
Severe Influenza Infection: Pathogenesis, Diagnosis, Management and Future Therapy

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Introduction

Severe influenza infection is an important cause of acute lung injury. Although other respiratory viruses (e. g., respiratory syncytial virus, human metapneumovirus) can also cause considerable pulmonary damage, influenza virus remains the main cause of respiratory failure in patients with suspected viral respiratory tract infection. In addition, influenza virus is the only respiratory virus that has caused four pandemics over the last 100 years, making it one of the most transmissible and virulent viruses in the world. Here, we review the pathogenesis, diagnosis, current management and future therapy of severe influenza infection.

Pathogenesis

Understanding the pathogenesis of severe influenza infection is the key to developing new therapeutic strategies. Although the basic process of a mild influenza infection is well understood, our understanding of how a mild illness progresses to a potentially lethal pulmonary infection remains poor. In this section, we will review recent advances in the immunopathology of severe influenza infection.

Pulmonary epithelial cells are the first target of invasion by influenza virus. Like most cells, epithelial cells constitutionally upregulate the interferon pathway in response to infection by viruses. Types I and III interferon pathways are the natural

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defense mechanism against influenza virus. Upon infection, epithelial cells upregulate interferon regulatory factors (IRF), such as IRF-3 and IRF-7. This leads to transcription and translation of a downstream interferon pathway, which in turn produces a family of interferon-stimulated genes/proteins. This vast family of interferon-stimulated genes/proteins (>300) provides a wide spectrum of anti-viral effects, ranging from inhibition of viral replication to sensing of influenza virus inside the host cells. This response is immediate and effective, making it a critical part of the innate immune response against influenza virus.

Whilst essential, the interferon response alone is not sufficient to prevent virus replication in severely infected cases. Multiple subsets of immune cells (e.g., macrophages, dendritic cells and neutrophils) are required to mount an effective immune response. The failure of this immune response is the hallmark of severe infection, which is characterized by multiple defects in immune cell recruitment, activation or proliferation, as described below.

Alveolar macrophages are among the early responders to influenza virus. They phagocytose infected cells containing influenza virus and initiate other cells of innate and adaptive immunity. Failure of alveolar macrophages to mount an effective early response is associated with increased viral dissemination and increased morbidity/mortality. Neutrophils are also early responders in severe influenza infection. Similar to alveolar macrophages, failure of this early neutrophil response is a prominent feature of severe influenza infection. Paradoxically, an exuberant or inappropriately exaggerated neutrophil response is also a feature of severe influenza infection. For example, in severe H1N1 and H5N1 infection, the large influx of neutrophils into the alveolar space is a classic feature [1]. During this massive neutrophil influx, the neutrophils release a large amount of cytokines, extracellular proteases and histones. This leads to a breakdown of the epithelial barrier, accumulation of reactive oxygen species (ROS), flooding of alveolar spaces by inflammatory fluid and increased barrier to oxygenation, all of which contribute to the clinical picture of acute lung injury commonly observed in patients with severe influenza infection.

Other immune cells are also involved in this early phase of infection (and contribute to pathogenesis). Monocytes, for example, traffic into the infected pulmonary tissue and participate in a pro-inflammatory response. Not surprisingly, inhibition of monocytes and preventing their subsequent participation in the pro-inflammatory response has been shown to decrease the extent of acute lung injury in animal models [2, 3]. Pulmonary dendritic cells are another important immune cell subset that contributes to pathogenesis. In a murine model of influenza infection, pulmonary dendritic depletion increased macrophage recruitment and enhanced pro-inflammatory responses (tumor necrosis factor [TNF]- α /interleukin [IL]-6 increased 5–35 fold) [4]. In another murine model, pulmonary dendritic cells induced T-regulatory cell responses that suppressed antigen-specific CD8 cells, thereby preventing an effective immune response [5]. Hence, the pathogenic role of dendritic cells seems to be to cause a dysregulated immune response, which either causes excessive lung injury (by causing increased inflammation) or impairs the effective clearance of influenza virus (by limiting CD8 cell response).

In the later phase of the host response, adaptive immunity becomes the dominant player. Here, activated CD8 T-lymphocytes cause lysis of the influenza-infected epithelial cells, which facilitates virus clearance. Impaired CD8 responses are a prominent feature of highly pathogenic influenza infection, such as the recently reported H7N9 outbreak in China [6]. In addition to cell lysis, CD8 cells also enhance the pro-inflammatory response, which could either contribute to host defense or, in some cases, worsen lung inflammation and cause further pulmonary damage.

