Chapter 22
Infectious and Inflammatory Disorders

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Abbreviations

BOM	Bilirubin oxidative metabolites
CNS	Central nervous system
CSF	Cerebrospinal fluid
ELISA	Enzyme-linked immunosorbent assay
GPX	Glutathione peroxidase
HIV	Human immunodeficiency virus
HO	Heme oxygenase
IAE	Influenza-associated acute encephalopathy
MDA	Malonaldehyde
MPO	Myeloperoxidase
NO	Nitric oxide
NOS	Nitric oxide synthase
8-OHdG	8-hydroxy-2′-deoxyguanosine
ROS	Reactive oxygen species
RSV	Respiratory syncytial virus
SOD	Superoxide dismutase
TAC	Total antioxidant capacity
TH	Total hydroperoxides

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22.1 Introduction

Reactive species with nitrogen-based or oxygen-based unpaired electrons appear to play diverse roles in many aspects of physiological and pathological events in the pediatric field [1]. This is readily evident in infectious and inflammatory disorders such as sepsis, meningitis, encephalopathy, pneumonia, gastritis, enterocolitis, urinary infection, skin infection, burn injury, and immune activation syndromes.

Nitric oxide (NO), produced endogenously in cells and tissues of various types [2, 3], is a biological messenger molecule involved in numerous homeostatic processes. Constitutive isoenzymes of nitric oxide synthase (NOS) contribute to important physiological processes such as vasorelaxation and neurotransmission. Inducible NOS, which is expressed in various cells including macrophages, neutrophils, epithelial cells, endothelial cells, and hepatocytes, produces excessive NO in infectious and inflammatory conditions. Furthermore, reactive oxygen species (ROS) including superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH) are produced through enzymes such as xanthine oxidoreductase and NADPH oxidases by infiltrating phagocytic cells and resident cells in inflamed tissues. Many effects of NO and O_2^- might be mediated by their reaction product peroxynitrite ($ONOO^-$). These reactive species, which have antimicrobial actions via their cytotoxic or cytostatic effects, contribute to innate (nonspecific) and immunological host defenses.

It is necessary to mention that NO and ROS act as a double-edged sword, having both beneficial and deleterious effects in biological systems [1–3]. They can attack various substrates in the body including lipids, proteins, nucleic acids, and carbohydrates. Oxidation of any of these substances, if unchecked, can contribute to the development of acute and chronic disorders. Biomarker research in this field can help guide the selection, efficiency, and sufficiency of medical interventions for pathological states that are characterized by the enhanced generation of NO and ROS [1].

This chapter presents a review of the medical literature and discusses the pathophysiological roles of NO and ROS in infectious and inflammatory disorders of children. It provides a detailed explanation of some devastating conditions affecting the brain (i.e., purulent meningitis and encephalopathy). We hope that this chapter will foster further investigation into the mechanisms by which oxidative stress influences the progression of these disorders, and that it will suggest potential therapeutic measures.

22.2 Oxidative Stress Biomarkers

Results of recent studies implicate oxidative stress in various acute and chronic disorders of children [1]. Accordingly, it is important to evaluate the stress conditions objectively and non-invasively in this population.

Any biomolecule can be damaged by NO and ROS. Direct measurement of these species is difficult because of their short half-life. Therefore, stress conditions are evaluated by measuring stable products of the oxidative modification processes

Table 22.1 Clinically important oxidative stress biomarkers of the two categories

(a) *Formation of modified molecules by nitric oxide and reactive oxygen species*
Lipid peroxidation: malondialdehyde,[a] 4-hydroxy-2,3-nonenal,[a] acrolein,[a] hexanoyl-lysine,[a] F2-isoprostane,[a] hydroperoxides[a]
Oxidative DNA damage: 8-hydroxy-2′-deoxyguanosine[a]
Glyco-oxidation: carboxymethyl-lysine, pentosidine, methylglyoxal
Nitro-oxidation: nitrotyrosine, nitrite/nitrate[a]
Others: hydrogen peroxide, o,o′-dityrosine, carbonyl protein,[a] bilirubin oxidative metabolites,[a] oxidized glutathione[a]
(b) *Antioxidative enzymes and molecules*
Enzymes: superoxide dismutase,[a] catalase,[a] glutathione peroxidase,[a] glutathione reductase, glutathione-S-transferase, thioredoxin reductase, heme oxygenase[a]
Proteins: albumin, transferrin, ceruloplasmin, thioredoxin, L-type fatty acid binding protein
Low molecular weight molecules: bilirubin,[a] retinol,[a] ascorbate,[a] tocopherols,[a] ubiquinol/ubiquinone (coenzyme Q_{10}),[a] reduced glutathione,[a] nitrite/nitrate,[a] selenium,[a] zinc[a]

[a]These markers are explained in this chapter

Only biomarkers that are determined in samples of blood or urine are shown. Nitric oxide behaves either as a prooxidant or as an antioxidant according to its environment. Therefore, its stable metabolites, nitrite/nitrate, are listed in both categories (a) and (b)

in vivo [1]. Oxidative stress is also evaluated by measuring the consumption or induction of certain enzymes or antioxidants. Measurement of specific biomarkers in blood or urine enables repeated monitoring of systemic oxidative stress status, which is not possible with invasive tests. Clinically applicable and reliable biomarkers are presented in Table 22.1.

The first category (a) includes molecules that are generated in reactions with NO and ROS. They are subjected to either scission, cross-linking, or covalent modification in these reactions. Accordingly, the amount of these molecules is increased when ROS is generated excessively. Some are removed or repaired rapidly, but others remain in the body for a long time. Major targets of NO and ROS in the molecular components of the cells are membrane lipids, proteins, nucleic acids, and carbohydrates.

The second category (b) includes antioxidative enzymes and molecules that are associated with NO and ROS metabolism. In most cases, these molecules are destroyed or modified and exhibit decreased activity or quantity. Conversely, they often show an overshooting response for a period of hours, days, or weeks.

