruses were isolated in 58 [5].

While coinfections with other enteroviruses did not appear to increase the risk of neurological complications, an association was found between patients who were coinfected with dengue viruses and neurological symptoms [5]. Similarly, in the retrospective study of 423 patients, those with CNS involvement were more likely to have EV71 (21%) instead of cox-sackie A virus infection (16%). In addition, rate of disease progression and severity was reported to be greater in EV71 infection [19].

4. Pathogenesis

The reservoir of human pathogenic enterovirus is humans and transmission of enteroviruses occurs through the fecal-oral route via droplets or in utero [28]. Infection starts in the gastro-intestinal system with proliferation in the pharynx or intestinal lymph nodes before disseminating to the rest of the body.

In vitro studies of EV71 show that the virus binds to DLD-1 intestinal cells which express sialic acid (SA) linked glycan on the cell surface [29]. Decreasing O-linked glycans or glycolipids on the cell surface decreased EV71 infection of DLD-1 intestinal cells but this was not reproducible on decreasing N-linked glycans. SA linked glycans isolated from human milk also inhibited EV71 infection of DLD-1 intestinal cells [29], suggesting potential therapeutic use.

The first step for a virus to infect the CNS is to cross the blood brain barrier (BBB). The BBB serves as a physical barrier, consisting of endothelial cells joined to each other by tight junctions and surrounded by foot processes of astrocytes, preventing access to the CNS. The meninges, choroid plexus and ependymal cells lining the ventricles also prevent access. Within the CNS are also dendritic cells and macrophages that detect pathogens and contribute to the host defense response. In utero, the BBB has not fully matured and viruses crossing into the placental circulation can also result in CNS infection.

Several RNA viruses causing neurological symptoms e.g. poliovirus, enter the CNS through axonal transport from the peripheral nervous system (PNS), circumventing the blood brain barrier. Coxsackie-virus B3 on the other hand targets nestin⁺ myeloid cells which subsequently migrate through ependymal cell layer of the BBB into the CNS [30]. Other enteroviruses such as EV71 cross the BBB by binding to receptors e.g. P-selectin glycoprotein ligand-1, infecting cells (leucocytes and lymphocytes) that normally cross the BBB [31], hitchhiking their way into the CNS. Enterovirus 71 and coxsackie-viruses have also been shown to bind to scavenger receptor class B member 2 (SCARB2) found on fibroblasts and GPI-anchored protein decay-accelerating factor found on epithelial cells in the CNS, gaining entry into the CNS [28, 32]. SCARB2 participates in membrane transportation and the re-organization of endosomal and lysosomal compartments [33]. The coxsackievirus and adeno-virus receptor (CAR) also facilitates viral entry in a caveolin-dependent or independent manner [32, 34] while human poliovirus receptor, an adhesion molecule, is used by human poliovirus in a caveolin independent manner but dynamin-dependent manner to gain entry [32, 35]. The receptors and varying method of entry in different cell types may explain for

the tropism to a certain degree. Human poliovirus receptors are found in high levels in the anterior horn cells of the spinal cord, accounting for the predilection of poliovirus for infection anterior horn cells [36]. However, there are other factors that contribute to the tropism of the viruses such as cell proliferation. It has been reported that coxsackievirus 3B targets neural progenitor and stem cells and viral replication increases markedly during cell division and when the cells are arrested at the G_1 or G_1/S phase while viral replication is reduced in quiescent cells in the G_0/G_2/M phase [37].

The human immune system consists of adaptive and innate immunity, both of which utilizes pattern recognition receptors (PRR) e.g. Toll-like receptor and RIG-I-like receptors that detect viral nucleic acids and initiate host defence [38], including modulating the release of chemokines, cytokines and interferons [39]. An appropriate host response to viral infection requires a complex interplay between the innate and adaptive immune system.

After entry into the CNS, glial cells which constitute part of the CNS innate immune system, detect the intracellular viral nucleic acid, and stimulate the release of IFN-1, causing apoptosis and inhibit viral replication. It is, however, important to note that collateral damage incurred upon activation of cytolytic T cells during an adaptive immune response within the CNS may be more damaging to neurons than the infection. Furthermore, both greater cytokine induced tissue destruction due to higher systemic levels of proinflammatory cytokines like IL-6, IL-1β, and TNF [40] and the pervasive infiltration of leukocytes into the CNS exacerbate the neuropathology linked to enteroviruses [41]. Due to this, the host may contain immune response towards viral infection within the CNS.

Even so, viruses have also evolved to escape host defence by producing viral proteins that inhibit host anti-viral response. For example, EV71 produces protein 3C which inhibits RIG-I like receptors and thus blocks host IFN-1, and protein 2C which inhibits IkB kinase beta phosphorylation, consequently blocking the TNF alpha activated NκB signaling pathway [42, 43]. *In vitro* studies also showed that protein 2C stimulates neuronal apoptosis via activation of the Abl-Cdk5 pathway [44]. Interestingly, *in vitro* studies of coxsackievirus A16 which is less neurovirulent than EV71 [45] may suggest that coxsackievirus A16 stimulates Abl to a smaller degree and does not stimulate Cdk5 [44]. Viruses can also dodge the adaptive immune system by binding to dendritic cell specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN). The DC-SIGN is a receptor present on macrophages and dendritic cells which recognizes and binds to pathogen associated molecular patterns (PAMPs) on viruses, bacteria and fungi. The binding itself stimulates phagocytosis and results in pathogen entry into dendritic cells and T-cells. Intracellular entry is not only mediated via DC-SIGN but also by other receptors such as CD36 and CD163. This results in suboptimal T and NK cell response as viral proteins inhibit IFN-1 synthesis and escape immune surveillance.

Neurological complications are reported at higher frequencies in younger children. The exact pathogenesis remains unknown, but research has elucidated a number of differences between patients who developed such complications and those who do not.

In a case-control study of 78 children who had EV71 infection, of which 31 children developed meningoencephalitis, expression of CD40-ligand on T cells was significantly lower in cases than in controls in the acute phase, but not in the convalescent phase of the disease [46]. CD40 ligand expression on T cells is recognized as a marker of T and B cell interaction [47]. Thus, a decrease in expression may suggest a decrease in stimulation of B cells, antibody class switching and antibody production. Yet, there was no significant difference in lymphocyte proliferation between cases and controls. In cases with meningoencephalitis, it was also noted that interleukin 4 production was significantly lower in cases than in controls during the acute phase, suggesting a decrease response from Th2 cells which stimulate the humoral immune system [46]. This suggests that a compromised immune status precipitates the fulminant progression of EV infections.