Diagnosis

The detection of influenza virus is the first step in establishing a diagnosis. Rapid antigen detection assays offer a low-cost approach with a short turn-around time. However, a recent review demonstrated that such assays have an unacceptably low sensitivity [7]. Nucleic acid amplification (e. g., multiplex viral polymerase chain reaction [PCR]) has recently gained a much greater prominence due to its high sensitivity and specificity. Currently, this is the most accepted gold standard for virus detection in the initial evaluation of suspected influenza infection. However, there are three important caveats regarding the clinical utility of nucleic acid amplification assay:

- (1) The reliability of such an assay is dependent on the fact that the viral genome is known. An unknown viral genome, mutant strain or new pandemic influenza virus will be difficult to detect.
- (2) The sensitivity is affected by the way the sample is collected. Poor sample collection, inability to access lower airway or reduced virus shedding (due to prior anti-viral administration) all reduce detection sensitivity.
- (3) Detection does not imply infection because the presence of influenza virus in the upper airway may be a co-incidental finding or active infection. In fact, 18% of exposed individuals show no clinical symptoms; therefore, the presence of the virus does not always imply that it is the causative agent. Furthermore, detection of an incomplete virus segment (by nucleic acid amplification) does not constitute sufficient proof that active viral replication is present.

In addition to virus detection, clinicians need to identify which patients are more likely to progress to severe disease or require admission to the intensive care unit (ICU). Table 1 summarizes virus-related and host factors that may contribute to progression to more severe disease. Some of these factors are clinically obvious (e. g., age, pre-existing medical conditions). Other factors (e. g., genetic susceptibility) require highly sophisticated laboratory testing (e. g., high-throughput genome sequencing), which are not yet available in the routine clinical setting.

Following the initial diagnostic work-up, the influenza infected patient needs to be continuously monitored for signs of bacterial co-infection. Several studies have shown that a significant proportion of influenza infected patients admitted to the

Table 1 Risk factors for progression to severe influenza infection

Viral factors	Host factors
Subtype of influenza virus (e. g., H7N9)	Genetic susceptibility (e. g., IFITM3)
Viral load (e. g., high viral load increases severity)	Pregnancy, obesity and extremes of age (elderly and neonates)
Mutation in viral genome (e. g., PB2 gene mutation enhances viral replication)	Pre-existing medical conditions (e. g., chronic lung diseases, cancer, chemotherapy)

IFITM3: interferon-induced transmembrane protein 3

ICU develop bacterial co-infection as a complication [8]. The causative bacterial co-pathogens are most likely to be *Streptococcus pneumoniae* or *Staphylococcus aureus*. The basis for increased susceptibility is thought to be due to production of type I interferon, which is increased initially in response to influenza virus infection, but also decreases the synthesis of IL-1B, IL-23, IL-17 and IL-22, which in turn inhibit the production of antimicrobial peptides [9]. Furthermore, the pro-inflammatory milieu caused by the influx of neutrophils also contributes towards increased susceptibility to bacterial super-infection. Other immune-related factors also contribute towards increased susceptibility including reduced type 17 immune response, impaired antimicrobial peptide (AMP) production by lung epithelia and reduced phagocyte function [9].

Host response biomarkers should form an important part of the diagnostic evaluation of an infected patient. Biomarkers assist clinical evaluation by providing additional information that is not available by conventional virus detection assay. This additional information includes an improved ability to distinguish between coincidental ‘bystander’ virus and true infection, to predict clinical risk for further deterioration and to monitor treatment response. Table 2 summarizes the host response biomarkers that have been recently investigated in the literature.

Gene expression biomarkers are the most recent development in biomarker research. These biomarkers differ from conventional biomarkers (e. g., C-reactive protein [CRP] or procalcitonin [PCT]) in that they are much more influenza specific, due to the fact that many of them are interferon derived genes, which are upregulated in response to respiratory virus infection. A recently published landmark study

