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Review

Adjunctive therapies and immunomodulatory agents in the management of severe influenza

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ABSTRACT

In addition to neuraminidase inhibitors and other drugs that directly target viral replication, a number of adjunctive and immunomodulatory therapies are currently under evaluation for the treatment of influenza. These novel treatments, which focus either on pathophysiological aspects of influenza virus infection or the neutralization of virus with antibodies, are the subject of this review. Cytokine dysregulation has been observed in patients with severe influenza, such as avian influenza A (H5N1) and pandemic 2009 influenza A (H1N1pdm09) virus infections, but the role of immunomodulatory therapy is unclear, due to lack of data from randomized controlled trials (RCTs). Convalescent plasma appears to be useful as an adjunctive therapy for the treatment of H5N1 and H1N1pdm09 infections. Until lately, data interpretation was limited to case reports and studies of non-randomized design, but a recent RCT found that patients with severe influenza A (H1N1pdm09) who were treated with hyperimmune immunoglobulin from persons who had survived the same disease had a lower peak viral load and lower mortality than controls, providing treatment was begun within 5 days of symptom onset. The efficacy of agents with potential immunomodulating effects, including intravenous immunoglobulin, *N*-acetylcysteine, acute use of statins, macrolides, peroxisome proliferator-activated receptors agonists, celecoxib and mesalazine, and the role of plasmapheresis and hemoperfusion as rescue therapy, deserve more investigation and where feasible, studies by RCTs. Prospective observational studies have shown that systemic corticosteroids increase morbidity (e.g., secondary infections) and mortality in H1N1pdm09 influenza. This article forms part of a symposium in *Antiviral Research* on "Treatment of influenza: targeting the virus or the host."

Contents

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1. Introduction

Ever since the first human cases of highly pathogenic H5N1 avian influenza were described in Hong Kong in 1997 (Chan, 2002) and the virus unexpectedly re-appeared in 2003 (Peiris et al., 2004), it has remained a major emerging disease of global concern, with a case fatality rate of about 60% and the potential for causing another pandemic (Hui, 2008). In early 2009, the novel swine-origin influenza A (H1N1) virus (H1N1pdm09) was first identified in Mexico and the United States, and it spread within a few weeks through international travel into a pandemic (Bautista et al., 2010). A prospective observational study in Hong Kong of adults hospitalized for H1N1pdm09 infection showed significant complications and mortality, even though the patients were younger than those with seasonal influenza. Antiviral treatment with oseltamivir administered within 96 h of illness onset improved survival, but without timely treatment, the mortality risk was higher for H1N1pdm09 than for seasonal influenza [9.0% vs. 5.8%, respectively; adjusted odds ratio (OR), 6.85; 95% CI, 1.64–28.65; $P = 0.008$] (Lee et al., 2011a).

Respiratory failure is the major complication in patients hospitalized with severe influenza, and some patients progress rapidly to acute respiratory distress syndrome (ARDS) and multi-organ dysfunction (Chan, 2002; Peiris et al., 2004; Bautista et al., 2010; Hui, 2008; Hui et al., 2010; Lee et al., 2011a). Severe cytokine storm, with marked elevations of interleukin (IL)-6, CXCL8/IL-8, CXCL10/IP-10, CCL2/MCP-1, and CXCL9/MIG, has been detected in patients with severe influenza A(H5N1) infection, in association with high viral load (Peiris et al., 2004; de Jong et al., 2006). Cytokine dysregulation has also been observed in patients with H1N1pdm09 influenza, with higher plasma levels of pro-inflammatory IL-6, CXCL8/IL-8, CCL2/MCP-1, and soluble tumor necrosis factor receptor-1 (sTNFR1) detected in those with severe infection, compared to mild disease; high cytokine levels correlated with the progression of pneumonia (Lee et al., 2011b; To et al., 2010). Antiviral therapy with a neuraminidase inhibitor (NAI) can improve the clinical outcome if administered to patients hospitalized with seasonal (Lee et al., 2010) or H1N1pdm09 influenza (Lee et al., 2011a) within 4–5 days of illness onset, and may reduce mortality when started within 6–8 days of symptom onset in all age groups with influenza A(H5N1) (Adisasmito et al., 2010). However, the final outcome is often compromised by delay in patient presentation and initiation of therapy.

This article reviews the potential role of immunomodulatory agents and adjunctive therapies in the management of patients hospitalized with severe influenza. We first discuss treatments for which there is evidence of clinical benefit, then review those for which current data are insufficient to prove either a positive or a negative effect, and conclude by summarizing therapies that have been predominantly associated with a worsened clinical outcome.

2. Therapies with evidence of improved patient outcome

2.1. Convalescent plasma

Convalescent plasma therapy uses plasma from patients who have fully recovered from an infection to treat those with the same infection. One meta-analysis that reviewed reports from the 1918 influenza pandemic suggested that early administration of convalescent blood products reduced the risk of death from pneumonia (overall mortality reduced from 37% to 16%, 95% CI 15–27%) (Luke et al., 2006). Passive immunotherapy in the form of convalescent plasma was administered as an adjunctive treatment with a favorable outcome in a patient in China with severe A(H5N1) influenza pneumonia and multi-organ failure, who did not respond initially to high-dose of NAI oseltamivir (Zhou et al., 2007). Animal data have shown that administration of anti-H5N1 specific antibodies, in the form of neutralizing monoclonal antibodies or polyclonal sera (convalescent or post-immunization), is effective in treating influenza A(H5N1) disease (Lu et al., 2006; Hanson et al., 2006).

Evidence of a beneficial effect of immunoglobulin therapy has been obtained during the recent influenza H1N1pdm09 pandemic. A prospective multicenter case-control study ($n = 93$) in Hong Kong in 2009 showed that convalescent plasma with a neutralizing antibody titer of >1:160 was effective in reducing mortality, respiratory tract viral load, and serum cytokine levels in 20 patients with severe H1N1pdm09 infection requiring intensive care support (Hung et al., 2011). More recently, a randomized controlled trial (RCT) in patients with severe H1N1pdm09 infection in five hospitals in Hong Kong assessed the value of treatment with hyperimmune intravenous gammaglobulin (H-IVIG) prepared from plasma of persons who had recovered from the disease in comparison to treatment with normal IVIG manufactured before 2009 (Hung et al., 2013). Patients who were on standard antiviral therapy and required intensive care support were randomized to receive H-IVIG ($n = 17$) or IVIG ($n = 18$). The H-IVIG group had significantly lower day 5 and 7 viral load, and a multivariate analysis of the 22 patients who received either H-IVIG or IVIG within 5 days of symptom onset found that H-IVIG treatment was the only factor which independently reduced mortality (OR:0.14, 95% CI, 0.02–0.92; $p = 0.04$).