In the same study, significant polymorphism of the cytotoxic T lymphocyte antigen-4 (CTLA-4) was noted, with cases having more G/G genotype at position 49 of exon 1 than in controls. CTLA-4 is involved in T cell anergy and apoptosis, and different polymorphisms has been linked to infectious and autoimmune conditions [46].

In enterovirus infection, autophagocytosis is subverted and induced in infected neurons, allowing for intracellular replication of viral particles before host cell death occurs. Autophagy is usually a protective process that occurs in cells to sequester and breakdown unwanted organelles or protein aggregates. In poliovirus [48] and coxsackievirus B4 [49] infections, however, it has been induced to assist in virus replication instead. It is postulated that enterovirus utilizes the autophagosome membrane for viral replication and increase in viral replication is associated with autophagy induction [49]. The exact mechanism by which autophagocytosis increases viral replication is currently unclear.

Non-structural 3C protein of EV71 has been reported to block polyadenylation of host messenger RNA while non-structural 2A protein impedes the host cap-dependent translation and simultaneously stabilizes polysomes enhancing translation of viral messenger RNA [50]. Non-structural 2A protein thus boosts viral protein synthesis, and it has been shown to be necessary for host cell apoptosis as well [51]. Accordingly, it is assumed that by deactivating the host cell translation, enteroviruses may directly cause apoptotic cell death in neurons. Enteroviruses bring about both anti-apoptotic (non-structural 3A and 2B proteins) and pro-apoptotic effects (VP2, non-structural 2A and 3C proteins) on the host cell [52]. Viral non-structural protein 2B is also known to be a viroporin that increases permeability of host cell resulting in eventual cell death.

The highest rates of infections occur in young children below 4 years of age, with fatal infections most commonly occurring at the ages of 6-11 months [13, 16, 53]. This age period is also associated with a decline in maternal antibodies within the child. Enterovirus 71 infects human peripheral blood monocytes, and it is postulated that in the presence of sub-neutralizing amounts of anti-EV71 antibodies, infectivity is enhanced [54]. This is also known as antibody-dependent enhancement (ADE), widely described in dengue infection as well as other viral infections such as human immunodeficiency virus infection [55]. Heterotypic non-neutralizing antibodies bind to the virions, forming EV71-antiEV71 antibody complexes, which subsequently bind to Fc-R on human monocytes and there-

fore, enhancing infectivity. *In vitro* experiments have shown that the addition of immune sera from patients increased EV71 infection of THP-1 cells, a leukemia cell line of macrophage lineage with monocytic markers, whereas addition of Fc-RI (CD64) significantly inhibited the infection [54].

Furthermore, patients who suffer from neurogenic pulmonary edema have been reported to have lower absolute monocyte counts, CD4, CD8 and NK cells counts as compared to patients who had autonomic nerve system abnormalities and uncomplicated brainstem encephalitis [56].

5. Pathogenesis of neurogenic pulmonary edema

Patients with enterovirus encephalitis who suffer from pulmonary edema, pulmonary hemorrhage and cardiopulmonary collapse usually have fairly normal premorbid cardiac function with normal pulmonary artery pressures and vascular resistance [56]. Myocarditis is also not evident on autopsy reports [57].

Involvement of the medulla and hence, the vagal nucleus and medial reticular nuclei is postulated to cause pulmonary edema [58, 59]. Neurogenic pulmonary edema occurs when there is pulmonary edema and CNS disease in the absence of underlying cardiopulmonary disease [58]. While the pathogenesis is not clear, it is believed that an insult to the medulla results in torrential release of catecholamine. This in turn, causes a rapid increase in total peripheral vasoconstriction and systemic hypertension, shifting blood from the systemic circulation to the pulmonary circulation. Since the pulmonary circulation is usually a low resistance system, it is unable to adapt to the sudden increase in hydrostatic pressure. The results are protein rich pulmonary edema and pulmonary hemorrhages. The resulting ''catecholamine storm'' induces catecholamine cardiotoxicity as well including coagulative myocytolysis, myofibrillar degeneration, and cardiomyocytes apoptosis [60]. This neurogenic nature is validated by MRI findings of brainstem involvement [61] and postmortem examinations of mortality cases of enterovirus encephalitis in which pathological lesions were predominantly located in the brainstem and the spinal cord, rather than in the lung or heart [21, 27, 62].

6. Pathogenesis in chronic infection

Chronic infection by enterovirus has been reported [63] and it is postulated that the persistence of infection alters normal neural stem cell migration and or differentiation. Although viral latency has yet to be established, there is evidence for their persistence in infected cells for years [64-66]. Therefore, enteroviral RNA may be reactivated upon stimulation. In the case of hypogammaglobulinaemia, reactivated enterovirus is not inactivated and can spread freely. In the same degree, persistent meningoencephalitis has been reported in patients

with agammaglobulinemia [67, 68]. In fact, the first case of enteroviral meningoencephalitis was reported in a patient with agammaglobulinemia [69].

7. Clinical signs and symptoms

Enteroviruses can cause a wide spectrum of clinical diseases, including but not limited to the common cold, gastroenteritis, hand, foot and mouth disease (HFMD), herpangina, myocarditis, severe neonatal sepsis-like disease, hepatoadrenal failure, aseptic meningitis, acute flaccid paralysis, meningoencephalitis, encephalitis, neurogenic pulmonary edema, pulmonary hemorrhage and shock induced sudden death especially in the young age group [13, 62, 70]. The neurological presentations include aseptic meningitis, benign intracranial hypertension, acute flaccid paralysis, opsoclonus-myoclonus syndrome, Guillain-Barre syndrome, transverse myelitis, encephalitis, cerebellitis, brainstem encephalitis, rhombencephalitis and encephalomyelitis [13, 25, 26, 71-76].