Table 2 Host response biomarkers for influenza infection

Biomarkers	Current evidence
Low antibody titer in serum	Could indicate increased risk of death
HLA-DR expression in monocytes	Suggests immune suppression
Procalcitonin (PCT) and C-reactive protein (CRP) in blood	May have some role in excluding bacterial co-infection
Mid-regional pro-adrenomedullin (MR-proADM)	May predict mortality or the need for mechanical ventilation
Gene expression biomarkers	May distinguish between virus detection and active infection

showed that these biomarkers could address several important clinical questions simultaneously (whereas conventional biomarkers could address only one question at a time) [10]. First, these biomarkers could assist clinicians to identify patients most likely to have infection (bacterial and viral) in a heterogeneous population of patients with undifferentiated respiratory illnesses. Second, among infected patients, the biomarkers could distinguish between bacterial and viral infection. Third, among infected patients, the biomarkers could prognosticate and predict clinical outcomes. In addition, the biomarkers could be easily measured in most clinical settings due to the ease of sampling (only 2.5 ml of whole blood is required) and the wide availability of PCR machines (to measure gene-expression). Importantly, because these biomarkers reflect changes in the immune pathway during influenza infection, they provide additional diagnostic information not offered by conventional pathogen detection assay (e. g., virus nucleic amplification). Although further validation studies are necessary before these biomarkers can be widely adopted in clinical practice, it is highly likely that they will be incorporated into the diagnostic armamentaria of modern laboratories in the future.

Management

The management of severe influenza infection is mainly supportive. Standard measures should include those used for the management of acute respiratory distress syndrome (ARDS). Therapeutic agents for severe influenza infection are limited, with oseltamivir being the most commonly used anti-viral agent. A recent meta-analysis showed that oseltamivir could reduce symptom duration and the risk of developing lower respiratory tract complications (e. g., viral pneumonia) [11]. However, its efficacy is dependent on oseltamivir being administered in the early phase of the illness. This may pose difficulty in the management of ICU patients, because these patients often present in the late phase of their illness. Regardless of the timing of presentation, oseltamivir should be considered in all high-risk patients. The current recommendation by the World Health Organization (WHO) indicates that it should be administered in immunocompromised patients, patients with severe comorbidities or underlying chronic lung diseases, age <2 or >65 years, morbid obesity, nursing home residents, women who are pregnant or post-partum, and patients with signs of severe respiratory disease.

Low-dose steroids are best avoided, as suggested by a recently published meta-analysis [12]. In this meta-analysis, the authors analyzed data from nine cohort studies (n = 1,405) and 14 case-control studies (n = 4,700). They found increased mortality associated with corticosteroid treatment in cohort studies (relative risk [RR] 1.85; 95% confidence interval [CI] 1.46–2.33; $p < 0.0001$) and in case-control studies (odds ratio [OR] 4.22; 95% CI 3.10–5.76; $p < 0.0001$). This increased mortality was consistent regardless of the quality of the included studies or the sample size of the individual studies. Other worrying findings are that corticosteroid use was associated with a higher incidence of hospital-acquired pneumonia, longer duration of mechanical ventilation and longer hospital stay. Therefore, the use of

corticosteroids in severe influenza infection is not recommended in routine clinical care and should be restricted to patients in the setting of clinical trials.

Future Therapy

Although conventional treatment for severe influenza infection is limited, novel therapeutic agents have shown great promise. These novel agents consist of mainly two classes: immune agents that modulate host response and anti-viral agents that inhibit viral replication.

Immune Agents that Modulate Host Response

Host Factors that Control Viral RNA Replication

In order to replicate successfully, the influenza virus mRNA undergoes transcription. Initiation of primary viral RNA transcription depends on the activity of host RNA polymerase. Inhibition of this transcription process provides a therapeutic opportunity to halt the commencement of viral RNA replication. Inhibitors of this process, such as CDK9 inhibitor, have undergone preclinical evaluation.

Host Signaling Pathways Influenced by Redox Balance

The influenza virus hijacks the host cell signaling pathway to benefit its own propagation. Phosphorylation of the mitogen-activated protein kinase (MAPK) pathway has been shown to facilitate viral nucleoprotein trafficking [13]. Therefore, inhibition of the MAPK pathway could potentially reduce spread of the influenza virus. Of particular relevance to the intensivist is the fact that the activity of the MAPK pathway is determined by the oxidative-reductive state of the host cell. *N*-acetylcysteine, a well-established drug already commonly used in ICU patients, could modulate the oxidative-reductive state of the host cell, thereby affecting influenza virus propagation. A recent study has demonstrated the potential efficacy of this agent in treating severe influenza infection in an animal model [14]. Other antioxidant agents, such as p38 inhibitor or glutathione, are also potential new host-based therapeutic agents that modulate the redox balance within the host cell. In addition to the MAPK pathway, the PI3K pathway is also sensitive to the effect of redox balance. PI3K is a signaling pathway implicated in influenza infection [15]. An *in vitro* study showed that inhibition of this pathway could reduce influenza virus replication [16]. Importantly, PI3K inhibitors have already been approved as anticancer drugs. Therefore, the possibility of extending their use as anti-influenza agent offers a promising new avenue for future investigation.