Comment: Provided suitable donors are available, passive immunotherapy with convalescent plasma or with hyperimmune globulin prepared from convalescent plasma is a potential option for the treatment of severe influenza.

2.2. Intravenous immunoglobulin (IVIG) preparations

IVIG contains concentrated globulin preparations made from pooled human plasma. In a study that analyzed prepandemic IVIG and sera from Kawasaki disease patients (who had received IVIG as the standard treatment for autoimmune vasculitis) for H1N1pdm09-specific antibodies by micro-neutralization and hemagglutination inhibition, investigators found that all six IVIG preparations tested had significant levels of cross-reactive specific antibody, at a concentration of 2.0 g/dL of immunoglobulin (Hong et al., 2011). Sera from 18 to 19 Kawasaki disease patients had significant increases in cross-reactive-specific antibody after receiving 2.0 g/kg of prepandemic IVIG. These data suggest a potential role for adjunctive IVIG therapy for severe influenza, especially in immunocompromised patients. A case report on a 59-year-old male patient with ARDS due to H1N1pdm09 influenza, who did not respond initially to twice-daily oseltamivir at a dose of 150 mg, described a favorable outcome following the administration of IVIG (Chong et al., 2011). Significant neutralizing activities against influenza A(H2N2) viruses have been observed in lots of human IVIG manufactured from 1993–2010 in Japan (Kubota-Koketsu et al., 2012).

Comment: The data suggest that evaluation of antibody titers may provide useful information about IVIG, which may be used for immunomodulation when a new influenza virus emerges in the human population. However, it is important to watch for thromboembolic complications, because when SARS patients were treated with IVIG in 2003, there were reports of thromboembolic disease despite the use of prophylactic low-molecular-weight heparin, which was likely related to increased blood viscosity (Lew et al., 2003; Chong et al., 2004; Umapathi et al., 2004).

3. Therapies of uncertain benefit

3.1. N-acetylcysteine (NAC)

N-acetylcysteine (NAC) is a modified form of the amino acid cysteine, with anti-oxidant properties. The production of reactive oxygen species (ROS) has been proposed as a mechanism of pulmonary damage caused by influenza virus infection (Han and Meydani, 2000). Leukocytes may be activated and primed by virus infection, producing ROS, and increased xanthine oxidase levels were found in influenza A virus-infected lungs (Akaike et al., 1990). Lung epithelial cells may also be a source of ROS, since influenza A virus infection induced an oxidant stress response in cultured airway epithelial cells (Knobil et al., 1998). NAC was shown to inhibit the production of pro-inflammatory molecules (CCL5, CXCL8, CXCL10, and IL6) in lung epithelial cells infected with the highly pathogenic influenza A (H5N1) virus (Geiler et al., 2010). High-dose NAC, administered at 100 mg/kg daily as a continuous IV infusion, appeared to be effective in reducing C-reactive protein and oxygen requirement and improving the clinical outcome in a 48-year-old, previously healthy woman, who had presented with severe pneumonia and septic shock due to H1N1pdm09 influenza. However, interpretation of the efficacy of NAC was limited by the concomitant use of NAI oseltamivir at 150 mg twice-daily (Lai et al., 2010).

Comment: More clinical data, based on RCTs are indicated to evaluate the role of NAC in the management of severe influenza.

3.2. Polymyxin B-immobilized fiber column hemoperfusion

The polymyxin B-immobilized fiber column, first introduced in Japan in 1994, is an extracorporeal device using polymyxin-B fixed to ⟨-chloroacetamide-methyl polystyrene-derived fibers, which can remove circulating endotoxin and reduce various cytokines through direct hemoperfusion (Takeda et al., 2010). In a 16-year-old girl with severe H1N1pdm09 influenza who did not initially respond to inhalation of dry powder NAI zanamivir 10 mg twice-daily, hypercytokinemia (elevated IL-6, IL-8, IFN-γ, and high-mobility group box-1) was successfully reduced with column hemoperfusion, resulting in a favorable clinical outcome (Takeda et al., 2010).

Comment: It is difficult to judge the effect of polymyxin B-immobilized fiber column hemoperfusion, due to the concomitant use of oseltamivir and Sivelestat (an inhibitor of human neutrophil elastase) in this patient.

3.3. Therapeutic plasma exchange

Therapeutic plasma exchange (TPE) or plasmapheresis is an extracorporeal blood purification technique designed to remove large-molecular-weight substances from the plasma. A pediatric case series described three children (aged 8, 11, and 17 years) with severe H1N1pdm09 influenza complicated by ARDS and hemodynamic instability who required invasive mechanical ventilation and inhaled nitric oxide, and one received extracorporeal membrane oxygenation (Patel et al., 2011). TPE was provided as a rescue strategy to these patients with three exchanges of 35–40 mL/kg on consecutive days. There was a dramatic subsequent reduction in blood lactate levels, oxygen requirement, inotropic support and pediatric logistic organ dysfunction scores. All patients survived with good functional recovery.

Comment: The case series suggests a role for TPE as a strategy for cytokine attenuation in severe shock and acute lung injury related to H1N1pdm09 influenza that is not responsive to traditional therapy. However, a further controlled study with a larger sample size is needed.

3.4. Statins

Statins are competitive inhibitors of the enzyme HMG-CoA reductase. Based on some experimental evidence of anti-inflammatory and immunomodulatory effects (e.g., by repressing the induction of MHC-II by IFN-γ and subsequent T-lymphocyte activation) and observational studies showing survival benefits in patients receiving statins who developed bacteremia, sepsis, or CAP, statins have been proposed to play a role in the prevention and treatment of pandemic influenza (Fedson, 2009).