Clinical manifestations can be classified according to 5 grades. In grade I, patients demonstrate clinical signs of HFMD and/or herpangina with erythematous vesicles on palms, soles, elbows and trunk and oral ulcers on mucosa of lips as well as palate. The majority of patients will display grade I symptoms as seen in the 1998 Taiwan epidemic where 5506 out of 5632 patients were classified to have grade I symptoms [61]. In grade II, patients suffer from fever, photophobia, vomiting, headache, and abdominal pain. Patients who initially exhibit grade II symptoms may subsequently progress onto grade III. The disease may take a fulminant course in patients younger than 2 years of age, deteriorating directly to grade IV in a short period of time. In grade III, patients may demonstrate lethargy, apathy, drowsiness, tachycardia, cranial nerve involvement (VI-XII), myoclonic jerks, monoparesis or hemiparesis, conjugate gaze disturbances, dyspnea and ataxia. Patients with grade III symptoms that are younger than 2 years of age usually progress to grade IV while older patients tend to recover completely after 1-2 weeks. In grade IV, patients experience hypothermia, pulmonary edema, respiratory failure, neurogenic shock and semicoma. In the last stage, grade V, there is pulmonary hemorrhage, respiratory distress syndrome, cardiorespiratory failure, coma and death. According to symptomatology, encephalitis is suspected in grade III-IV.

HFMD and herpangina are generally mild, self-limiting illnesses that occur in infants and young children. The culprit virus for HFMD and herpangina is usually coxsackie-virus A16 or EV71 [8, 14, 19]. Despite this, a small percentage of patients can rapidly decompensate and die within days. In cases where neurological complications occur, the culprit viruses isolated are usually EV71 and coxsackie-virus A16 [19] in some instances other echovirus 7 [77, 78].

Patients who had CNS complications were usually younger and more likely to have symptoms of fever, vomiting, breathlessness and signs of shock that includes cold peripheries and poor urinary output [5]. The exact symptoms and signs depend on the extent of CNS involvement. For example, in EV71 encephalitis, clinical signs of lethargy and cranial nerve palsies such as conjugate gaze disturbance, dyspnea and tachycardia suggest involvement of the brain stem, [61] and this is further substantiated by the magnetic resonance imaging.

8. Investigations and diagnosis

In general, profound leucopenia is usually noted in patients with severe EV71 infections [56]. This is attributed to T-cell apoptosis as EV71 infection may increase FasL expression.

• Investigations: Lumbar puncture

A common investigation in the presence of neurological symptoms would be a lumbar puncture. In aseptic meningitis, there is usually lymphocytic cerebrospinal fluid (CSF) pleocytosis [20] and normal glucose levels [79]. Yet in some of the patients, neutrophilia was observed with low glucose levels less than half of that of plasma glucose instead [5].

• Investigations: Magnetic resonance imaging

Magnetic resonance imaging of the brain is also a useful investigation. Reports of magnetic resonance imaging of polioencephalitis are rare as poliovirus is currently rarely seen in developed countries. The few imaging reports of polioencephalitis reveal involvement of the midbrain and posterior medulla and pons [61].

During the 1998 Taiwan EV71 epidemic, magnetic resonance features were described by Shen et al [61]. Out of 15 patients classified in grade III with clinical encephalitis, 10 had abnormal magnetic resonance imaging scans. Of the patients with abnormal scans, all 10 demonstrated hyperintense lesions of the posterior medulla and pons on T2 weighted images but not on T1 weighted images, which implies acute inflammation. The majority showed involvement of the mesencephalon and dentate nuclei of cerebellum, and in severe cases, the ventral horns of the spinal cord and deep supratentorial nuclei as well [61]. The inclusion of the brainstem is supported by pathological findings on autopsy [26, 80], with inflammation limited to the gray matter of the spinal cord and medulla as well as the tegmentum of the midbrain and pons [80]. It is of note that EV71 and poliovirus affect the same areas of the brain and the areas of involvement demonstrated on the nuclei correlated with the clinical symptoms and signs. A marked difference would be that although the inflammation of inferior olives is reported in EV71 infection, it is absent in bulbar poliomyelitis [80].

Newer techniques in magnetic resonance imaging such as fluid attenuated inversion recovery and diffusion weighted imaging allow detection of subtle meningeal and cortical abnormalities that can occur in meningoencephalitis [81]. Consequently, in patients stable enough to undergo scans, magnetic resonance imaging can reveal areas of CNS involvement. This allows greater diagnostic accuracy and perhaps predicts the need for cardiothoracic support before patients deteriorate too rapidly.

9. Diagnostic methods

Diagnosis normally depends on (a) rise in virus specific acute and convalescent antibody titers, (b) isolation of the virus via viral cultures from throat swabs, stool specimens and CSF samples (c) visual identification of virus via (i) electron microscopy (ii) unique histological

features and/or (iii) histochemical staining, (d) in situ hybridization assays or (e) polymerase chain reaction (PCR) to amplify viral nucleic acids [82, 83]. Since some enteroviruses such as coxsackievirus A [84] do not grow in standard cell cultures [85], the extensive use of PCR with its generally high specificity and sensitivity has greatly improved diagnosis for numerous pathogens. The overall sensitivity, specificity, positive and negative predictive values have been reported to be 85.7%, 93.9%, 61.7% and 98.3%,respectively, using viral culture as the gold standard [86]. However, the majority of clinically suspected viral encephalitis are still of unknown etiology. It is presumed as well that enterovirus will be detectable in the gastrointestinal (GI) tract, but in the case of chronic encephalitis, by the time the disease surfaces, the virus may have cleared from the GI tract and be undetectable in the stool. Additionally, due to low viral concentration, detection of viral RNA in the CSF early after manifestation may be challenging as well.

In EV71 encephalomyelitis, inflammation is stereotypical. Common areas of involvement are the spinal cord, brainstem, hypothalamus, cerebellar dentate nucleus, cerebral cortex and meninges. The anterior pons, corpus striatum, temporal lobe, hippocampus and cerebellar cortex are spared. These areas of involvement facilitate the differentiation of encephalitis due to EV71 from Japanese encephalitis virus [87]. The nature of the lesions, however, is non-specific, with inflammatory infiltration of perivascular and parenchymal tissue, edema, necrosis, stimulation of microglial cells and phagocytic destruction of neurons[83].