22.3 Infectious and Inflammatory Disorders Associated with Oxidative Stress

Numerous infectious and inflammatory disorders appear to be linked to oxidative damage attributable to NO and ROS in their pathogenesis and progression [1]. In most studies, oxidative stress biomarkers were determined in samples of blood (such as whole blood serum, plasma, erythrocytes, and leukocytes) or urine [4–36] (Table 22.2). In other studies, the parameters were measured using tissues or

Table 22.2 Infectious and inflammatory diseases associated with enhanced oxidative stress

Condition	Primary findings
Bacterial or fungal infection:	
Purulent meningitis	Higher urinary nitrite/nitrate [4]; higher serum MDA, ascorbate, lower serum-reduced glutathione [16]; higher serum nitrite/nitrate, lipid hydroperoxides, SOD [29]; higher serum MDA, MPO, lower serum TAC [31]
Sepsis	Lower whole blood-reduced glutathione [6]
Cholera	Higher plasma nitrite/nitrate, MPO [8]
Purulent skin infection	Higher urinary acrolein, 8-OHdG, bilirubin oxidative metabolites [11]
Chronic tonsillitis	Higher plasma MDA [12]
Chronic nail candidiasis	Higher serum MDA, 4-hydroxy-2,3-nonenal [15]
Chronic otitis media	Higher plasma MDA, lower plasma-reduced glutathione [18]
Acute otitis media, acute tonsillitis	Higher whole blood MDA, lower whole blood reduced glutathione [20]
Lower urinary tract infection	Higher urinary MDA, TAC [21]
Acute pneumonia	Higher whole blood MDA, lower whole blood reduced glutathione, GPX, SOD [22]; higher plasma MDA [23]
Viral infection:	
Human immunodeficiency virus infection	
	Lower serum TAC [5]; higher serum MDA [9]; lower plasma ascorbate, lower serum tocopherol [28]; lower serum ascorbate [34]
Hepatitis C virus infection	Higher leukocyte 8-OHdG [7]
Influenza encephalopathy	Higher serum nitrite/nitrate [10]
Viral bronchiolitis	Higher serum MDA, lower serum selenium [14]
Rotavirus-associated convulsion	Higher serum nitrite/nitrate [17]
Measles	Higher whole blood MDA, lower whole blood-reduced glutathione, lower serum retinol, ascorbate, tocopherol [25]
Human herpes virus 6 encephalopathy	Higher urinary 8-OHdG [26]
Pandemic influenza (H1N1)	Lower serum TAC, ubiquinol/ubiquinone, zinc [35]
Protozoan infection:	
Cutaneous leishmaniasis	Higher erythrocyte MDA, SOD [13]; higher serum nitrite/nitrate, MDA, lower serum SOD, GPX [19]
Schistosoma mansoni infection	Higher serum hydroperoxides, lower serum retinol, tocopherol [24]
Fasciola hepatica infection	Higher serum "total oxidant status," lower plasma TAC [32]
Others:	
Acute rheumatic fever	Lower serum TAC [27]
Burn	Higher plasma MDA [30]
Kawasaki disease	Higher plasma hydroperoxides [33]; higher serum hydroperoxides [36]

GPX glutathione peroxidase, *MDA* malondialdehyde, *MPO* myeloperoxidase, *8-OHdG* 8-hydroxy-2′-deoxyguanosine, *SOD* superoxide dismutase, *TAC* total antioxidant capacity

Only studies in which systemic oxidative stress status was evaluated are listed here. In these studies, specific biomarkers were determined in blood or urine samples from the patients. Conditions associated with bacterial or fungal infection are described in the early part of the table

Numbers in square brackets indicate cited references

different body fluids, either alone or in combination with samples of blood or urine. The results are the following: higher levels of myeloperoxidase (MPO) and inducible NO synthase in rectal tissue in cholera [8]; higher leukocyte levels and lower urinary levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) in *Helicobacter pylori* infection [37]; higher levels of malondialdehyde (MDA) and catalase in tonsillar and adenoid tissue in chronic adenotonsillitis [38]; lower "total antioxidant capacity (TAC)" in saliva in human immunodeficiency virus (HIV) infection [39]; higher 8-isoprostane, carbonyl protein, and glutathione peroxidase (GPX) in bronchoalveolar lavage fluid in postinfectious bronchiolitis obliterans [40]; and higher MDA and 8-isoprostane in nasopharyngeal secretion in hypoxic respiratory syncytial virus (RSV) bronchiolitis [41]. The results for cerebrospinal fluid (CSF) examination are presented in the next section.

Recently, enzyme-linked immunosorbent assay (ELISA) systems have been constructed for various stress biomarkers [11, 26, 40, 41]. Onerous pretreatments and expensive apparatus are virtually obviated by ELISA, which is a labor-saving, cost-saving method [42]. Examination using a few sample aliquots is possible. Furthermore, compact machines by which serum/plasma total hydroperoxides (TH) and "TAC" [24, 33, 36, 43] or urinary 8-OHdG [44] can be measured are available. These machines provide highly reproducible results quickly.

It is noteworthy that oxidative damage might take place in a selective manner. For instance, lipid peroxidation and oxidative DNA damage are not always accompanied by overproduction of NO. Detection of more than one marker is an important key because a single marker might yield misleading results. It might also be crucial to determine which particular markers, alone or in combination with others, can serve as a precise indicator of the contribution of oxidative stress to a disease, thereby allowing the success (or failure) of the treatment to be monitored.

22.4 Biomarkers in CSF

Purulent meningitis and acute encephalopathy are severe central nervous system (CNS) disorders that can result in sudden death or development of neurological sequelae [45, 46]. These diseases, which are not rare in children, persist as an important public health problem worldwide.

Computed tomography and magnetic resonance imaging might be useful to evaluate the severity of brain injury, but it is often difficult to perform such radiological examinations during the critical period when key therapeutic decisions are made. Therefore, assessing ongoing brain injury and predicting outcomes using CSF samples is extremely valuable. These markers might be of various kinds, including cytokines/chemokines (such as tumor necrosis factor-α, interleukins-1β, -6, -8, -10, -13, granulocyte colony-stimulating factor, monocyte chemotactic protein-1, macrophage inflammatory protein-1, transforming growth factor-β, interferon-γ, interferon-γ-inducible protein-10), brain injury marker (S-100B protein, neuron-specific enolase, glial fibrillary acidic protein, neurofilaments, tau

protein), tissue degradation enzymes (matrix metalloproteinases-8, -9), and oxidative stress markers (as described below).

The brain is regarded as vulnerable to free radical damage because of high oxygen consumption, in addition to its consequent generation of high levels of NO and ROS, high contents of unsaturated lipids and cellular iron, and weakened antioxidant defense system [47]. Oxidative stress is a predisposing factor for neuronal destruction in meningitis and encephalopathy, and for neuronal degeneration in developmental brain disorders [48].