In BALB/c mice infected with H5N1, H3N2 or H1N1 influenza virus, the combination of 50 g statin + 200 g caffeine ameliorated lung damage and inhibited viral replication, and appeared to be at least as effective as oseltamivir and ribavirin (Liu et al., 2009). However, the statin/caffeine combination seemed to be more effective when administered preventatively, rather than as treatment. These findings provide justification for further research into this novel antiviral formulation (Liu et al., 2009). However, in a murine model of influenza A virus infection, the administration of rosuvastatin had no effect on viral clearance after infection or on mortality (Radigan et al., 2012).

Epidemiologic studies of patients who were taking statins when they developed influenza have given conflicting results:

1. In a population-based cohort study over 10 influenza seasons (1996–2006) in Ontario, Canada with propensity-based matching, the chronic use of statins showed small protective effects against pneumonia hospitalization ([OR] 0.92; 95% CI 0.89–0.95), 30 day pneumonia mortality (0.84; 95% CI 0.77–0.91), and all-cause mortality (0.87; 95% CI 0.84–0.89). However, these positive effects were reduced substantially following multivariate adjustment for confounding factors (Kwong et al., 2009).
2. In the United Kingdom Influenza Clinical Information Network database of 1520 patients hospitalized with H1N1pdm09 influenza from April, 2009 to January, 2010, a retrospective case-control study revealed no statistically significant association between pre-admission statin use and the severity of outcome in patients aged $\geqslant$35 years [adjusted OR: 0.81 (95% CI 0.46–1.38); $n = 571$]. Following adjustment for age, sex, obesity and indication for statins, there was no statistically significant association between pre-admission statin use and the severity of outcome (Brett et al., 2011).
3. In a study of hospitalized adults in 10 states of the USA during the 2007–08 influenza season, which was analyzed to evaluate the association between receiving statins and influenza-related death, treatment before or during hospitalization was associated with a protective adjusted odds-of-death of 0.59 (95% CI 0.38–0.92), following adjustment for age, race, comorbid diseases, influenza vaccination and antiviral administration (Vandermeer et al., 2012).

Comment: As there are currently no data from RCTs on the acute use of statins in the management of severe influenza, and the drugs are relatively cheap and readily available, further research is warranted on their role as an adjunctive therapy, in animal models or in human clinical studies.

3.5. Macrolides

Macrolides belong to a class of organic compounds that contain a large lactone ring made up of twelve or more members, and act by inhibiting protein synthesis. They are produced by actinomycete bacteria of the genus *Streptomyces*. Macrolide antibiotics such as clarithromycin and azithromycin are known to possess anti-inflammatory effects and immunomodulatory properties, in addi-

tion to their antibacterial actions (Zarogoulidis et al., 2012). Evidence of a beneficial effect has been seen for influenza, respiratory syncytial virus (RSV) and rhinovirus infections.

In cultured human tracheal epithelial cells infected with influenza A (H3N2) virus, clarithromycin treatment reduced viral titers and cytokines in supernatant fluids, viral RNA in the cells, and susceptibility to influenza virus infection (Yamaya et al., 2010). It also reduced expression of sialic acid α2, 6Gal on the tracheal mucosal surface, and the number and fluorescence intensity of acidic endosomes in the cells, from which viral RNPs enter the cytoplasm (Yamaya et al., 2010). There are in vitro data showing that clarithromycin can act on a middle to late stage of the viral replication cycle, inhibiting progeny influenza virus production (Miyamoto et al., 2008). However, in a RCT of young patients in Japan with mild seasonal influenza A who had received early NAI therapy, the addition of clarithromycin did not result in a better outcome, apart from a shorter duration of cough in patients who were without cough at the onset of pyrexia (Ishii et al., 2012). In respiratory syncytial virus bronchiolitis, treatment with clarithromycin had beneficial effects on the length of hospital stay, the duration of need for supplemental oxygen and the need for beta(2)-agonist treatment, and there were significant decreases in plasma IL-4, IL-8 and eotaxin levels after 3 weeks of treatment. Readmission to hospital within 6 months after discharge was also significantly lower in the clarithromycin group (Tahan et al., 2007).

Azithromycin had anti-rhinoviral activity in bronchial epithelial cells, and during infection, it significantly increased rhinovirus-1B and rhinovirus-16-induced interferons and interferon-stimulated gene mRNA expression and protein production. Treatment also significantly reduced viral replication and release (Gielen et al., 2010). In a mouse model of parainfluenza bronchiolitis, azithromycin attenuated acute and chronic airway inflammation (Beigelman et al., 2010).

Comment: These findings demonstrate anti-inflammatory effects of clarithromycin and azithromycin that are not related to antiviral activity. More data based on RCTs are needed on the role of macrolides in patients hospitalized with severe influenza.

3.6. Peroxisome proliferator-activated receptors agonists

The PPAR agonists are a group of medications that act on the peroxisome proliferator-activated receptor. Apart from its clinically useful lipid-lowering activity, there is evidence that gemfibrozil (a PPARα agonist) can inhibit production of pro-inflammatory cytokines.

Survival in BALB/c mice infected with influenza A/Japan/305/57 (H2N2) virus increased from 26% in vehicle-treated mice ($n = 50$) to 52% in mice given gemfibrozil intraperitoneally at 60 mg/kg once daily ($n = 46$) from days 4–10 following intranasal infection (Budd et al., 2007). In a study examining the effects of systemic corticosteroids (dexamethasone) versus the PPARγ agonist pioglitazone on the outcome of influenza, C57BL/6 mice were exposed to room air or cigarette smoke for 4 days, then inoculated with an H1N1 virus, to mimic influenza and COPD. Smoke-exposed mice were noted to have an exacerbated inflammatory response following infection. Dexamethasone treatment reduced mononuclear cells in the broncho-alveolar lavage (BAL) of smoke-exposed, virus-infected mice, while pioglitazone reduced mononuclear cells and neutrophils in the BAL and increased peripheral CD4+ and CD8+ T cells (Bauer et al., 2010).

In another study of mice challenged with virulent influenza A viruses, including currently circulating avian H5N1 strains, an increased selective accumulation of a particular dendritic cell (DC) subset, the TNF/iNOS-producing (tip) DCs, was noted in airways of mice with pneumonia (Aldridge et al., 2009). These tipDCs appear to be required for the further proliferation of influenza-specific CD8+ T cells in the infected lung, because blocking their recruitment in CCR2-knockout mice decreased the numbers of CD8+ effectors and reduced virus clearance. However, the investigators found that treatment with the PPARγ agonist pioglitazone reduced tipDC trafficking, and concluded that this might moderate the potentially lethal consequences of excessive tipDC recruitment, without abrogating CD8+ T cell expansion or compromising virus control (Aldridge et al., 2009).