The tests currently available have a low diagnostic yield, even in the case of PCR which has high specificity and high sensitivity. This is shown in a meta-analysis conducted in 2010 which reviewed 41 studies on the etiology of encephalitis [88]. In 26 of the studies, more than 50% of the cases were of unknown etiology [88]. Identifying the viral etiological agent enables effective preventive measures and treatments to be implemented.

The European Union Concerted Action on Virus Meningitis and Encephalitis conducted a multicenter retrospective study to evaluate the Amplicor Enterovirus PCR test [86]. 476 CSF samples were collected from 9 laboratories in 5 European countries and analysed via cultures and PCR [86]. Out of 476 samples, 50 were positive via cultures and 66 via PCR. Relative increase in rate of positivity via PCR in relation to culture is thus 32%. Among the 50 samples positive by cultures, 38 were positive while 12 were negative by PCR. On repeat testing of the 12 samples that were culture positive but PCR negative with a different set of primers and probes, 4 of the samples became PCR positive [86]. On the other hand, in the 66 samples positive via PCR, 28 were negative via cultures [86]. Interestingly, in samples from patients with meningitis following the case definition of CSF pleocytosis (more than 10 leukocytes/mm3), 25 were positive via cultures and 45 via PCR, thus there were samples from patients who did not satisfy the criteria for pleocytosis and yet had enterovirus infection.

The California Encephalitis Project (CEP) conducted from 1998-2000 evaluated samples from 334 patients with case definition of encephalitis, which is encephalopathy requiring hospitalization plus one of the following: fever, seizure, focal neurologic findings, cerebrospinal fluid pleocytosis and electroencephalographic or neuroimaging findings consistent with encephalitis [89]. Encephalopathy is defined as depressed or altered level of consciousness lasting 24 hours, lethargy and/or change in personality [89]. 9% of the cases had a confirmed

viral agent, 3% a confirmed bacterial agent, 1% a confirmed parasitic agent, 10% a non-infectious etiology and 12% a possible etiology identified. 3% had a non-encephalitis infection identified. Nevertheless, CEP is not population based and the study group consisted of diagnostically challenging cases. Therefore, the rate of unknown etiology cases may be an overestimate when extrapolated to the general population.

Diagnostic strategies that have emerged recently include MassTagPCR, panmicrobial DNA microarrays and high-throughput DNA pyrosequencing [82]. MassTag PCR is a multiplex PCR assay utilizing primer pairs targeting highly conserved gene sequences that represent a wide variety of potential pathogens. The primer pairs have been tagged with MassCodes that are used to identify the etiological agent. There are different MassTag PCR systems with different primers for different clinical specimens and presentations. Clinical use of this method has demonstrated effectiveness in identification of pathogens [90-93]. Panmicrobial DNA microarrays utilize a single chip with numerous highly conserved gene sequences, permitting the swift identification of pathogens similar to that of MassTag PCR [94, 95]. Clinical use of this diagnostic method has also demonstrated efficacy in pathogen identification [96-98].

High-throughput DNA pyrosequencing on the other hand, does not make use of highly conserved gene sequences. Instead, it uses random primers to amplify all RNA after removing human chromosomal DNA from the sample [99]. Amplification products are then sequenced via pyrosequencing wherein DNA polymerases synthesize complementary strands to the amplified products and each enzymatic attachment of a complementary nucleotide results in an emission of a light signal. The light signal is recorded and the sequences are identified and subsequently analyzed to look for pathogens. This technique allows for identification of novel pathogens [100].

10. Treatment

At present, only herpes simplex encephalitis, one of the more prevalent infective encephalitis, has a specific treatment validated by scientific research. It is treated with aciclovir [101]. Effective treatment is lacking for other viruses and mainly symptomatic in nature.

Currently, intravenous immunoglobulin (IVIG) is administered to patients with severe HFMD [5]. Enteroviruses are cleared from the host by antibody-mediated mechanisms (22), and IVIG is an effective treatment option. Various routes of administration have been documented including intravenous, intrathecal and intraventricular with different degrees of success [102-104].

11. Potential treatments in the future – vaccines

However, the best defence would be prevention through vaccination, especially in the case of rabies, polio, mumps and measles. Vaccination may be an option to prevent infection by

enteroviruses as well, but the potential ADE phenomenon is an important consideration in the development of a safe and effective vaccine. The genetic diversity of enterovirus strains therefore complicates the development of vaccines [105].

12. Potential treatments in the future – anti-virals

Ribavirin, a broad-spectrum antiviral synthesized by ICN pharmaceuticals, Inc., USA inhibits the replication of a variety of enteroviruses. Studies on EV71-infected mice has shown that ribavirin can reduce mortality by reducing the viral loads in tissues. The required dosage of ribavirin is close to the initial dose of the drug administered intravenously to treat patients with encephalitis caused by Nipah virus [106]. Given these results, ribavirin may be, in combination with interferon, deployed to combat potentially fatal EV71 infection. Interferon has a synergist effect and this combination is already adopted as a standard therapy for HCV-infected patients [107].

Pyridyl imidazolidinone is a novel class of EV71 inhibitor [108]. It was first identified using computer-assisted drug design. It targets EV71 capsid protein VP1 and time course experiments on one of the pyridyl imidazolidinones, BPR0Z-194, have shown that viral replication is effectively inhibited in early stages, suggesting that the compound inhibits adsorption of virions and/or viral RNA uncoating [108]. Resistant strains do exist, and sequence analysis has demonstrated that a single amino acid alteration at position 192 of VP1 confers resistance to BPR0Z-194 [108].

Pleconaril, an anti-viral produced by Sterling-Winthrop, Inc., USA incorporates itself into the capsid of enteroviruses and blocks the virus from docking to cellular receptors and uncoating to release RNA into the cell. It targets VP1 and has already passed the last stage of clinical trials [109]. Results are promising with pleconaril showing antiviral effects for most enteroviruses [109, 110]. Presently, there is an ongoing study on the efficacy of pleconaril in enteroviral sepsis syndrome in neonates [111]. The National Health Research Institutes (NHRI) in Taiwan has reported a number of virtual compounds with similar stable conformations and preliminary studies have identified a few promising imidazolidinone derivatives.