Host Factors that Regulate Inflammation

Nuclear factor kappa B (NF- κ B) is a family of transcription factors that initiate inflammation. Influenza virus benefits from the activation of the NF- κ B pathway as the virus exploits the pathway machinery to facilitate viral replication. NF- κ B path-

way inhibitors, such as acetyl-salicylic acid, could block influenza virus replication and propagation. Other pathway inhibitors, such as SC75741, also decrease viral replication. This agent has the unique feature of having a low potential in selecting viral resistant variants, therefore making it unlikely to result in anti-viral resistance [17]. Furthermore, SC75741 has recently been shown to reduce viral replication and cytokine expression in highly pathogenic strains (e. g., H5N1 and H7N7), making it a potential candidate for further investigation in severe influenza infection [18].

The cyclooxygenase (COX) pathway is another pro-inflammatory pathway that has been implicated in influenza virus infection. Highly pathogenic influenza strains, such as H5N1, strongly upregulate COX-2 mediated pro-inflammatory signaling that causes hypercytokinemia during severe H5N1 infection. A non-steroidal COX-2 inhibitor has been shown to inhibit H5N1 infection in human macrophages, making it another potential agent for severe influenza infection [19].

Host Interferon Pathway

The interferon pathways (type I and type II) are the most potent defense of the host cell against influenza virus infection. Activation of interferon pathways leads to upregulation of more than 300 interferon-stimulated genes. Many of these interferon-stimulated genes have potent anti-influenza activity, such as *MX1* (anti-influenza), *ISG15* (inhibits influenza virus replication), *OAS1*, *OAS2*, *OAS3* (degrades viral RNA), *EIF2AK2* (inhibits viral replication), *HERC5* (positive regulator of anti-viral response) and *IFIT2* (inhibits expression of viral mRNA). In addition, these genes activate the adaptive immune response and induce programmed cell death of virally infected cells.

Novel therapeutic strategies take advantage of this endogenous anti-influenza defense by identifying trigger points that activate the interferon pathway. Several molecular pathways are known to trigger the interferon pathway. For example, Toll-like receptor (TLR) 3 and 7 are known to activate the interferon pathway in lung epithelium and immune cells. In plasmacytoid dendritic cells, TLR7 activation produces massive interferon release at 1,000 times that of any other immune cell in the human host. Ligands that selectively target TLR7 in plasmacytoid dendritic cells could be potential therapeutic targets. Other TLR ligands, such as CpG oligodeoxynucleotides (TLR9), have been shown to protect against lethal influenza infection in experimental settings [20]. In lung epithelium, TLR3 is the dominant pathway leading to interferon pathway activation. A large number of TLR3 and TLR9 agonists are currently in clinical trial phase for the treatment of autoimmune conditions, cancer and viruses. It is possible to extend the application of these agents to treat severe influenza infection. Further investigation on these promising new agents may open the door for developing new treatments in severe influenza infection.

Host Factors Implicated in Virus Entry into Human Cells

Before influenza virus replicates in human cells, it needs to gain entry successfully into the cells. The influenza virus harnesses host proteolytic enzymes to achieve this process. One example of such an enzyme is the transmembrane protease serine

S1 (TMPRSS) that belongs to the type II transmembrane serine protease family. This enzyme is located in the human airway epithelium and plays an important role in permitting influenza virus to gain entry into the host cell. Consequently, a protease inhibitor that binds to the TMPRSS molecule is a potential drug target in the treatment of influenza infection. Recent studies have identified three TMPRSS molecules, namely TMPRSS2, TMPRSS4 and TMPRSS11D, as potential drug targets [21]. These molecules have been detected in multiple locations within the human respiratory tract, including nasal mucosa, the trachea, the distal airway and the lung. Aprotinin, a drug familiar to most intensivists, is a protease inhibitor and has been shown to reduce influenza virus replication. In addition to reducing viral replication, aprotinin has also been shown to reduce inflammatory cytokines, suggesting a further benefit other than its impact on viral replication. So far, findings with the TMPRSS molecule have been derived mainly from *in vitro* models. Further studies in animal models and human clinical trials are needed in the future.