Comment: These studies suggest that alternative anti-inflammatory drugs such as PPARγ agonists should be further explored for the treatment of severe viral infections such as influenza.

3.7. Combination of celecoxib and mesalazine

The effect of delayed NAI therapy in combination with immunomodulatory agents was assessed in BALB/c mice challenged with highly pathogenic influenza A/Vietnam/1194 /2004 (H5N1) virus (Zheng et al., 2008). Drug treatment was deliberately initiated at 48 h after viral challenge, to imitate a real-life scenario. Survival rates and survival time were improved in the group that received combinations of zanamivir, celecoxib and mesalazine, compared to zanamivir alone. Significantly higher levels of CD4+ and CD8+ T cells and less pulmonary inflammation were also noted in the group receiving the triple therapy. Zanamivir alone reduced viral load, but without any significant effect on lung inflammation or mortality. Celecoxib is well known as a cyclooxygenase-2 (COX-2) inhibitor. Mesalazine can inhibit both lipoxygenase and COX pathways, leading to reductions in pro-inflammatory cytokines and eicosanoids, and therefore deactivation of inflammatory cells such as macrophages and neutrophils. Mesalazine also inhibits NF-κB activation and promotes phosphatidic acid synthesis, inhibiting the stimulatory effects of ceramides on apoptosis (Zheng et al., 2008).

Comment: More clinical studies are needed on the therapeutic role of these immunomodulating agents in severe influenza.

4. Therapies with evidence of worsened patient outcome

4.1. Systemic corticosteroids

Data from several observational studies have shown that systemic corticosteroid therapy was associated with a higher risk of death, an increased rate of nosocomial pneumonia and an increased risk of developing critical disease in patients hospitalized with H1N1pdm09 influenza, especially those with initiation of oseltamivir after day 3–4 of illness, or who did not receive NAI therapy:

1. A prospective observational study of 147 adults hospitalized with seasonal influenza A(H3N2) in Hong Kong showed that systemic corticosteroids, which had been administered for acute exacerbations of either chronic obstructive pulmonary disease (COPD) or asthma, was an independent risk factor (adjusted OR 5.44, 95% CI 1.86–15.89) associated with delayed viral clearance beyond the first week of illness (Lee et al., 2009).
2. In the European Society of Intensive Care H1N1pdm09 influenza cohort ($n = 220$), there was a delay of NAI therapy (mainly oseltamivir) of an average of 4–5 days from influenza-like illness onset, and 67 patients (30.5%) died. Those who received early systemic corticosteroids ($n = 126$) were older and more likely to have coexisting asthma, COPD, or chronic steroid use. After adjusting for severity and potential confounding factors, Cox regression analysis showed that early use of systemic corticosteroids was not significantly associated with mortality [hazard ratio (HR) 1.3, 95% CI 0.7–2.4, $p = 0.4$], but systeic ste-

roid therapy was associated with an increased rate of hospital-acquired pneumonia (HAP) (OR 2.2, 95% CI 1.0–4.8, $p < 0.05$) (Martin-Loeches et al., 2011).

3. Of the 208 patients with ARDS in the French H1N1pdm09 ICU Registry, 83 (40%) received systemic corticosteroids for ARDS, in a median initial dose of 270 mg of hydrocortisone daily, for a median of 11 days. Systemic corticosteroid therapy was associated with a fatal outcome, both in crude analysis (33.7 vs. 16.8%; HR, 2.4; 95% CI, 1.3–4.3; $P = 0.004$) and after propensity score-adjusted analysis (aHR, 2.82; 95% CI, 1−5.4; $P = 0.002$), more hospital-acquired pneumonia and a trend towards a greater duration of ventilation (Brun-Buisson et al., 2011).
4. In the Korean cohort of critically ill patients with H1N1pdm09 infection ($N = 245$), NAI (mainly oseltamivir) was given at mean 4.5 ± 4.0 days from illness onset, whereas 107 (44%) received adjuvant systemic corticosteroids treatment for ARDS. Systemic steroid treatment was associated with an increased 90 day mortality, when independent predictors and propensity score were considered (adjusted OR, 2.20; 95% CI, 1.03–4.71). The patients were also more likely to develop secondary bacterial pneumonia or invasive fungal infections, and had more prolonged ICU stays than the no-steroid group (Kim et al., 2011).
5. Among 83 hospitalized patients with H1N1pdm09 infection in four Chinese hospitals, there was a median delay of 5 days from the onset of influenza-like illness to hospital admission; 46% developed critical illness, of whom 17% died and 37% recovered. Of 17 patients who received glucocorticoid therapy beginning less than 72 h after illness onset, 71% subsequently developed critical disease, as compared to 39% of 66 patients who received late or no glucocorticoids (risk ratio by Mantel–Haenszel testing = 1.8, 95% CI 1.2–2.8, after adjusting for underlying diseases and risk factors). Proportional hazards modeling showed that use of glucocorticoids tripled the hazard of developing critical illness (Han et al., 2011).
6. In contrast, a retrospective review of Japanese patients in a national hospital, who had received NAI mostly within 2 days of illness onset, showed that the administration of systemic corticosteroids for H1N1pdm09-related pneumonia with acute wheezing was not associated with an adverse outcome (Kudo et al., 2012).

The 2003 SARS outbreak provides some important lessons as regards the widespread use of systemic corticosteroids and related complications, which may have implications for the management of severe influenza. Systemic corticosteroids were widely used in SARS patients with progressive respiratory failure, based on evidence of bronchiolitis obliterans organizing pneumonia (Lee et al., 2003; Tse et al., 2004). One uncontrolled study showed a favorable clinical response with reduction of inflammatory cytokines (Wong et al., 2004). However, in a study of hospitalized patients randomized to receive either early intravenous hydrocortisone or normal saline, plasma levels of SARS-CoV RNA were considerably higher during weeks 2–3 of illness among those who had received hydrocortisone, suggesting that systemic corticosteroids might prolong viremia (Lee et al., 2004).