Measurement of oxidative stress biomarkers in CSF samples has been introduced into the pediatric field. These markers presumably permit sequential biological monitoring of the CNS of the patients. Clinically applicable CSF markers are the following: lipid hydroperoxides, MDA, acrolein, TH, 8-OHdG, nitrite/nitrate, bilirubin oxidative metabolites (BOM), and TAC [17, 29, 49–62]. All these markers have also been determined in blood or urine samples for evaluation of systemic oxidative stress status (Table 22.2). ELISA [53, 54, 62] and rapid analysis methods [50, 57, 58, 60, 61] are applied to practice in CSF analyses.

22.5 Acute Purulent Meningitis

Acute purulent meningitis is a severe bacterial infection of the CNS that occurs especially in children younger than 5 years of age. Although the introduction of antibiotics has made it curable, mortality and morbidity from the disease remain high [46, 63]. The mortality rate is about 5 %. Long-term morbidity, consisting mainly of persistent neurological sequelae, is about 20 %.

It is apparent that the excessive host immune response is incapable of controlling infection within the CNS, particularly the CSF within the subarachnoid space, and that this host inflammatory response contributes to many adverse events that can occur during purulent meningitis [63]. A complex series of events involving host cytokines/chemokines, proteolytic enzymes, and NO/ROS is responsible for meningitis-induced brain damage, at least during the early phase of the disease (Fig. 22.1).

In this section, we highlight the ROS-mediated brain damage and antioxidant defenses in pediatric patients with acute purulent meningitis. Previous results for the CSF analyses from the patients are presented in Table 22.3 [29, 31, 49–54, 58]. They show enhanced production of NO and ROS in the CNS of patients with purulent meningitis compared with those with aseptic meningitis as well as non-meningitis subjects. The pathogenic bacteria were *Streptococcus pneumoniae*, *Haemophilus influenzae*, Group B streptococcus, and others. These findings also suggest the contribution of elevated oxidative stress to disease severity and occurrence of neurological complications [29, 31, 49, 51, 52, 58]. Our earlier observations [53, 54], which are presented in Fig. 22.2, suggest that clinical and laboratory improvement is linked closely to the decrease in oxidant activation in CNS.

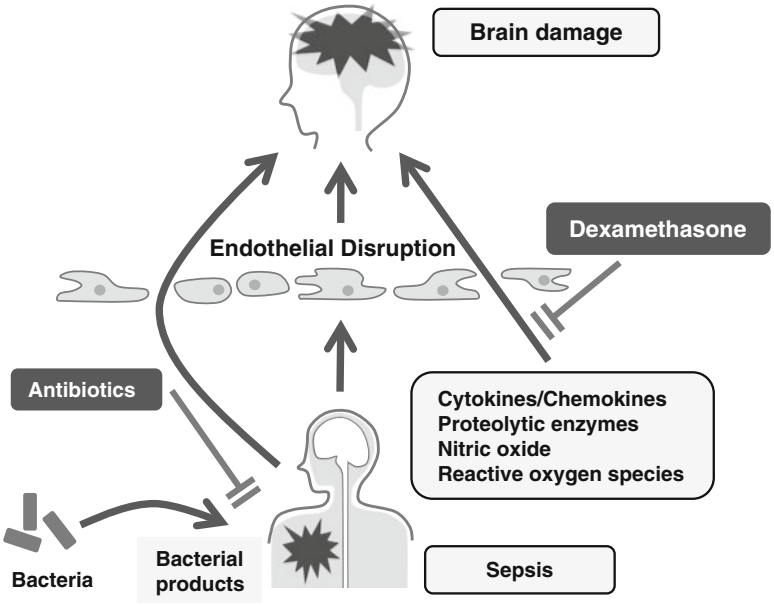

Fig. 22.1 Mechanisms of brain damage in purulent meningitis. Meningitis-associated brain damage and neuronal death is not mediated simply by the presence of viable bacterial components. Widely diverse inflammatory host factors (cytokines/chemokines, nitric oxide/reactive oxygen species, proteolytic enzymes) are now known to be involved in the complex pathophysiological cascade of purulent meningitis. Further exploration of these pathways is expected to contribute to the development of therapeutic adjunctive strategies in purulent meningitis

Table 22.3 CSF levels of oxidative stress biomarkers in acute purulent meningitis

Authors	Primary findings
Hamed et al. [29]	Higher nitrite/nitrate, lipid hydroperoxides
Miric et al. [31]	Higher malondialdehyde, myeloperoxidase, lower "total antioxidant capacity"
van Furth et al. [49]	Higher nitrite/nitrate
Tsukahara et al. [50]	Higher nitrite (levels were also determined using "urine reagent strips")[a]
Ray et al. [51]	Higher superoxide, malondialdehyde, superoxide dismutase
Murawska-Ciałowicz et al. [52]	Higher nitrite/nitrate
Tsukahara et al. [53]	Higher 8-hydroxy-2′-deoxyguanosine
Tsukahara et al. [54]	Higher acrolein-lysine, nitrite, bilirubin oxidative metabolites
Yamanaka et al. [58]	Higher hydroperoxides (levels were determined using the free radical analytical system; Diacron International, Italy)[a]

Numbers in square brackets indicate cited references
[a]Rapid analytical methods

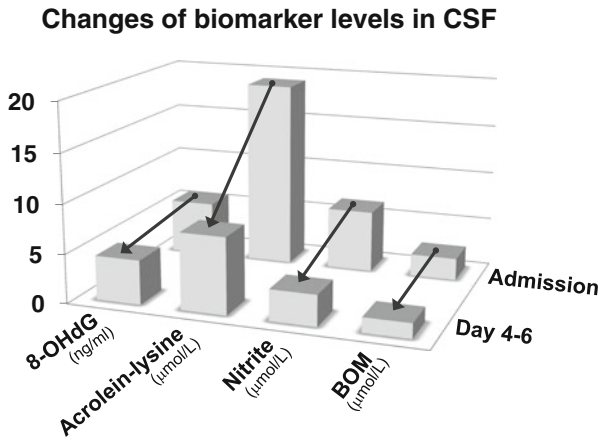

Fig. 22.2 Changes in cerebrospinal fluid (CSF) oxidative stress biomarker levels in children with purulent meningitis. All these markers were several times higher in children during the early phase of purulent meningitis than they were in children without meningitis. In the purulent meningitis group, the CSF levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG), acrolein-lysine, nitrite, and BOM decreased significantly from 5.6 ng/mL, 19.0 μmol/L, 6.3 μmol/L, and 2.3 μmol/L on admission to 4.5 ng/mL, 7.8 μmol/L, 3.2 μmol/L, and 1.5 μmol/L at 4–6 hospital days, respectively, as patients responded to intravenous β-lactam antibiotics and dexamethasone administration [53, 54]. Presented data are mean values of the markers

The pathological hallmark of purulent meningitis is the dense inflammatory infiltrates in the subarachnoid and ventricular spaces, predominantly composed of neutrophils. Activated neutrophils secrete proteolytic enzymes and liberate NO and ROS. Excessive NO and ROS can exert various cytotoxic effects through lipid peroxidation, DNA strand breakage, mitochondrial damage, or potassium channel activity alterations [63, 64]. Oxidative alterations to vital macromolecules are observed in brain samples (most prominently in blood vessels and inflammatory cells) of patients who have died of meningitis [64, 65].