13. Potential treatments in the future – RNA interference

Another promising therapy is the use of RNA interference (RNA-i) in silencing viral gene expression [28, 112]. As aforementioned, viruses penetrate the BBB and the very nature of the BBB makes it difficult for large and charged molecules to cross it. RNA-i on the other hand, are small and have the potential to cross the BBB and exert a therapeutic effect. RNA-i can bind to specific viral mRNA, causing degradation and preventing the translation and synthesis of viral proteins that enable the virus to inhibit the host IFN-1 response [112]. To synthesize RNA-i for therapeutic use, the viral proteins and subsequent viral mRNA have to

be identified in advance. *In vitro* experiments performed have shown effective inhibition of EV71 infection [113] however *in vivo* experiments utilizing murine models have yet to replicate similar results [114].

14. Prognosis

There are cases where spontaneous recoveries do not occur and neurological symptoms persist. A retrospective study of 105 patients from 1966 to 1972, with documented enterovirus infection and CNS complications, revealed that half, 9 out of 18, of the children not lost to follow up still displayed signs after 1-5 years [78]. Magnetic resonance imaging of two cases with neurological sequelae demonstrated hypointense lesions on T1-weighted images and hyperintense lesions on T2-weighted images, implying tissue destruction [61]. In a more recent retrospective study of 177 cases with enterovirus isolated via throat swab or stool specimen, 92 patients (52%) had nervous system involvement, out of which 13 patients (7%) had persistent neurological deficits at discharge [19]. Out of the 92 patients with neurological involvement, 67 (73%) had EV71 isolated and of the 13 patients with deficits at discharge, 11 (85%) had EV71 isolated. The persistent neurological deficits ranged from dysphagia and weakness to lack of regular, spontaneous respiration despite presence of brain function [19]. Studies have also linked EV71 CNS infections to increased symptoms of inattention, hyperactivity, oppositional defiance, internalizing problems, and greater likelihood of the diagnosis of attention deficit hyperactivity disorder [115].

Overall, mortality rates for HFMD is reported to be at 0.05% in China [19]. In the aforementioned retrospective study of 177 cases, 5 mortalities were reported and in all these 5 cases, EV71 was isolated [19]. Of the 5 mortalities, 2 were attributed to neurogenic pulmonary edema, 2 to shock and 1 to brain-death. Mortalities in EV71 infection is generally due to neurogenic pulmonary edema secondary to medulla destruction [13, 80, 116, 117]. In Taiwan, the Department of Health has recorded a decrease of incidence in recent years, however the mortality rate is still high (9 deaths in 1999, 41 deaths in 2000, 58 deaths in 2001) [60].

15. Conclusion

The reemergence and emergence of viral infections with involvement of the neurological system is a challenge to public health officers, clinicians and researchers. Enterovirus infection is a major concern with changing circulating genotypes in Asia. While CNS complications of encephalitis are being increasingly reported in enterovirus infections, further research is needed to understand whether this is due to increased virulence or due to undetected immune system defects. The epidemiology continuously evolves with shifting population and hence, there is also a need to have better diagnostic methods and treatment options.

16. Acknowledgements

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References

[1]   Olival, K.J. and P. Daszak, The ecology of emerging neurotropic viruses. J Neurovirol, 2005. 11(5): p. 441-6.

[2]   Oberste, M.S., et al., Molecular evolution of the human enteroviruses: correlation of serotype with VP1 sequence and application to picornavirus classification. J Virol, 1999. 73(3): p. 1941-8.

[3]   Schmidt, N.J., E.H. Lennette, and H.H. Ho, An apparently new enterovirus isolated from patients with disease of the central nervous system. J Infect Dis, 1974. 129(3): p. 304-9.

[4]   Kitamura, N., et al., Primary structure, gene organization and polypeptide expression of poliovirus RNA. Nature, 1981. 291(5816): p. 547-53.

[5]   Ooi, M.H., et al., Human enterovirus 71 disease in Sarawak, Malaysia: a prospective clinical, virological, and molecular epidemiological study. Clin Infect Dis, 2007. 44(5): p. 646-56.

[6]   Whitley, R.J., et al., Diseases that mimic herpes simplex encephalitis. Diagnosis, presentation, and outcome. NIAD Collaborative Antiviral Study Group. JAMA, 1989. 262(2): p. 234-9.

[7]   Whitley, R.J., et al., Herpes simplex encephalitis: vidarabine therapy and diagnostic problems. N Engl J Med, 1981. 304(6): p. 313-8.

[8]   Chan, K.P., et al., Epidemic hand, foot and mouth disease caused by human enterovirus 71, Singapore. Emerg Infect Dis, 2003. 9(1): p. 78-85.

[9] Kim, K.H., Enterovirus 71 infection: An experience in Korea, 2009. Korean J Pediatr, 2010. 53(5): p. 616-22.

[10] McMinn, P., et al., Phylogenetic analysis of enterovirus 71 strains isolated during linked epidemics in Malaysia, Singapore, and Western Australia. J Virol, 2001. 75(16): p. 7732-8.

[11] Fujimoto, T., 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. Microbiol Immunol, 2002. 46(9): p. 621-7.

[12] Tu, P.V., et al., Epidemiologic and virologic investigation of hand, foot, and mouth disease, southern Vietnam, 2005. Emerg Infect Dis, 2007. 13(11): p. 1733-41.

[13] Chen, S.C., et al., An eight-year study of epidemiologic features of enterovirus 71 infection in Taiwan. Am J Trop Med Hyg, 2007. 77(1): p. 188-91.

[14] Yang, F., et al., Survey of enterovirus infections from hand, foot and mouth disease outbreak in China, 2009. Virol J, 2011. 8: p. 508.

[15] Podin, Y., et al., Sentinel surveillance for human enterovirus 71 in Sarawak, Malaysia: lessons from the first 7 years. BMC Public Health, 2006. 6: p. 180.

[16] Ho, M., et al., An epidemic of enterovirus 71 infection in Taiwan. Taiwan Enterovirus Epidemic Working Group. N Engl J Med, 1999. 341(13): p. 929-35.

[17] Thivierge, B. and G. Delage, [Infections of the central nervous system caused by enterovirus: 223 cases seen at a pediatric hospital between 1973 and 1981]. Can Med Assoc J, 1982. 127(11): p. 1097-102.

