ORIGINAL ARTICLE

Immunological Features in Patients With Pneumonitis Due to Influenza A H1N1 Infection

Y Wen, BC Deng, Y Zhou, Y Wang, W Cui, W Wang, P Liu

Department of Infectious Diseases, The First Affiliated Hospital, China Medical University, Shenyang, China

■ Abstract

Background: Pneumonitis induced by pandemic influenza A H1N1 has a potential to cause respiratory failure, which is a risk factor for death. The underlying immunopathological mechanisms, however, have not yet been fully elucidated.

Patients and Methods: We investigated changes in plasma cytokines, T cell subsets, and C-reactive protein (CRP) in 16 hospitalized patients with pneumonia caused by 2009 H1N1 influenza infection. The patients were classified into a severe disease group and a mild disease group according to PaO$_2$.

Results: Cytokine profiles showed no changes in interferon γ (IFN-γ), interleukin 6 (IL-6), IL-8, or transforming necrosis factor α (TNF-α) levels throughout the observation period. Transforming growth factor ß (TGF-ß1) was overproduced in the severe group but not in the mild group. Accordingly, we also found some signs of pulmonary fibrosis during the recovery period. Elevated CRP levels and lymphopenia were common in both the severe and the mild group. After treatment, there was a significant elevation in lymphocytes in both groups, but a significant decrease in CRP in the mild group. Lymphocyte counts and CRP levels rapidly recovered to normal levels in all survivors posttreatment; otherwise it seemed to be related to poor prognosis.

Conclusions: Serial measurements of cytokines showed that only TGF-ß1 was overproduced, possibly in relation to the early use of corticosteroids, which may have downregulated immune responses to H1N1 infection. Pretreatment TGF-ß1 plasma concentrations and absolute lymphocyte counts were independent predictors of severity. However, the role of elevated TGF-ß1 in H1N1 infection-associated pulmonary fibrosis requires further investigation.

Key words: Cytokines. Immunological FEATURES. Influenza A H1N1. Pneumonitis.

■ Resumen

Antecedentes: La neumonitis inducida por la gripe A pandémica, H1N1, puede causar insuficiencia respiratoria, un factor de riesgo de muerte. Sin embargo, todavía no están claros los mecanismos inmunopatológicos subyacentes.

Pacientes y métodos: Se estudiaron los cambios en las citocinas plasmáticas, los subconjuntos de linfocitos T y la proteína C-reactiva (PCR) en 16 pacientes hospitalizados con neumonía causada por una infección por gripe H1N1 en 2009. Los pacientes se clasificaron en un grupo de enfermedad grave y un grupo de enfermedad leve en función de la PaO$_2$.

Resultados: Los perfiles de citocinas no mostraron cambios en los niveles de interferón γ (IFN-γ), interleucina 6 (IL-6), IL-8 o factor de necrosis tumoral α (TNF-α) a lo largo del período de observación. Se observó una sobreproducción de factor transformador de crecimiento ß (TGF-ß1) en el grupo de enfermedad grave pero no en el de enfermedad leve. En consecuencia, también se detectaron algunos signos de fibrosis pulmonar durante el período de recuperación. Los niveles elevados de PCR y la linfopenia fueron frecuentes tanto en el grupo de enfermedad grave como en el de enfermedad leve. Tras el tratamiento, se produjo un aumento significativo de los linfocitos en ambos grupos y una reducción significativa de la PCR en el grupo de enfermedad leve. Los recuentos linfocitarios y los niveles de PCR volvieron rápidamente a la normalidad en todos los supervivientes después del tratamiento; en caso contrario, se asoció con un pronóstico desfavorable.

Conclusiones: Las mediciones seriadas de citocinas únicamente mostraron sobreproducción de TGF-ß1, posiblemente debido al uso temprano de corticoesteroides, que pueden reducir las respuestas inmunitarias frente a la infección por H1N1. Las concentraciones plasmáticas de TGF-ß1 y los recuentos linfocitarios absolutos previos al tratamiento fueron factores predictivos de gravedad independientes. No obstante, se requieren más estudios para determinar la función de los niveles elevados de TGF-ß1 en la fibrosis pulmonar asociada a infección por H1N1.

Palabras clave: Citocinas. Características inmunológicas. Gripe A (H1N1). Neumonitis.