The presence of increased CSF levels of BOM in children with purulent meningitis is noteworthy here [54]. The levels were nearly eight times higher in these patients than in individuals of the non-meningitis group (Fig. 22.2). That significant increase of BOM is assumed to be attributable to local or systemic high production of bilirubin, mainly reflecting the overexpression of inducible heme oxygenase (HO) [66]. The HO induction and the subsequent bilirubin biosynthesis might be part of a common defense mechanism aimed at reducing oxidative brain injury in several neurological conditions [67].

Corticosteroids are beneficial for the early treatment of both human and experimental purulent meningitis [68]. Dexamethasone attenuates neuronal tissue damage by intervening at various levels of meningeal inflammatory cascade (Fig. 22.1). The proposed neuroprotective mechanisms include inhibition of ROS production, lipid peroxidation, leukocyte-endothelial interaction, and of transcription of various proinflammatory cytokines and inducible NOS. Dexamethasone treatment

significantly lowered the nitrite concentration in the CSF of purulent meningitis children compared with those of non-treated children on the second day of the disease [52]. An additional piece of evidence for the beneficial effects of dexamethasone on purulent meningitis might be reduction in the levels of 8-OHdG, acrolein, and nitrite as the patients started to recover from the disease [53, 54] (Fig. 22.2).

Studies in rats have provided substantial evidence that antioxidant therapy (such as *N*-acetyl-L-cysteine, phenyl tert-butyl nitrone, and Mn(III) tetrakis(4-benzoic acid) porphyrin) is protective against vascular, cortical, and cochlear injury in pneumococcal meningitis [63, 64]. The adjunctive use of these antioxidants appears to offer promising future treatment options for purulent meningitis in humans.

22.6 Acute Encephalopathy

Acute encephalopathy is a severe CNS complication of common infections (such as influenza, exanthem subitum, and acute viral gastroenteritis) with high mortality and neurological sequelae [69]. As explained in previous sections, assessing brain injury and predicting outcomes using bedside point-of-care testing is valuable in these patients.

Here, we briefly highlight the ROS-mediated brain damage and antioxidant defenses in children with acute encephalopathy. Previous results for the CSF analyses from the patients are described in Table 22.4 [55–57, 59–62]. They indicate enhanced production of NO and ROS in the CNS of children with acute encephalopathy compared with those of non-encephalopathy subjects. These reports described the pathogenic viruses of influenza, enterovirus, RSV, and other viruses. Pleocytosis and high protein levels in CSF were found only occasionally in the patients. It remains to be clarified whether the NO/ROS production levels are related to the development of sequelae because available data in this research area remain limited.

Table 22.4 CSF levels of oxidative stress biomarkers in acute encephalopathy

Authors	Primary findings
Kawashima et al. [55]	Higher nitrite/nitrate in influenza encephalopathy
Kawashima et al. [56]	Higher nitrite/nitrate in influenza encephalopathy
Yamanaka et al. [57]	Higher hydroperoxides (free radical analytical system)[a] in influenza encephalopathy
Morichi et al. [59]	Higher nitrite/nitrate in respiratory syncytial virus encephalopathy
Kawashima et al. [60]	Higher hydroperoxides in enterovirus encephalopathy (free radical analytical system)[a]
Kawashima et al. [61]	Higher nitrite/nitrate, hydroperoxides (free radical analytical system)[a] in influenza encephalopathy
Miyata et al. [62]	Higher 8-hydroxy-2′-deoxyguanosine, hexanoyl-lysine in “clinically mild encephalopathy with a reversible splenial lesion”

Numbers in square brackets indicate cited references

[a]Rapid analytical method

It is noteworthy that in influenza encephalopathy patients, the increase of CSF NO levels was linked to the low serum zinc levels [56]. Trace elements are micronutrients that are present in small amounts in the body and which are necessary for the normal functioning of immune and antioxidant systems. The role of micronutrients in the pathogenesis, course, and outcome of encephalopathy warrants further study.

Stratification of acute encephalopathy is important clinically. Strategies for severe encephalopathy should be established in the near future. Prevention (or modulation) of excessive host inflammatory response including NO/ROS release, a possible therapeutic approach, is explained in the following section.

22.7 Redox Modulation Strategy for Influenza Encephalopathy

Influenza-associated acute encephalopathy (IAE) is an abrupt disorder of the CNS triggered by influenza virus infection, often engendering severe sequelae or death [70]. The Centers for Disease Control and Prevention have regarded IAE as an important public health problem at least since 2003, citing our report on IAE [71]. The 2009 pandemic influenza A (H1N1) virus emerged in Mexico in April 2009, thereafter spreading rapidly worldwide. In June 2009, The World Health Organization declared that the spreading novel influenza virus constituted a global pandemic. IAE of severe type also occurred following the 2009 pandemic influenza [72, 73].

Because influenza virus infection occurs predominantly in younger generations, great concern has arisen in relation to the severity of complications, such as IAE or severe pneumonia, among children [70]. Pathological findings, including the lack of viral antigen and sparse, if any, inflammatory infiltrates in the brain, imply that direct viral invasion and subsequent inflammation are unlikely to cause this encephalopathy. A prevailing theory is that excessive host inflammatory response characterized by massive production of pro-inflammatory cytokines/chemokines and NO/ROS and excessive apoptosis exacerbate IAE [74–79]. As a consequence, widespread vascular endothelial activation, dysfunction, and damage occur, ultimately resulting in multiple organ failure and death (Fig. 22.3).