[18] Koskiniemi, M., et al., Epidemiology of encephalitis in children: a 20-year survey. Ann Neurol, 1991. 29(5): p. 492-7.

[19] Xu, W., et al., Distribution of enteroviruses in hospitalized children with hand, foot and mouth disease and relationship between pathogens and nervous system complications. Virol J, 2012. 9(1): p. 8.

[20] Servais, S., et al., Enteroviral meningoencephalitis as complication of Rituximab therapy in a patient treated for diffuse large B-cell lymphoma. Br J Haematol, 2010. 150(3): p. 379-81.

[21] Nagy, G., et al., 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): p. 217-27.

[22] Zheng, Z.M., et al., Enterovirus 71 isolated from China is serologically similar to the prototype E71 BrCr strain but differs in the 5'-noncoding region. J Med Virol, 1995. 47(2): p. 161-7.

[23] Samuda, G.M., et al., Monoplegia caused by Enterovirus 71: an outbreak in Hong Kong. Pediatr Infect Dis J, 1987. 6(2): p. 206-8.

[24] Hagiwara, A., et al., Genetic and phenotypic characteristics of enterovirus 71 isolates from patients with encephalitis and with hand, foot and mouth disease. Arch Virol, 1984. 79(3-4): p. 273-83.

[25] Chang, L.Y., et al., Clinical features and risk factors of pulmonary oedema after enterovirus-71-related hand, foot, and mouth disease. Lancet, 1999. 354(9191): p. 1682-6.

[26] Huang, C.C., et al., Neurologic complications in children with enterovirus 71 infection. N Engl J Med, 1999. 341(13): p. 936-42.

[27] Lum, L.C., et al., Neurogenic pulmonary oedema and enterovirus 71 encephalomyelitis. Lancet, 1998. 352(9137): p. 1391.

[28] Denizot, M., J.W. Neal, and P. Gasque, Encephalitis due to emerging viruses: CNS innate immunity and potential therapeutic targets. J Infect, 2012.

[29] Yang, B., H. Chuang, and K.D. Yang, Sialylated glycans as receptor and inhibitor of enterovirus 71 infection to DLD-1 intestinal cells. Virol J, 2009. 6: p. 141.

[30] Tabor-Godwin, J.M., et al., A novel population of myeloid cells responding to coxsackievirus infection assists in the dissemination of virus within the neonatal CNS. J Neurosci, 2010. 30(25): p. 8676-91.

[31] Yamayoshi, S., et al., Scavenger receptor B2 is a cellular receptor for enterovirus 71. Nat Med, 2009. 15(7): p. 798-801.

[32] Coyne, C.B. and J.M. Bergelson, Virus-induced Abl and Fyn kinase signals permit coxsackievirus entry through epithelial tight junctions. Cell, 2006. 124(1): p. 119-31.

[33] Yamayoshi, S., K. Fujii, and S. Koike, Scavenger receptor b2 as a receptor for hand, foot, and mouth disease and severe neurological diseases. Front Microbiol, 2012. 3: p. 32.

[34] Patel, K.P., C.B. Coyne, and J.M. Bergelson, Dynamin- and lipid raft-dependent entry of decay-accelerating factor (DAF)-binding and non-DAF-binding coxsackieviruses into nonpolarized cells. J Virol, 2009. 83(21): p. 11064-77.

[35] Brandenburg, B., et al., Imaging poliovirus entry in live cells. PLoS Biol, 2007. 5(7): p. e183.

[36] Gromeier, M., et al., Expression of the human poliovirus receptor/CD155 gene during development of the central nervous system: implications for the pathogenesis of poliomyelitis. Virology, 2000. 273(2): p. 248-57.

[37] Feuer, R., et al., Cell cycle status affects coxsackievirus replication, persistence, and reactivation in vitro. J Virol, 2002. 76(9): p. 4430-40.

[38] Kawai, T. and S. Akira, Toll-like receptor and RIG-I-like receptor signaling. Ann N Y Acad Sci, 2008. 1143: p. 1-20.

[39] Hosking, M.P. and T.E. Lane, The role of chemokines during viral infection of the CNS. PLoS Pathog, 2010. 6(7): p. e1000937.

[40] Lin, T.Y., et al., Proinflammatory cytokine reactions in enterovirus 71 infections of the central nervous system. Clin Infect Dis, 2003. 36(3): p. 269-74.

[41] Feuer, R., et al., Viral persistence and chronic immunopathology in the adult central nervous system following Coxsackievirus infection during the neonatal period. J Virol, 2009. 83(18): p. 9356-69.

[42] Zheng, Z., et al., Enterovirus 71 2C protein inhibits TNF-alpha-mediated activation of NF-kappaB by suppressing IkappaB kinase beta phosphorylation. J Immunol, 2011. 187(5): p. 2202-12.

[43] Lei, X., et al., The 3C protein of enterovirus 71 inhibits retinoid acid-inducible gene I-mediated interferon regulatory factor 3 activation and type I interferon responses. J Virol, 2010. 84(16): p. 8051-61.

[44] Chen, T.-C., et al., Enterovirus 71 triggering of neuronal apoptosis through activation of Abl-Cdk5 signalling. Cellular Microbiology, 2007. 9(11): p. 2676-2688.

[45] Chang, L.Y., et al., Comparison of enterovirus 71 and coxsackie-virus A16 clinical illnesses during the Taiwan enterovirus epidemic, 1998. Pediatr Infect Dis J, 1999. 18(12): p. 1092-6.

[46] Yang, K.D., et al., Altered cellular but not humoral reactions in children with complicated enterovirus 71 infections in Taiwan. J Infect Dis, 2001. 183(6): p. 850-6.

[47] Grewal, I.S. and R.A. Flavell, The role of CD40 ligand in costimulation and T-cell activation. Immunol Rev, 1996. 153: p. 85-106.

[48] Jackson, W.T., et al., Subversion of Cellular Autophagosomal Machinery by RNA Viruses. PLoS Biol, 2005. 3(5): p. e156.

[49] Yoon, S.Y., et al., Coxsackievirus B4 uses autophagy for replication after calpain activation in rat primary neurons. J Virol, 2008. 82(23): p. 11976-8.

[50] Rhoades, R.E., et al., Enterovirus infections of the central nervous system. Virology, 2011. 411(2): p. 288-305.