Introduction

Pneumonitis caused by the pandemic H1N1 2009 influenza virus infection may develop into life-threatening acute lung injury and even acute respiratory distress syndrome (ARDS). The underlying molecular mechanisms are very complex. Some patients have a stronger host response to virus infection than others with similar viral replication levels [1]. In addition, patients continue to manifest lung injury when the viral load falls after antivirus therapy, providing further support of the immune nature of the lung damage that occurs in such cases. However, virus-specific antibody(Ab) does not seem to play a role in the early stages of disease because it has not been detected 2 to 3 weeks after severe acute respiratory syndrome-coronavirus (SARS-CoV) infection [2]. Cytokine activation, which usually occurs much earlier than clinically observable responses, may be an early event. We wished to investigate cytokine induction in this context as we believed it might play an important role in stimulating an immune response to the H1N1 virus and subsequently be responsible for extensive parenchymal damage. We also wished to investigate changes in C-reactive protein (CRP) and lymphocytes counts, which are vital predictors for prognosis in SARS [3,4]. The main aim of this study was to characterize the immunological features of hospitalized pneumonia patients with 2009 H1N1 influenza.

Methods

Patients

From November 9, 2009 to December 31, 2009 nasopharyngeal swab samples from suspected cases were tested for 2009 H1N1 influenza virus infection by reverse transcription polymerase chain reaction. Sixteen adults, diagnosed with H1N1 pneumonitis and admitted to the First Affiliated Hospital of China Medical University with profound hypoxemia, were included in the study. According to the World Health Organization, the main characteristics of H1N1 pneumonitis include a history of close contact with a person diagnosed with H1N1, persistent high fever (temperature >38°C), dry cough or sputum production, dyspnea, and a new atypical form of pneumonia verified by multiple spot films of chest computed tomography (CT) scans. Patients were classified into 2 disease severity groups according to pretreatment PaO_2 levels: a severe disease group (PaO_2 <60 mm Hg, n=9) and a mild disease group (PaO_2 >60 mm Hg, n=7). A control group was formed by healthy volunteer doctors and nurses (n=8). The study protocol was approved by the Clinical Research Ethics Committee of the Chinese University of China, and informed consent was obtained from all participants.

Demographics

The age of the patients, sex, body mass index (BMI), and underlying diseases were recorded. According to the criteria established by the Working Group On Obesity In China in 2005, patients were classified as overweight if their BMI was 24 to 27.9 kg/m² and obese if their BMI was ≥28 kg/m².

Measurement of Plasma Cytokines

Blood samples coated with EDTA were collected from patients on days 7 and 14 after disease onset. Plasma was separated by centrifugation (2000 g for 10 minutes, 4°C) and stored at –70°C until analyzed. Plasma concentrations of interleukin 6 (IL-6), IL-8, interferon γ (IFN-γ), tumor necrosis factor α (TNF-α) and transforming growth factor ß (TGF-ß) were determined by an ABC-enzyme-linked immunosorbent assay (ELISA) kit (Research & Diagnostics, Minneapolis, Minnesota, USA).

Measurement of Lymphocytes, T Cell Subsets and CRP

Lymphocyte counts, T cell subset counts, and CRP levels were determined using an automatic hematology analyzer (XT1800i; Sysmex, Kobe, Japan), a flow cytometer (Becton Dickinson, California, USA) and nephelometry and turbidimetry-chemical analysis (Nycocard READERII; AxisShield PoC AS, Oslo, Norway).

Statistical Analysis

The most important data were presented as means (SD) and analyzed using the Statistical Package for Social Sciences (SPSS) 9·0 software (SPSS Inc., Chicago, Illinois, USA). Differences between the 3 groups were analyzed by 1-way analysis of variance followed by the least-squares difference test, and differences between 2 groups were analyzed by 2 independent t tests. Changes from pretreatment to posttreatment were analyzed using the paired-samples t test. To identify independent predictors for disease severity, Pearson correlation analysis was applied. A 2-sided value of P<.05 was considered statistically significant.

Results

Demographics

No pregnant woman or children were enrolled in the study. The median age of the patients was 47 years (range, 19-66 years). Fourteen (87.5%) of the 16 patients were aged between 33 and 59 years. There was just 1 patient (6.25%) over 60 years old and another under 30 years old. The male to female ratio was 1.67 to 1 (10:6). Based on BMI calculations, 3 patients (18.75%) were overweight and 8 (50%) were obese (P=.1). Seven patients (43.75%) had an underlying medical condition and 1 (6.25%) had 2 underlying conditions. The conditions included diabetes, nephrotic syndrome, hypertension, hyperthyroidism, pituitary tumor, fatty liver, and old tuberculosis.