In Japan, the guideline for diagnosis and management of pediatric IAE was formulated in 2005 by the collaborating study group on IAE, which was organized by the Japanese Ministry of Health, Labour, and Welfare. Dr. Morishima (an author of this chapter) has been the supervisor of the study since its inception. The guideline has been used widely among general and pediatric hospitals in our country. In addition to neuraminidase inhibitors, pulse steroid therapy, high-dose immunoglobulin, antioxidative agent (edaravone), coagulation modifying agent (thrombomodulin), plasma exchange, and hypothermia are listed as selectable treatments for severe IAE. These treatments are expected to impede excessive inflammatory host response and enhanced oxidative stress in the patients [57, 61, 78, 79] (Fig. 22.3).

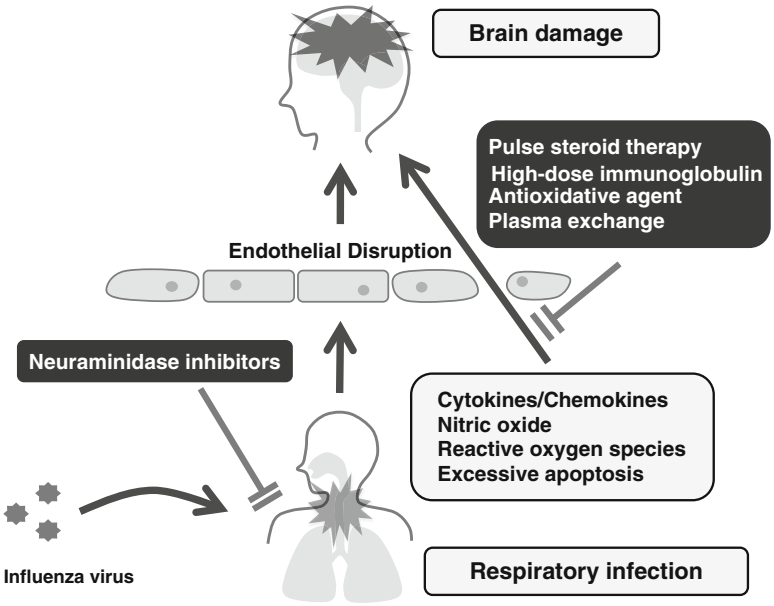

Fig. 22.3 Mechanisms of brain damage in influenza-associated acute encephalopathy (IAE). The IAE pathogenesis remains to be clarified. Viral RNA has not been detected in the CSF of most patients with IAE. The findings presented in recent reports suggest that, in cases of severe IAE, either seasonal or 2009 pandemic, pathological manifestations similarly result from complex biological phenomena including overproduction of cytokines/chemokines and nitric oxide/reactive oxygen species, apoptosis induction, and vascular endothelial disruption. Additional exploration of these pathways is expected to contribute to the development of more effective adjunctive strategies in IAE

For pediatric patients with IAE, the mortality rate was about 30 % in the pre-guideline era in Japan, when no efficient strategy had been proposed. Thereafter, the mortality rate decreased to about the one-fourth along with the nationwide distribution of this practical guideline (Fig. 22.4). Nevertheless, the incidence of poor outcomes of pediatric IAE has not been ideally low: 7 % for death and about 20 % for neurological sequelae. More global studies using edaravone and/or other antioxidants with sequential monitoring of oxidative stress biomarkers must be conducted to identify more effective strategies for IAE of severe type.

22.8 Summary and Conclusions

Infectious and inflammatory disorders are common and often severe in young generations. Severe forms of these disorders are occasionally fatal or leave severe sequelae, for which effective treatment is currently either insufficient or

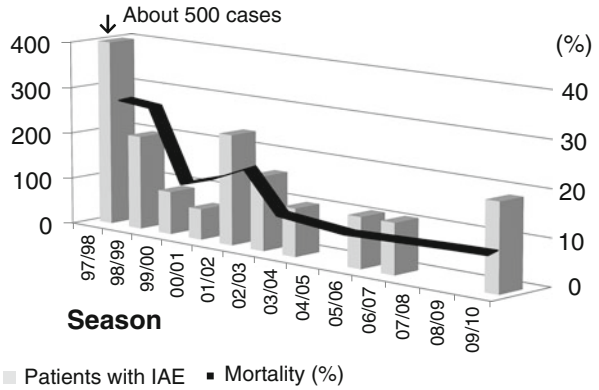

Fig. 22.4 Annual incidence (*vertical bars*) and mortality (*line graph*) of pediatric IAE in Japan. The mortality rate was about 30 % in the pre-guideline era in Japan, when no efficient strategy had been proposed. Thereafter, the mortality rate decreased to about one-fourth along with the nationwide distribution of this practical guideline. Nevertheless, the incidence of poor outcomes of pediatric IAE has not been ideally low: 7 % for death and about 20 % for neurological sequelae

unavailable. The excessive host response and enhanced oxidative stress are inferred to play an important role in the progression and deterioration of these disorders.

Nitric oxide and ROS are unstable molecules produced by various cells and tissues. Using biomarkers will be of great importance for the diagnosis and management of infectious and inflammatory disorders. Those levels are expected to reflect the severity of clinical illness and to predict long-term consequences (if appropriately efficient strategies are not selected). Measurement of oxidative stress biomarkers in CSF samples is especially valuable in patients with purulent meningitis and acute encephalopathy.

Anti-oxidative strategies represent a potential adjunctive and an effective approach to ameliorate these disorders in the pediatric field. Determination of these parameters might identify the disease state and enhance the timing of therapeutic approaches in future medical applications.