[51] Shih, S.R., et al., Viral protein synthesis is required for Enterovirus 71 to induce apoptosis in human glioblastoma cells. J Neurovirol, 2008. 14(1): p. 53-61.

[52] Whitton, J.L., C.T. Cornell, and R. Feuer, Host and virus determinants of picornavirus pathogenesis and tropism. Nat Rev Microbiol, 2005. 3(10): p. 765-76.

[53] Chang, L.Y., 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): p. e88.

[54] Wang, S.M., et al., Enterovirus 71 infection of monocytes with antibody-dependent enhancement. Clin Vaccine Immunol, 2010. 17(10): p. 1517-23.

[55] Takada, A. and Y. Kawaoka, Antibody-dependent enhancement of viral infection: molecular mechanisms and in vivo implications. Rev Med Virol, 2003. 13(6): p. 387-98.

[56] Wang, S.M., et al., Pathogenesis of enterovirus 71 brainstem encephalitis in pediatric patients: roles of cytokines and cellular immune activation in patients with pulmonary edema. J Infect Dis, 2003. 188(4): p. 564-70.

[57] Wu, J.M., et al., Cardiopulmonary manifestations of fulminant enterovirus 71 infection. Pediatrics, 2002. 109(2): p. E26-.

[58] Brown, R.H., Jr., et al., Medulla oblongata edema associated with neurogenic pulmonary edema. Case report. J Neurosurg, 1986. 64(3): p. 494-500.

[59] Baker, A.B., Poliomyelitis. 16. A study of pulmonary edema. Neurology, 1957. 7(11): p. 743-51.

[60] Fu, Y.C., et al., Cardiac complications of enterovirus rhombencephalitis. Arch Dis Child, 2004. 89(4): p. 368-73.

[61] Shen, W.C., et al., MR imaging findings of enteroviral encephaloymelitis: an outbreak in Taiwan. AJNR Am J Neuroradiol, 1999. 20(10): p. 1889-95.

[62] Wang, S.M., 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): p. 184-90.

[63] Valcour, V., et al., A case of enteroviral meningoencephalitis presenting as rapidly progressive dementia. Nat Clin Pract Neurol, 2008. 4(7): p. 399-403.

[64] Dunn, J.J., et al., Stable enterovirus 5' nontranslated region over a 7-year period in a patient with agammaglobulinemia and chronic infection. J Infect Dis, 2000. 182(1): p. 298-301.

[65] Feuer, R., et al., Coxsackievirus replication and the cell cycle: a potential regulatory mechanism for viral persistence/latency. Med Microbiol Immunol, 2004. 193(2-3): p. 83-90.

[66] Galbraith, D.N., C. Nairn, and G.B. Clements, Evidence for enteroviral persistence in humans. J Gen Virol, 1997. 78 (Pt 2): p. 307-12.

[67] Rudge, P., et al., Encephalomyelitis in primary hypogammaglobulinaemia. Brain, 1996. 119 (Pt 1): p. 1-15.

[68] McKinney, R.E., Jr., S.L. Katz, and C.M. Wilfert, Chronic enteroviral meningoencephalitis in agammaglobulinemic patients. Rev Infect Dis, 1987. 9(2): p. 334-56.

[69] Quartier, P., et al., Enteroviral meningoencephalitis in X-linked agammaglobulinemia: intensive immunoglobulin therapy and sequential viral detection in cerebrospinal fluid by polymerase chain reaction. Pediatr Infect Dis J, 2000. 19(11): p. 1106-8.

[70] Choi, C.S., et al., Clinical manifestations of CNS infections caused by enterovirus type 71. Korean J Pediatr, 2011. 54(1): p. 11-6.

[71] McMinn, P., et al., 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): p. 236-42.

[72] Chang, L.Y., et al., Transmission and clinical features of enterovirus 71 infections in household contacts in Taiwan. JAMA, 2004. 291(2): p. 222-7.

[73] Melnick, J.L., Enterovirus type 71 infections: a varied clinical pattern sometimes mimicking paralytic poliomyelitis. Rev Infect Dis, 1984. 6 Suppl 2: p. S387-90.

[74] Chumakov, M., et al., Enterovirus 71 isolated from cases of epidemic poliomyelitis-like disease in Bulgaria. Arch Virol, 1979. 60(3-4): p. 329-40.

[75] Ishimaru, Y., et al., 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): p. 583-8.

[76] Alexander, J.P., Jr., et al., Enterovirus 71 infections and neurologic disease--United States, 1977-1991. J Infect Dis, 1994. 169(4): p. 905-8.

[77] Lum, L.C., et al., Echovirus 7 associated encephalomyelitis. J Clin Virol, 2002. 23(3): p. 153-60.

[78] Zuckerman, M.A., et al., Fatal case of echovirus type 9 encephalitis. J Clin Pathol, 1993. 46(9): p. 865-6.

[79] Tyler, K.L., Emerging viral infections of the central nervous system: part 1. Arch Neurol, 2009. 66(8): p. 939-48.

[80] Wong, K.T., L.C. Lum, and S.K. Lam, Enterovirus 71 infection and neurologic complications. N Engl J Med, 2000. 342(5): p. 356-8.

[81] Zimmerman, R.D., MR imaging findings of enteroviral encephalomyelitis: an outbreak in Taiwan. AJNR Am J Neuroradiol, 1999. 20(10): p. 1775-6.

[82] Wilson, M.R. and K.L. Tyler, Issues and updates in emerging neurologic viral infections. Semin Neurol, 2011. 31(3): p. 245-53.

[83] Wong, K.T., Emerging and re-emerging epidemic encephalitis: a tale of two viruses. Neuropathol Appl Neurobiol, 2000. 26(4): p. 313-8.

[84] Rotbart, H.A., et al., Factors affecting the detection of enteroviruses in cerebrospinal fluid with coxsackievirus B3 and poliovirus 1 cDNA probes. J Clin Microbiol, 1985. 22(2): p. 220-4.

[85] Chonmaitree, T., et al., Comparison of cell cultures for rapid isolation of enteroviruses. J Clin Microbiol, 1988. 26(12): p. 2576-80.