Invasive fungal infection with fatal outcome occurred in patients with SARS (Wang et al., 2003) and H1N1pdm09 (Lat et al., 2010) who had received systemic corticosteroids for ARDS, whereas higher rate of ventilator-associated pneumonia during the SARS period was observed than before and after the SARS outbreak (Yap et al., 2004). Osteonecrosis occurred in patients who had received systemic corticosteroids for SARS (Griffith et al., 2005; Hong and Du, 2004); the risk was 0.6% for a cumulative dose of <3 g versus 13% for >3 g of cumulative prednisolone equivalent dose (Griffith et al., 2005).

Although numerous RCTs have suggested that systemic corticosteroids improve the clinical outcome in patients hospitalized with community-acquired pneumonia (CAP) (Nie et al., 2012), it must be pointed out that these studies included predominantly CAP cases with bacterial that had received appropriate antibiotic therapy; the favorable results therefore cannot be generalized to CAP with viral (Lee and Hui, 2011).

Comments: These data call for caution against the use of systemic corticosteroids in managing patients with severe viral pneumonia, including influenza, especially when there is a delay or lack of effective antiviral therapy. However, low-dose systemic corticosteroids may be considered in the treatment of refractory septic shock related to severe influenza, as recommended by the World Health Organization (Bautista et al., 2010).

5. Conclusion

Convalescent plasma appears to be useful as an adjunctive therapy for influenza A (H5N1) and H1N1pdm09 infections, but it is limited by its availability and timing of administration. Until recently, interpretation of the efficacy of convalescent plasma was

Table 1
General comments related to adjunctive therapies and immunomodulatory agents for the treatment of severe influenza.

	Therapeutic approach	Summary of findings
I. Agents with some evidence of patient benefit	Passive immunotherapy such as convalescent plasma and hyperimmune immunoglobulin	Case reports, a non-randomized study and a RCT have shown benefit if given early in the disease course. Efficacy may be limited by the availability of donors and timing of administration.
	IVIG	May have neutralizing activities against influenza viruses but caution with thromboembolic side effects.
II. Agents of uncertain value	*N*-acetylcysteine	Data limited to a case report related to H1N1pdm09 influenza and in vitro testing.
	PPAR agonists	Data limited to animal studies.
	Macrolides	Favorable in vitro data but limited human data for influenza.
	Statins	Cheap and readily available. Conflicting epidemiological data for outcome of influenza in chronic users. No data on acute use of statins for severe influenza.
	Combination of Cox II inhibitors and mesalazine	Data limited to animal studies.
	Plasmapharesis	May play a role as rescue therapy but need more data than case series.
	Haemoperfusion	May play a role as rescue therapy but need more data than a case report.
III. Agents with evidence of harm	Systemic corticosteroids	The risks of mortality and morbidity (e.g. secondary infections) were increased by administration of systemic corticosteroids in severe H1N1pdm09 influenza, especially when there was delay or lack of effective antiviral therapy. Systemic corticosteroids may prolong viremia

limited by data based on case reports and studies of non-randomized design. However, a newly published RCT has shown that patients with severe H1N1pdm09 influenza who received hyperimmune-IVIG had a lower viral load and a lower risk of death, if treatment was begun within 5 days of symptom onset. Evaluation of antibody titers may provide useful information about IVIG, which may be used for immunomodulation when a new influenza virus emerges in the human population. More animal experiments and detailed human observational studies and (where feasible) RCTs are needed to evaluate the efficacy of other agents with potential immunomodulatory effects, such as the acute use of statins, NAC, macrolides, PPAR agonists, celecoxib and mesalazine. In particular, the role of plasmapheresis and hemoperfusion as rescue therapy requires more investigation (Table 1). Systemic corticosteroids may increase the risk of mortality and morbidity, including the hospital length of stay and secondary bacterial or fungal infections in patients with severe influenza, especially when there is a delay or lack of effective antiviral therapy.

References

Adisasmito, W., Chan, P.K., Lee, N., Oner, A.F., Gasimov, V., Zaman, M., Bamgboye, E., Dogan, N., Starzyk, K., Dreyer, N.A., Toovey, S., 2010. Effectiveness of antiviral treatment in human influenza A(H5N1) infections: analysis of a global patient registry. J. Infect. Dis. 202, 1154–1160.

Akaike, T., Ando, M., Oda, T., Doi, T., Ijiri, S., Araki, S., Maeda, H., 1990. Dependence on O2-generation by xanthine oxidase of pathogenesis of influenza virus infection in mice. J. Clin. Invest. 85, 739–745.

Aldridge Jr., J.R., Moseley, C.E., Boltz, D.A., Negovetich, N.J., Reynolds, C., Franks, J., Brown, S.A., Doherty, P.C., Webster, R.G., Thomas, P.G., 2009. TNF/iNOS-producing dendritic cells are the necessary evil of lethal influenza virus infection. Proc. Natl. Acad. Sci. USA 106, 5306–5311.

Bauer, C.M., Zavitz, C.C., Botelho, F.M., Lambert, K.N., Brown, E.G., Mossman, K.L., Taylor, J.D., Stämpfli, M.R., 2010. Treating viral exacerbations of chronic obstructive pulmonary disease: insights from a mouse model of cigarette smoke and H1N1 influenza infection. PLoS One 5, e13251.

Bautista, E., Chotpitayasunondh, T., Gao, Z.C., Harper, S.A., Hayden, F.G., Hui, D.S., Kettner, J.D., Kumar, A., Lim, M., Nicholson, K.G., Penn, C., Shaw, M., Shindo, N., Uyeki, T.M., 2010. Clinical aspects of pandemic influenza a (H1N1) 2009. N. Engl. J. Med. 362, 1708–1719.

Beigelman, A., Mikols, C.L., Gunsten, S.P., Cannon, C.L., Brody, S.L., Walter, M.J., 2010. Azithromycin attenuates airway inflammation in a mouse model of viral bronchiolitis. Respir. Res. 11, 90.

Brett, S.J., Myles, P., Lim, W.S., Enstone, J.E., Bannister, B., Semple, M.G., Read, R.C., Taylor, B.L., McMenamin, J., Nicholson, K.G., Nguyen-Van-Tam, J.S., Openshaw, P.J., Influenza Clinical Information Network (FLU-CIN), 2011. Pre-admission statin use and in-hospital severity of pandemic influenza A(H1N1) disease. PLoS One 6, e18120.