References

1. Tsukahara H (2007) Biomarkers for oxidative stress: clinical application in pediatric medicine. Curr Med Chem 14:339–351
2. Akaike T, Maeda H (2000) Nitric oxide and virus infection. Immunology 101:300–308
3. Goligorsky MS, Brodsky SV, Noiri E (2004) NO bioavailability, endothelial dysfunction, and acute renal failure: new insights into pathophysiology. Semin Nephrol 24:316–323
4. Nishimura M, Tsukahara H, Hiraoka M, Osaka Y, Ohshima Y, Tanizawa A, Mayumi M (2000) Systemic inflammatory response syndrome and acute renal failure associated with *Hemophilus influenzae* septic meningitis. Am J Nephrol 20:208–211
5. de Martino M, Chiarelli F, Moriondo M, Torello M, Azzari C, Galli L (2001) Restored antioxidant capacity parallels the immunologic and virologic improvement in children with perinatal

human immunodeficiency virus infection receiving highly active antiretroviral therapy. Clin Immunol 100:82–86
6. Lyons J, Rauh-Pfeiffer A, Ming-Yu Y, Lu XM, Zurakowski D, Curley M, Collier S, Duggan C, Nurko S, Thompson J, Ajami A, Borgonha S, Young VR, Castillo L (2001) Cysteine metabolism and whole blood glutathione synthesis in septic pediatric patients. Crit Care Med 29:870–877
7. Cardin R, Saccoccio G, Masutti F, Bellentani S, Farinati F, Tiribelli C (2001) DNA oxidative damage in leukocytes correlates with the severity of HCV-related liver disease: validation in an open population study. J Hepatol 34:587–592
8. Qadri F, Raqib R, Ahmed F, Rahman T, Wenneras C, Das SK, Alam NH, Mathan MM, Svennerholm AM (2002) Increased levels of inflammatory mediators in children and adults infected with *Vibrio cholerae* O1 and O139. Clin Diagn Lab Immunol 9:221–229
9. Jareño EJ, Romá J, Romero B, Marín N, Muriach M, Johnsen S, Bosch-Morell F, Marselou L, Romero FJ (2002) Serum malondialdehyde correlates with therapeutic efficiency of high activity antiretroviral therapies (HAART) in HIV-1 infected children. Free Radic Res 36:341–344
10. Kawashima H, Watanabe Y, Ichiyama T, Mizuguchi M, Yamada N, Kashiwagi Y, Takekuma K, Hoshika A, Mori T (2002) High concentration of serum nitrite/nitrate obtained from patients with influenza-associated encephalopathy. Pediatr Int 44:705–707
11. Tsukahara H, Shibata R, Ohshima Y, Todoroki Y, Sato S, Ohta N, Hiraoka M, Yoshida A, Nishima S, Mayumi M (2003) Oxidative stress and altered antioxidant defenses in children with acute exacerbation of atopic dermatitis. Life Sci 72:2509–2516
12. Kaygusuz I, Ilhan N, Karlidag T, Keles E, Yalçin S, Cetiner H (2003) Free radicals and scavenging enzymes in chronic tonsillitis. Otolaryngol Head Neck Surg 129:265–268
13. Kocyigit A, Gurel M, Ulukanligil M (2003) Erythrocyte antioxidative enzyme activities and lipid peroxidation levels in patients with cutaneous leishmaniasis. Parasite 10:277–281
14. Gurkan F, Atamer Y, Ece A, Kocyigit Y, Tuzun H, Mete M (2004) Relationship among serum selenium levels, lipid peroxidation, and acute bronchiolitis in infancy. Biol Trace Elem Res 100:97–104
15. Gangemi S, Saija A, Minciullo PL, Tomaino A, Cimino F, Bisignano G, Briuglia S, Merlino MV, Dallapiccola B, Salpietro DC (2004) Serum levels of malondialdehyde and 4-hydroxy-2,3-nonenal in patients affected by familial chronic nail candidiasis. Inflamm Res 53:601–603
16. Caksen H, Cemek M, Dede S, Dulger H, Cemek F (2004) Brief clinical study: lipid peroxidation and antioxidant status in children with acute purulent meningitis and encephalitis. Int J Neurosci 114:105–111
17. Kawashima H, Inage Y, Ogihara M, Kashiwagi Y, Takekuma K, Hoshika A, Mori T, Watanabe Y (2004) Serum and cerebrospinal fluid nitrite/nitrate levels in patients with rotavirus gastroenteritis induced convulsion. Life Sci 74:1397–1405
18. Yilmaz T, Koçan EG, Besler HT, Yilmaz G, Gürsel B (2004) The role of oxidants and antioxidants in otitis media with effusion in children. Otolaryngol Head Neck Surg 131:797–803
19. Serarslan G, Yilmaz HR, Söğüt S (2005) Serum antioxidant activities, malondialdehyde and nitric oxide levels in human cutaneous leishmaniasis. Clin Exp Dermatol 30:267–271
20. Cemek M, Dede S, Bayiroğlu F, Caksen H, Cemek F, Yuca K (2005) Oxidant and antioxidant levels in children with acute otitis media and tonsillitis: a comparative study. Int J Pediatr Otorhinolaryngol 69:823–827
21. Pavlova EL, Lilova MI, Savov VM (2005) Oxidative stress in children with kidney disease. Pediatr Nephrol 20:1599–1604
22. Cemek M, Caksen H, Bayiroğlu F, Cemek F, Dede S (2006) Oxidative stress and enzymic—non-enzymic antioxidant responses in children with acute pneumonia. Cell Biochem Funct 24:269–273
23. Mahalanabis D, Basak M, Paul D, Gupta S, Shaikh S, Wahed MA, Khaled MA (2006) Antioxidant vitamins E and C as adjunct therapy of severe acute lower-respiratory infection in infants and young children: a randomized controlled trial. Eur J Clin Nutr 60:673–680