Anti-Viral Agents that Inhibit Viral Replication

Neuraminidase

Neuraminidase is a glycoside hydrolase that removes a sialic acid residue of the host cellular receptor recognized by influenza virus hemagglutinin. Therefore, it is an essential component of a process that allows virus penetration through mucosal barriers and subsequently to gain entry into the host cell. In addition, after virus replication, neuraminidase detaches the virion from the infected cells, thereby facilitating release and subsequent spread of the viral progeny. Consequently, neuraminidase is essential for viral infectivity to host cells. Therefore, inhibiting neuraminidase is the primary therapeutic strategy currently used in clinical practice. Most clinicians will be familiar with two neuraminidase inhibitors, zanamivir and oseltamivir.

Unfortunately, the true efficacy of these agents in treating patients with severe influenza infection in the ICU is yet to be established. The vast majority of the clinical trials on these drugs were performed in non-ICU patients. Furthermore, to be effective, these drugs need to be administered during the very early phase of the disease. Consequently, the clinical utility of current neuraminidase inhibitors is limited in ICU patients. To improve the clinical utility of these drugs, a recently developed strategy has been used to increase the efficacy of the approved neuraminidase inhibitors. This strategy involved use of multivalent inhibitors and conjugating the compounds to a biocompatible polymer. Using this innovative approach, recent studies have shown that neuraminidase inhibitors significantly increase their antiviral potency, to 1,000–10,000 times higher than their predecessors [22]. If proven in clinical trials, these newer formulations could become extremely valuable in treating patients with severe influenza infection.

Table 3 Drugs that block the two critical processes in hemagglutinin function

Virus interacting with cell surface	Virus fusion with cell membrane
Carbohydrate-binding agents that recognize glycosylation sites on hemagglutinin	Molecules that inhibit conformation change in hemagglutinin
Peptides against hemagglutinin	Neutralizing antibodies directed against the stem region of hemagglutinin
Decoy receptor or sialic acid-containing inhibitors	
Neutralizing monoclonal antibodies directed against the globular head domain of hemagglutinin	

Hemagglutinin

Hemagglutinin is pivotal for the interaction between influenza virus and the sialic acid on the surface of the host cells. In addition, it is required for the fusion between the viral envelop and the endosomal membrane of the host cell, which is the final step in the virus's entry into the host cell. Inhibiting hemagglutinin could be achieved by two methods: (1) preventing the interaction between viral surface molecules and the host cell surface receptor; and (2) blocking the fusion of the viral envelop with the host cell membrane. Table 3 summarizes the recent development in the new drugs that utilize the above two strategies.

M2 Ion Channel

The M2 protein is a proton channel inside the influenza virus. After gaining entry into the host cell, the influenza virus activates the M2 protein by sensing a drop in the pH value inside the enveloped vesicle (the endosome). The activation of the M2 proton channel results in a proton flux from the endosome into the virion core. Acidification of the virus interior leads to dissociation of the viral ribonucleoprotein complexes. Subsequent membrane fusion releases the ribonucleoprotein into the cytoplasm. This release allows the virus to be imported into the nucleus to start viral replication. Other important functions of the M2 protein are: formation of the filamentous strains of the virus; release of the budding virion; and stabilization of the virion budding site. Due to these important functions, inhibition of M2 protein represents an ideal therapeutic target. A well-known licensed antiviral drug, amantadine, is an M2 blocker that binds the N-terminal channel lumen of the M2 pore resulting in repulsion of protons and subsequently prevent virus uncoating. Unfortunately, this class of drug is not active against all strains of influenza virus (e.g., influenza B). In addition, the emergence of drug-resistant virus variants has been reported. These drawbacks have significantly limited the use of M2 blockers.

Conclusion

Severe influenza infection remains an important clinical challenge for intensivists. The potentially high morbidity and mortality of this condition has remained un-

changed over the last few decades, due mainly to a lack of effective new therapies with which to treat such patients.

However, we have gained a much better understanding of the mechanisms of the disease in recent years. This improved understanding points to the pivotal roles played by immune dysregulation in causing severe disease. Furthermore, our ability to diagnose influenza infection, to stratify high-risk patients and to prognosticate clinical outcomes has also improved thanks to recent advances in genomic science. Importantly, a large number of novel therapeutic agents are currently under investigation. These novel agents target multiple critical points of the host response pathway. Agents that modulate the host response hold particularly great promise since dysregulated immunity is the main driver towards more severe infection. In the future, clinical trials will be an important next step to demonstrate the efficacy of these novel agents.

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