Brun-Buisson, C., Richard, J.C., Mercat, A., Thiébaut, A.C., Brochard, L., REVA-SRLF A/H1N1v 2009 Registry group, 2011. Early corticosteroids in severe influenza A/H1N1 pneumonia and acute respiratory distress syndrome. Am. J. Respir. Crit. Care Med. 183, 1200–1206.

Budd, A., Alleva, L., Alsharifi, M., Koskinen, A., Smythe, V., Müllbacher, A., Wood, J., Clark, I., 2007. Increased survival after gemfibrozil treatment of severe mouse influenza. Antimicrob. Agents Chemother. 51, 2965–2968.

Chan, P.K., 2002. Outbreak of avian influenza A(H5N1) virus infection in Hong Kong in 1997. Clin. Infect. Dis. 34 (Suppl. 2), S58–S64.

Chong, P.Y., Chui, P., Ling, A.E., Franks, T.J., Tai, D.Y., Leo, Y.S., Kaw, G.J., Wansaicheong, G., Chan, K.P., Ean Oon, L.L., Teo, E.S., Tan, K.B., Nakajima, N., Sata, T., Travis, W.D., 2004. Analysis of deaths during the severe acute respiratory syndrome (SARS) epidemic in Singapore: challenges in determining a SARS diagnosis. Arch. Pathol. Lab. Med. 128, 195–204.

Chong, J.L., Sapari, S., Kuan, Y.C., 2011. A case of acute respiratory distress syndrome associated with novel H1N1 treated with intravenous immunoglobulin G. J. Microbiol. Immunol. Infect. 44, 319–322.

de Jong, M.D., Simmons, C.P., Thanh, T.T., Hien, V.M., Smith, G.J., Chau, T.N., Hoang, D.M., Chau, N.V., Khanh, T.H., Dong, V.C., Qui, P.T., Cam, B.V., Ha do, Q., Guan, Y., Peiris, J.S., Chinh, N.T., Hien, T.T., Farrar, J., 2006. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. Nat. Med. 12, 1203–1207.

Fedson, D.S., 2009. Confronting the next influenza pandemic with anti-inflammatory and immunomodulatory agents: why they are needed and how they might work. Influenza Other Respi. Viruses 3, 129–142.

Geiler, J., Michaelis, M., Naczk, P., Leutz, A., Langer, K., Doerr, H.W., Cinatl Jr., J., 2010. *N*-acetyl-l-cysteine (NAC) inhibits virus replication and expression of pro-inflammatory molecules in A549 cells infected with highly pathogenic H5N1 influenza A virus. Biochem. Pharmacol. 79, 413–420.

Gielen, V., Johnston, S.L., Edwards, M.R., 2010. Azithromycin induces anti-viral responses in bronchial epithelial cells. Eur. Respir. J. 36, 646–654.

Griffith, J.F., Antonio, G.E., Kumta, S.M., Hui, D.S., Wong, J.K., Joynt, G.M., Wu, A.K., Cheung, A.Y., Chiu, K.H., Chan, K.M., Leung, P.C., Ahuja, A.T., 2005. Osteonecrosis of hip and knee in patients with severe acute respiratory syndrome treated with steroids. Radiology 235, 168–175.

Han, S.N., Meydani, S.N., 2000. Antioxidants, cytokines, and influenza infection in aged mice and elderly humans. J. Infect. Dis. 182 (Suppl. 1), S74–S80 (Review).

Han, K., Ma, H., An, X., Su, Y., Chen, J., Lian, Z., Zhao, J., Zhu, B.P., Fontaine, R.E., Feng, Z., Zeng, G., 2011. Early use of glucocorticoids was a risk factor for critical disease and death from pH1N1 infection. Clin. Infect. Dis. 53, 326–333.

Hanson, B., Boon, A.C., Lim, A.P., Webb, A., Ooi, E.E., Webby, R.J., 2006. Passive immunoprophylaxis and therapy with humanized monoclonal antibody specific for influenza A H5 hemagglutinin in mice. Respir. Res. 7, 126.

Hong, N., Du, X.K., 2004. Avascular necrosis of bone in severe acute respiratory syndrome. Clin. Radiol. 59, 602–608.

Hong, D.K., Tremoulet, A.H., Burns, J.C., Lewis, D.B., 2011. Cross-reactive neutralizing antibody against pandemic 2009 H1N1 influenza A virus in intravenous immunoglobulin preparations. Pediatr. Infect. Dis. J. 30, 67–69.

Hui, D.S., 2008. Review of clinical symptoms and spectrum in humans with influenza A/H5N1 infection. Respirology 13 (Suppl. 1), S10–S13.

Hui, D.S., Lee, N., Chan, P.K., 2010. Clinical management of pandemic (H1N1) infection. Chest 137, 916–925.

Hung, I.F., To, K.K., Lee, C.K., Lee, K.L., Chan, K., Yan, W.W., Liu, R., Watt, C.L., Chan, W.M., Lai, K.Y., Koo, C.K., Buckley, T., Chow, F.L., Wong, K.K., Chan, H.S., Ching, C.K., Tang, B.S., Lau, C.C., Li, I.W., Liu, S.H., Chan, K.H., Lin, C.K., Yuen, K.Y., 2011. Convalescent plasma treatment reduced mortality in patients with severe pandemic influenza A (H1N1) 2009 virus infection. Clin. Infect. Dis. 52, 510–519.

Hung, I.F., To, K.K., Lee, C.K., Lee, K.L., Yan, W.W., Chan, K., Chan, W.M., Ngai, C.W., Law, K.I., Chow, F.L., Liu, R., Lai, K.Y., Lau, C.C., Liu, S.H., Chan, K.H., Lin, C.K., Yuen, K.Y., 2013. Hyperimmune intravenous immunoglobulin treatment: a multicentre double-blind randomized controlled trial for patients with severe A(H1N1)pdm09 infection. Chest 28 (Feb). http://dx.doi.org/10.1378/chest.12-2907 (Epub ahead of print).

Ishii, H., Komiya, K., Yamagata, E., Yatera, K., Chojin, Y., Yamamoto, H., Mukae, H., Kadota, J., 2012. Clarithromycin has limited effects in non-elderly, non-severe patients with seasonal influenza virus A infection. J. Infect. 64, 343–345.