24. Berhe N, Halvorsen BL, Gundersen TE, Myrvang B, Gundersen SG, Blomhoff R (2007) Reduced serum concentrations of retinol and alpha-tocopherol and high concentrations of hydroperoxides are associated with community levels of *S. mansoni* infection and schistosomal periportal fibrosis in Ethiopian school children. Am J Trop Med Hyg 76:943–949
25. Cemek M, Dede S, Bayiroglu F, Caksen H, Cemek F, Mert N (2007) Oxidant and non-enzymatic antioxidant status in measles. J Trop Pediatr 53:83–86
26. Fukuda M, Yamauchi H, Yamamoto H, Aminaka M, Murakami H, Kamiyama N, Miyamoto Y, Koitabashi Y (2008) The evaluation of oxidative DNA damage in children with brain damage using 8-hydroxydeoxyguanosine levels. Brain Dev 30:131–136
27. Kurban S, Mehmetoglu I, Oran B, Kiyici A (2008) Homocysteine levels and total antioxidant capacity in children with acute rheumatic fever. Clin Biochem 41:26–29
28. Srinivas A, Dias BF (2008) Antioxidants in HIV positive children. Indian J Pediatr 75:347–350
29. Hamed SA, Hamed EA, Zakary MM (2009) Oxidative stress and S-100B protein in children with bacterial meningitis. BMC Neurol 9:51
30. Barbosa E, Faintuch J, Machado Moreira EA, Gonçalves da Silva VR, Lopes Pereima MJ, Martins Fagundes RL, Filho DW (2009) Supplementation of vitamin E, vitamin C, and zinc attenuates oxidative stress in burned children: a randomized, double-blind, placebo-controlled pilot study. J Burn Care Res 30:859–866
31. Miric D, Katanic R, Kisic B, Zoric L, Miric B, Mitic R, Dragojevic I (2010) Oxidative stress and myeloperoxidase activity during bacterial meningitis: effects of febrile episodes and the BBB permeability. Clin Biochem 43:246–252
32. Karsen H, Sunnetcioglu M, Ceylan RM, Bayraktar M, Taskin A, Aksoy N, Erten R (2011) Evaluation of oxidative status in patients with *Fasciola hepatica* infection. Afr Health Sci 11:S14–S18
33. Yahata T, Suzuki C, Hamaoka A, Fujii M, Hamaoka K (2011) Dynamics of reactive oxygen metabolites and biological antioxidant potential in the acute stage of Kawasaki disease. Circ J 75:2453–2459
34. Oliveira KF, Cunha DF, Weffort VR (2011) Analysis of serum and supplemented vitamin C and oxidative stress in HIV-infected children and adolescents. J Pediatr (Rio J) 87:517–522
35. Kelekçi S, Evliyaoğlu O, Sen V, Yolbaş I, Uluca U, Tan I, Gürkan MF (2012) The relationships between clinical outcome and the levels of total antioxidant capacity (TAC) and coenzyme Q (CoQ10) in children with pandemic influenza (H1N1) and seasonal flu. Eur Rev Med Pharmacol Sci 8:1033–1038
36. Kaneko K, Takahashi M, Yoshimura K, Kitao T, Yamanouchi S, Kimata T, Tsuji S (2012) Intravenous immunoglobulin counteracts oxidative stress in Kawasaki disease. Pediatr Cardiol 33:1086–1088
37. Siomek A, Rytarowska A, Szaflarska-Poplawska A, Gackowski D, Rozalski R, Dziaman T, Czerwionka-Szaflarska M, Olinski R (2006) *Helicobacter pylori* infection is associated with oxidatively damaged DNA in human leukocytes and decreased level of urinary 8-oxo-7,8-dihydroguanine. Carcinogenesis 27:405–408
38. Kiroglu AF, Noyan T, Oger M, Kara T (2006) Oxidants and antioxidants in tonsillar and adenoidal tissue in chronic adenotonsillitis and adenotonsillar hypertrophy in children. Int J Pediatr Otorhinolaryngol 70:35–38
39. Padmanabhan V, Rai K, Hegde AM, Shetty S (2010) Total antioxidant capacity of saliva in children with HIV. J Clin Pediatr Dent 34:347–350
40. Mallol J, Aguirre V, Espinosa V (2011) Increased oxidative stress in children with post infectious bronchiolitis obliterans. Allergol Immunopathol (Madr) 39:253–258
41. Hosakote YM, Jantzi PD, Esham DL, Spratt H, Kurosky A, Casola A, Garofalo RP (2011) Viral-mediated inhibition of antioxidant enzymes contributes to the pathogenesis of severe respiratory syncytial virus bronchiolitis. Am J Respir Crit Care Med 183:1550–1560
42. Noiri E, Tsukahara H (2005) Parameters for measurement of oxidative stress in diabetes mellitus: applicability of enzyme-linked immunosorbent assay for clinical evaluation. J Investig Med 53:167–175

43. Nakatsukasa Y, Tsukahara H, Tabuchi K, Tabuchi M, Magami T, Yamada M, Fujii Y, Yashiro M, Tsuge M, Morishima T (2013) Thioredoxin-1 and oxidative stress status in pregnant women at early third trimester of pregnancy: relation to maternal and neonatal characteristics. J Clin Biochem Nutr 52:27–31
44. Kaneko K, Kimata T, Tsuji S, Ohashi A, Imai Y, Sudo H, Kitamura N (2012) Measurement of urinary 8-oxo-7,8-dihydro-2-deoxyguanosine in a novel point-of-care testing device to assess oxidative stress in children. Clin Chim Acta 413:1822–1826
45. Ichiyama T (2010) Acute encephalopathy/encephalitis in childhood: a relatively common and potentially devastating clinical syndrome. Brain Dev 32:433–434
46. Woehrl B, Klein M, Grandgirard D, Koedel U, Leib S (2011) Bacterial meningitis: current therapy and possible future treatment options. Expert Rev Anti Infect Ther 9:1053–1065
47. Tsukahara H, Fujii Y, Hayashi Y, Morishima T (2012) Multiple biomarkers for oxidative stress in patients with brain disorders. J Neurol Sci 313:196
48. Hayashi M, Miyata R, Tanuma N (2012) Oxidative stress in developmental brain disorders. Adv Exp Med Biol 724:278–290
49. van Furth AM, Seijmonsbergen EM, Groeneveld PH, van Furth R, Langermans JA (1996) Levels of nitric oxide correlate with high levels of tumor necrosis factor alpha in cerebrospinal fluid samples from children with bacterial meningitis. Clin Infect Dis 22:876–878
50. Tsukahara H, Haruta T, Hori C, Matsuda M, Ono N, Hiraoka M, Shigematsu Y, Mayumi M (1999) Evaluation of a rapid reagent strip test for the diagnosis of childhood meningitis. Pediatr Int 41:443–446
51. Ray G, Aneja S, Jain M, Batra S (2000) Evaluation of free radical status in CSF in childhood meningitis. Ann Trop Paediatr 20:115–120
52. Murawska-Ciałowicz E, Szychowska Z, Tr busiewicz B (2000) Nitric oxide production during bacterial and viral meningitis in children. Int J Clin Lab Res 30:127–131
53. Tsukahara H, Haruta T, Ono N, Kobata R, Fukumoto Y, Hiraoka M, Mayumi M (2000) Oxidative stress in childhood meningitis: measurement of 8-hydroxy-2′-deoxyguanosine concentration in cerebrospinal fluid. Redox Rep 5:295–298
54. Tsukahara H, Haruta T, Todoroki Y, Hiraoka M, Noiri E, Maeda M, Mayumi M (2002) Oxidant and antioxidant activities in childhood meningitis. Life Sci 71:2797–2806
55. Kawashima H, Watanabe Y, Morishima T, Togashi T, Yamada N, Kashiwagi Y, Takekuma K, Hoshika A, Mori T (2003) NOx (nitrite/nitrate) in cerebral spinal fluids obtained from patients with influenza-associated encephalopathy. Neuropediatrics 34:137–140
56. Kawashima H, Amaha M, Ioi H, Yamanaka G, Kashiwagi Y, Sasamoto M, Takekuma K, Hoshika A, Watanabe Y (2005) Nitrite/nitrate (NOx) and zinc concentrations in influenza-associated encephalopathy in children with different sequela. Neurochem Res 30:311–314
57. Yamanaka G, Kawashima H, Suganami Y, Watanabe C, Watanabe Y, Miyajima T, Takekuma K, Oguchi S, Hoshika A (2006) Diagnostic and predictive value of CSF d-ROM level in influenza virus-associated encephalopathy. J Neurol Sci 243:71–75
58. Yamanaka G, Ishii C, Kawashima H, Oana S, Miyajima T, Hoshika A (2008) Cerebrospinal fluid diacron-reactive oxygen metabolite levels in pediatric patients with central nervous system diseases. Pediatr Neurol 39:80–84
59. Morichi S, Kawashima H, Ioi H, Ushio M, Yamanaka G, Kashiwagi Y, Takekuma K, Hoshika A, Watanabe Y (2009) Cerebrospinal fluid NOx (nitrite/nitrate) in RSV-infected children with CNS symptoms. J Infect 59:299–301
60. Kawashima H, Suzuki K, Yamanaka G, Kashiwagi Y, Takekuma K, Amaha M, Takahashi Y (2010) Anti-glutamate receptor antibodies in pediatric enteroviral encephalitis. Int J Neurosci 120:99–103
61. Kawashima H, Yamanaka G, Ishii C, Kashiwagi Y, Takekuma K, Watanabe Y (2010) NOx as new targets of treatment in influenza-associated encephalopathy. J Pediatr Infect Dis 5:171–176
62. Miyata R, Tanuma N, Hayashi M, Imamura T, Takanashi J, Nagata R, Okumura A, Kashii H, Tomita S, Kumada S, Kubota M (2012) Oxidative stress in patients with clinically mild encephalitis/encephalopathy with a reversible splenial lesion (MERS). Brain Dev 34:124–127