[86] van Vliet, K.E., et al., Multicenter evaluation of the Amplicor Enterovirus PCR test with cerebrospinal fluid from patients with aseptic meningitis. The European Union

Concerted Action on Viral Meningitis and Encephalitis. J Clin Microbiol, 1998. 36(9): p. 2652-7.

[87] Wong, K.T., et al., Enterovirus 71 encephalomyelitis and Japanese encephalitis can be distinguished by topographic distribution of inflammation and specific intraneuronal detection of viral antigen and RNA. Neuropathol Appl Neurobiol, 2012.

[88] Granerod, J., et al., Challenge of the unknown. A systematic review of acute encephalitis in non-outbreak situations. Neurology, 2010. 75(10): p. 924-32.

[89] Fowlkes, A.L., et al., Enterovirus-associated encephalitis in the California encephalitis project, 1998-2005. J Infect Dis, 2008. 198(11): p. 1685-91.

[90] Lamson, D., et al., MassTag polymerase-chain-reaction detection of respiratory pathogens, including a new rhinovirus genotype, that caused influenza-like illness in New York State during 2004-2005. J Infect Dis, 2006. 194(10): p. 1398-402.

[91] Briese, T., et al., Diagnostic system for rapid and sensitive differential detection of pathogens. Emerg Infect Dis, 2005. 11(2): p. 310-3.

[92] Palacios, G., et al., MassTag polymerase chain reaction for differential diagnosis of viral hemorrhagic fever. Emerg Infect Dis, 2006. 12(4): p. 692-5.

[93] Dominguez, S.R., et al., Multiplex MassTag-PCR for respiratory pathogens in pediatric nasopharyngeal washes negative by conventional diagnostic testing shows a high prevalence of viruses belonging to a newly recognized rhinovirus clade. J Clin Virol, 2008. 43(2): p. 219-22.

[94] Wang, D., et al., Microarray-based detection and genotyping of viral pathogens. Proc Natl Acad Sci U S A, 2002. 99(24): p. 15687-92.

[95] Wang, D., et al., Viral discovery and sequence recovery using DNA microarrays. PLoS Biol, 2003. 1(2): p. E2.

[96] Chiu, C.Y., et al., Utility of DNA microarrays for detection of viruses in acute respiratory tract infections in children. J Pediatr, 2008. 153(1): p. 76-83.

[97] Ksiazek, T.G., et al., A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med, 2003. 348(20): p. 1953-66.

[98] Urisman, A., et al., Identification of a novel Gammaretrovirus in prostate tumors of patients homozygous for R462Q RNASEL variant. PLoS Pathog, 2006. 2(3): p. e25.

[99] Gunson, R.N., T.C. Collins, and W.F. Carman, Practical experience of high throughput real time PCR in the routine diagnostic virology setting. J Clin Virol, 2006. 35(4): p. 355-67.

[100] Quan, P.L., et al., Astrovirus encephalitis in boy with X-linked agammaglobulinemia. Emerg Infect Dis, 2010. 16(6): p. 918-25.

[101] Pouplin, T., et al., Valacyclovir for herpes simplex encephalitis. Antimicrob Agents Chemother, 2011. 55(7): p. 3624-6.

[102] Erlendsson, K., T. Swartz, and J.M. Dwyer, Successful reversal of echovirus encephalitis in X-linked hypogammaglobulinemia by intraventricular administration of immunoglobulin. N Engl J Med, 1985. 312(6): p. 351-3.

[103] Johnson, P.R., Jr., K.M. Edwards, and P.F. Wright, Failure of intraventricular gamma globulin to eradicate echovirus encephalitis in a patient with X-linked agammaglobulinemia. N Engl J Med, 1985. 313(24): p. 1546-7.

[104] Dwyer, J.M. and K. Erlendsson, Intraventricular gamma-globulin for the management of enterovirus encephalitis. Pediatr Infect Dis J, 1988. 7(5 Suppl): p. S30-3.

[105] Hsu, B.M., C.H. Chen, and M.T. Wan, Genetic diversity of epidemic enterovirus 71 strains recovered from clinical and environmental samples in Taiwan. Virus Res, 2007. 126(1-2): p. 69-75.

[106] Li, Z.H., et al., Ribavirin reduces mortality in enterovirus 71-infected mice by decreasing viral replication. J Infect Dis, 2008. 197(6): p. 854-7.

[107] Poordad, F. and D. Dieterich, Treating hepatitis C: current standard of care and emerging direct-acting antiviral agents. J Viral Hepat, 2012. 19(7): p. 449-64.

[108] Shih, S.R., et al., Mutation in enterovirus 71 capsid protein VP1 confers resistance to the inhibitory effects of pyridyl imidazolidinone. Antimicrob Agents Chemother, 2004. 48(9): p. 3523-9.

[109] Pevear, D.C., et al., Activity of pleconaril against enteroviruses. Antimicrob Agents Chemother, 1999. 43(9): p. 2109-15.

[110] Romero, J.R., Pleconaril: a novel antipicornaviral drug. Expert Opin Investig Drugs, 2001. 10(2): p. 369-79.

[111] National Institute of Allergy and Infectious Diseases (NIAID). A Double-Blind, Placebo-Controlled, Virologic Efficacy Trial of Pleconaril in the Treatment of Neonates With Enteroviral Sepsis Syndrome, in ClinicalTrials.gov.

[112] Tan, E.L. and Chu, J.J., RNA interference (RNAi) - An antiviral Strategy for Enteroviruses. . J Antivir Antiretrovir S2. doi:10.4172/jaa.S2-001, 2011.

[113] Tan, E.L., et al., Enhanced potency and efficacy of 29-mer shRNAs in inhibition of Enterovirus 71. Antiviral Res, 2007. 74(1): p. 9-15.

[114] Tan, E.L., et al., Inhibition of enterovirus 71 in virus-infected mice by RNA interference. Mol Ther, 2007. 15(11): p. 1931-8.

[115] Gau, S.S., et al., Attention-deficit/hyperactivity-related symptoms among children with enterovirus 71 infection of the central nervous system. Pediatrics, 2008. 122(2): p. e452-8.

[116] Wang, S.M. and C.C. Liu, Enterovirus 71: epidemiology, pathogenesis and management. Expert Rev Anti Infect Ther, 2009. 7(6): p. 735-42.

[117] Chan, L.G., 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): p. 678-83.