Kim, S.H., Hong, S.B., Yun, S.C., Choi, W.I., Ahn, J.J., Lee, Y.J., Lee, H.B., Lim, C.M., Koh, Y., Korean Society of critical care medicine H1N1 collaborative, 2011. Corticosteroid treatment in critically ill patients with pandemic influenza A/H1N1 2009 infection: analytic strategy using propensity scores. Am. J. Respir. Crit. Care Med. 183, 1207–1214.

Knobil, K., Choi, A.M., Weigand, G.W., Jacoby, D.B., 1998. Role of oxidants in influenza virus-induced gene expression. Am. J. Physiol. 274, L134–142 (1 Pt 1).

Kubota-Koketsu, R., Yunoki, M., Okuno, Y., Ikuta, K., 2012. Significant neutralizing activities against H2N2 influenza A viruses in human intravenous immunoglobulin lots manufactured from 1993 to 2010. Biologics 6, 245–247.

Kudo, K., Takasaki, J., Manabe, T., Uryu, H., Yamada, R., Kuroda, E., Kobayashi, N., Matsushita, T., 2012. Systemic corticosteroids and early administration of antiviral agents for pneumonia with acute wheezing due to influenza A(H1N1)pdm09 in Japan. PLoS One 7 (2), e32280.

Kwong, J.C., Li, P., Redelmeier, D.A., 2009. Influenza morbidity and mortality in elderly patients receiving statins: a cohort study. PLoS One 4 (11), e8087.

Lai, K.Y., Ng, W.Y., Osburga Chan, P.K., Wong, K.F., Cheng, F., 2010. High-dose N-acetylcysteine therapy for novel H1N1 influenza pneumonia. Ann. Intern. Med. 152, 687–688.

Lat, A., Bhadelia, N., Miko, B., Furuya, E.Y., Thompson 3rd., G.R., 2010. Invasive aspergillosis after pandemic (H1N1) 2009. Emerg. Infect. Dis. 16, 971–973.

Lee, N., Hui, D.S., 2011. Dexamethasone in community-acquired pneumonia. Lancet 378, 979–980.

Lee, N., Hui, D.S., Wu, A., Chan, P., Cameron, P., Joynt, G.M., Ahuja, A., Yung, M.Y., Leung, C.B., To, K.F., Lui, S.F., Szeto, C.C., Chung, S., Sung, J.J., 2003. A major outbreak of severe acute respiratory syndrome in Hong Kong. N. Engl. J. Med. 348, 1986–1994.

Lee, N., Allen Chan, K.C., Hui, D.S., Ng, E.K., Wu, A., Chiu, R.W., Wong, V.W., Chan, P.K., Wong, K.T., Wong, E., Cockram, C.S., Tam, J.S., Sung, J.J., Lo, Y.M., 2004. Effects of early corticosteroid treatment on plasma SARS-associated Coronavirus RNA concentrations in adult patients. J. Clin. Virol. 31, 304–309.

Lee, N., Chan, P.K., Hui, D.S., Rainer, T.H., Wong, E., Choi, K.W., Lui, G.C., Wong, B.C., Wong, R.Y., Lam, W.Y., Chu, I.M., Lai, R.W., Cockram, C.S., Sung, J.J., 2009. Viral loads and duration of viral shedding in adult patients hospitalized with influenza. J. Infect. Dis. 200, 492–500.

Lee, N., Choi, K.W., Chan, P.K., Hui, D.S., Lui, G.C., Wong, B.C., Wong, R.Y., Sin, W.Y., Hui, W.M., Ngai, K.L., Cockram, C.S., Lai, R.W., Sung, J.J., 2010. Outcomes of adults hospitalized with severe influenza. Thorax 65, 510–515.

Lee, N., Chan, P.K., Lui, G.C., Wong, B.C., Sin, W.W., Choi, K.W., Wong, R.Y., Lee, E.L., Yeung, A.C., Ngai, K.L., Chan, M.C., Lai, R.W., Yu, A.W., Hui, D.S., 2011a. Complications and outcomes of pandemic 2009 influenza A (H1N1) in hospitalized adults – how are they different from seasonal influenza? J. Infect. Dis. 203, 1739–1747.

Lee, N., Chan, P.K., Wong, C.K., Wong, K.T., Choi, K.W., Joynt, G.M., Lam, P., Chan, M.C., Wong, B.C., Lui, G.C., Sin, W.W., Wong, R.Y., Lam, W.Y., Yeung, A.C., Leung, T.F., So, H.Y., Yu, A.W., Sung, J.J., Hui, D.S., 2011b. Viral clearance and inflammatory response patterns in adults hospitalized for pandemic 2009 influenza A(H1N1)Virus pneumonia. Antivir. Ther. 16, 237–247.

Lew, T.W., Kwek, T.K., Tai, D., Earnest, A., Loo, S., Singh, K., Kwan, K.M., Chan, Y., Yim, C.F., Bek, S.L., Kor, A.C., Yap, W.S., Chelliah, Y.R., Lai, Y.C., Goh, S.K., 2003. Acute