63. Klein M, Koedel U, Kastenbauer S, Pfister HW (2008) Nitrogen and oxygen molecules in meningitis-associated labyrinthitis and hearing impairment. Infection 36:2–14
64. Koedel U, Klein M, Pfister HW (2010) Modulation of brain injury as a target of adjunctive therapy in bacterial meningitis. Curr Infect Dis Rep 12:266–273
65. Kastenbauer S, Koedel U, Becker BF, Pfister HW (2002) Oxidative stress in bacterial meningitis in humans. Neurology 58:186–191
66. Balla J, Vercellotti GM, Jeney V, Yachie A, Varga Z, Jacob HS, Eaton JW, Balla G (2007) Heme, heme oxygenase, and ferritin: how the vascular endothelium survives (and dies) in an iron-rich environment. Antioxid Redox Signal 9:2119–2137
67. Syapin PJ (2008) Regulation of haeme oxygenase-1 for treatment of neuroinflammation and brain disorders. Br J Pharmacol 155:623–640
68. Borchorst S, Møller K (2012) The role of dexamethasone in the treatment of bacterial meningitis: a systematic review. Acta Anaesthesiol Scand 56:1210–1221
69. Hoshino A, Saitoh M, Oka A, Okumura A, Kubota M, Saito Y, Takanashi J, Hirose S, Yamagata T, Yamanouchi H, Mizuguchi M (2012) Epidemiology of acute encephalopathy in Japan, with emphasis on the association of viruses and syndromes. Brain Dev 34:337–343
70. Wang GF, Li W, Li K (2010) Acute encephalopathy and encephalitis caused by influenza virus infection. Curr Opin Neurol 23:305–311
71. Morishima T, Togashi T, Yokota S, Okuno Y, Miyazaki C, Tashiro M, Okabe N, Collaborative Study Group on Influenza-Associated Encephalopathy in Japan (2002) Encephalitis and encephalopathy associated with an influenza epidemic in Japan. Clin Infect Dis 35:512–517
72. Kawashima H, Morichi S, Okumara A, Nakagawa S, Morishima T, Collaborating study group on influenza-associated encephalopathy in Japan (2012) National survey of pandemic influenza A (H1N1) 2009-associated encephalopathy in Japanese children. J Med Virol 84:1151–1156
73. Okumura A, Nakagawa S, Kawashima H, Morichi S, Muguruma T, Saito O, Fujimoto J, Toida C, Kuga S, Imamura T, Shimizu T, Kondo N, Morishima T (2013) Severe form of encephalopathy associated with 2009 pandemic influenza A (H1N1) in Japan. J Clin Virol 56:25–30
74. Kawada J, Kimura H, Ito Y, Hara S, Iriyama M, Yoshikawa T, Morishima T (2003) Systemic cytokine responses in patients with influenza-associated encephalopathy. J Infect Dis 188:690–869
75. Ichiyama T, Isumi H, Ozawa H, Matsubara T, Morishima T, Furukawa S (2003) Cerebrospinal fluid and serum levels of cytokines and soluble tumor necrosis factor receptor in influenza virus-associated encephalopathy. Scand J Infect Dis 35:59–61
76. Hosoya M, Nunoi H, Aoyama M, Kawasaki Y, Suzuki H (2005) Cytochrome c and tumor necrosis factor-alpha values in serum and cerebrospinal fluid of patients with influenza-associated encephalopathy. Pediatr Infect Dis J 24:467–470
77. Hasegawa S, Matsushige T, Inoue H, Shirabe K, Fukano R, Ichiyama T (2011) Serum and cerebrospinal fluid cytokine profile of patients with 2009 pandemic H1N1 influenza virus-associated encephalopathy. Cytokine 54:167–172
78. Kawashima H, Togashi T, Yamanaka G, Nakajima M, Nagai M, Aritaki K, Kashiwagi Y, Takekuma K, Hoshika A (2005) Efficacy of plasma exchange and methylprednisolone pulse therapy on influenza-associated encephalopathy. J Infect 51:E53–E56
79. Kawashima H, Morichi S, Okumara A, Nakagawa S, Morishima T, The Collaborating Study Group on Influenza-Associated Encephalopathy in Japan (2012) Treatment of pandemic influenza A (H1N1) 2009-associated encephalopathy in children. Scand J Infect Dis 44:941–947