respiratory distress syndrome in critically ill patients with severe acute respiratory syndrome. JAMA 290, 374–380.
Liu, Z., Guo, Z., Wang, G., Zhang, D., He, H., Li, G., Liu, Y., Higgins, D., Walsh, A., Shanahan-Prendergast, L., Lu, J., 2009. Evaluation of the efficacy and safety of a statin/caffeine combination against H5N1, H3N2 and H1N1 virus infection in BALB/c mice. Eur. J. Pharm. Sci. 38, 215–223.
Lu, J., Guo, Z., Pan, X., Wang, G., Zhang, D., Li, Y., Tan, B., Ouyang, L., Yu, X., 2006. Passive immunotherapy for influenza A H5N1 virus infection with equine hyperimmune globulin F(ab′)2 in mice. Respir. Res. 7, 43.
Luke, T., Kilbane, E., Jackson, J., Hoffman, S., 2006. Meta-analysis: convalescent blood products for Spanish influenza pneumonia: a future H5N1 treatment? Ann. Intern. Med. 145, 599–609.
Martin-Loeches, I., Lisboa, T., Rhodes, A., Moreno, R.P., Silva, E., Sprung, C., Chiche, J.D., Barahona, D., Villabon, M., Balasini, C., Pearse, R.M., Matos, R., Rello, J., ESICM H1N1 Registry Contributors, 2011. Use of early corticosteroid therapy on ICU admission in patients affected by severe pandemic (H1N1)v influenza A infection. Intensive Care Med. 37, 272–283.
Miyamoto, D., Hasegawa, S., Sriwilaijaroen, N., Yingsakmongkon, S., Hiramatsu, H., Takahashi, T., Hidari, K., Guo, C.T., Sakano, Y., Suzuki, T., Suzuki, Y., 2008. Clarithromycin inhibits progeny virus production from human influenza virus-infected host cells. Biol. Pharm. Bull. 31, 217–222.
Nie, W., Zhang, Y., Cheng, J., Xiu, Q., 2012. Corticosteroids in the treatment of community-acquired pneumonia in adults: a meta-analysis. PLoS One 7, e47926.
Patel, P., Nadwani, V., Vanchiere, J., Conrad, S.A., Scott, L.K., 2011. Use of therapeutic plasma exchange as a rescue therapy in 2009 pH1N1 influenza-An associated respiratory failure and hemodynamic shock. Pediatr. Crit. Care Med. 12, e87–9.
Peiris, J.S., Yu, W.C., Leung, C.W., Cheung, C.Y., Ng, W.F., Nicholls, J.M., Ng, T.K., Chan, K.H., Lai, S.T., Lim, W.L., Yuen, K.Y., Guan, Y., 2004. Re-emergence of fatal human influenza A subtype H5N1 disease. Lancet 363, 617–619.
Radigan, K.A., Urich, D., Misharin, A.V., Chiarella, S.E., Soberanes, S., Gonzalez, A., Perlman, H., Wunderink, R.G., Budinger, G.R., Mutlu, G.M., 2012. The effect of rosuvastatin in a murine model of influenza A infection. PLoS One 7, e35788.
Tahan, F., Ozcan, A., Koc, N., 2007. Clarithromycin in the treatment of RSV bronchiolitis: a double-blind, randomised, placebo-controlled trial. Eur. Respir. J. 29, 91–97.
Takeda, S., Munakata, R., Abe, S., Mii, S., Suzuki, M., Kashiwada, T., Azuma, A., Yamamoto, T., Gemma, A., Tanaka, K., 2010. Hypercytokinemia with 2009 pandemic H1N1 (pH1N1) influenza successfully treated with polymyxin B-immobilized fiber column hemoperfusion. Intensive Care Med. 36, 906–907.
To, K.K., Hung, I.F., Li, I.W., Lee, K.L., Koo, C.K., Yan, W.W., Liu, R., Ho, K.Y., Chu, K.H., Watt, C.L., Luk, W.K., Lai, K.Y., Chow, F.L., Mok, T., Buckley, T., Chan, J.F., Wong, S.S., Zheng, B., Chen, H., Lau, C.C., Tse, H., Cheng, V.C., Chan, K.H., Yuen, K.Y., 2010. Delayed clearance of viral load and marked cytokine activation in severe cases of pandemic H1N1 2009 influenza virus infection. Clin. Infect. Dis. 50, 850–859.
Tse, G.M., To, K.F., Chan, P.K., Lo, A.W., Ng, K.C., Wu, A., Lee, N., Wong, H.C., Mak, S.M., Chan, K.F., Hui, D.S., Sung, J.J., Ng, H.K., 2004. Pulmonary pathological features in coronavirus associated severe acute respiratory syndrome (SARS). J. Clin. Pathol. 57, 260–265.
Umapathi, T., Kor, A.C., Venketasubramanian, N., Lim, C.C., Pang, B.C., Yeo, T.T., Lee, C.C., Lim, P.L., Ponnudurai, K., Chuah, K.L., Tan, P.H., Tai, D.Y., Ang, S.P., 2004. Large artery ischemic stroke in severe acute respiratory distress syndrome (SARS). J. Neurol. 251, 1227–1231.
Vandermeer, M.L., Thomas, A.R., Kamimoto, L., Reingold, A., Gershman, K., Meek, J., Farley, M.M., Ryan, P., Lynfield, R., Baumbach, J., Schaffner, W., Bennett, N., Zansky, S., 2012. Association between use of statins and mortality among patients hospitalized with laboratory-confirmed influenza virus infections: a multistate study. J. Infect. Dis. 205, 13–19.
Wang, H., Ding, Y., Li, X., Yang, L., Zhang, W., Kang, W., 2003. Fatal aspergillosis in a patient with SARS who was treated with corticosteroids. N. Engl. J. Med. 349, 507–508.
Wong, C.K., Lam, C.W., Wu, A.K., Ip, W.K., Lee, N.L., Chan, I.H., Lit, L.C., Hui, D.S., Chan, M.H., Chung, S.S., Sung, J.J., 2004. Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. Clin. Exp. Immunol. 136, 95–103.
Yamaya, M., Shinya, K., Hatachi, Y., Kubo, H., Asada, M., Yasuda, H., Nishimura, H., Nagatomi, R., 2010. Clarithromycin inhibits type a seasonal influenza virus infection in human airway epithelial cells. J. Pharmacol. Exp. Ther. 333, 81–90.
Yap, F.H., Gomersall, C.D., Fung, K.S., Ho, P.L., Ho, O.M., Lam, P.K., Lam, D.T., Lyon, D.J., Joynt, G.M., 2004. Increase in methicillin-resistant *Staphylococcus aureus* acquisition rate and change in pathogen pattern associated with an outbreak of severe acute respiratory syndrome. Clin. Infect. Dis. 39, 511–516.
Zarogoulidis, P., Papanas, N., Kioumis, I., Chatzaki, E., Maltezos, E., Zarogoulidis, K., 2012. Macrolides: from in vitro anti-inflammatory and immunomodulatory properties to clinical practice in respiratory diseases. Eur. J. Clin. Pharmacol. 68, 479–503.
Zheng, B.J., Chan, K.W., Lin, Y.P., Zhao, G.Y., Chan, C., Zhang, H.J., Chen, H.L., Wong, S.S., Lau, S.K., Woo, P.C., Chan, K.H., Jin, D.Y., Yuen, K.Y., 2008. Delayed antiviral plus immunomodulator treatment still reduces mortality in mice infected by high inoculum of influenza A/H5N1 virus. Proc. Natl. Acad. Sci. USA 105, 8091–8096.
Zhou, B., Zhong, N., Guan, Y., 2007. Treatment with convalescent plasma for influenza A (H5N1) infection. N. Engl. J. Med. 357 (14), 1450–1451.