Treatment and Prognosis

All 16 patients received oseltamivir treatment and oxygen supplementation. The median±SEM time from symptom onset to the initiation of antiviral therapy was 7 days (range, 2-13 days). The corresponding figures for the severe disease group and the mild disease group were 8±3 days and 5.71±2.93
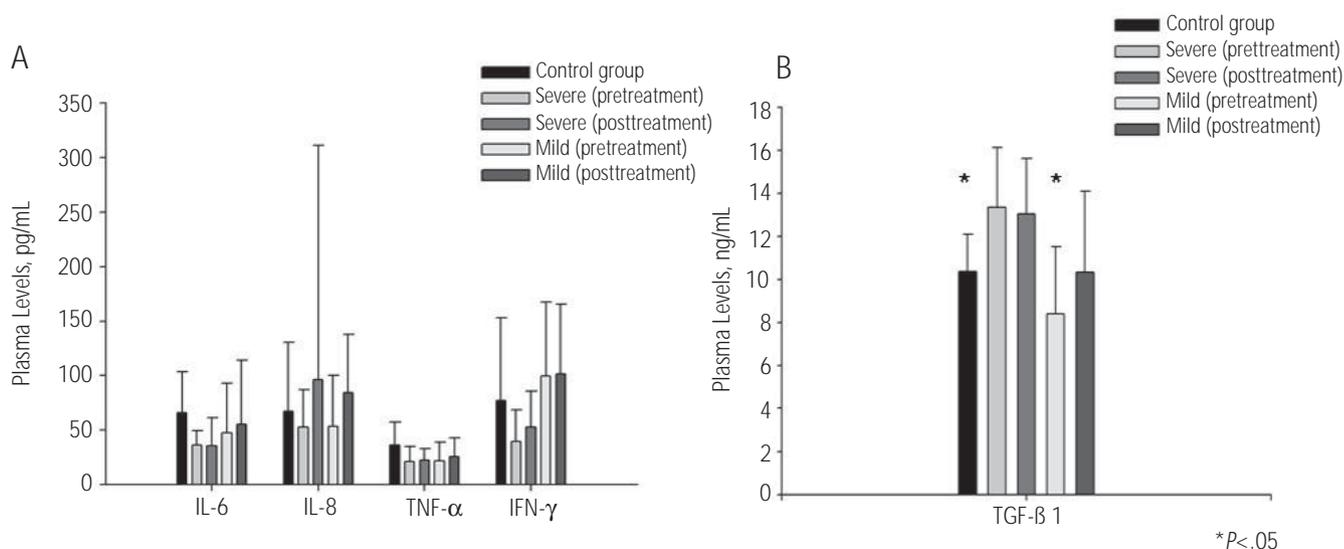
Figure 1. MA, Plasma cytokine levels. There were no significant differences in tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), IL-8, or interferon γ (IFN-γ) levels in patients with either severe disease (PaO$_2$ <60 mm Hg) or mild disease (PaO$_2$ >60 mm Hg). Pretreatment and posttreatment differences were also insignificant in both groups (P>.05). B, Transforming growth factor ß1 (TGF-ß1) levels. Pretreatment TGF-ß1 levels were lower in the mild disease group and the control group than in the severe group (*P<.001 and *P=.027, respectively). There were no significant changes in TGF-ß1 levels with treatment (P>.05).

days, respectively (P=.149). Only 2 patients (12.5%) were treated with oseltamivir within 48 hours of symptom onset; the remaining 14 (87.5%) were treated after 48 hours. All patients received preventive antibiotic therapy, with 14 (87.5%) receiving more than 1 antibiotic. The median duration of antibiotic treatment was 7 days. Commonly used antibiotics were cefminox and levofloxacin. Of the 16 patients, 14 (87.5%) received corticosteroids by means of an intravenous injection. The average dose of methylprednisolone was 1 mg·kg-1·d-1. Fourteen patients (87.5%) received extrasin alpha1 by means of a subcutaneous injection. The number of patients who survived was also 14. The median length of hospitalization in the survival group was 14.43±6.27 days. Two patients were admitted to an intensive care unit (ICU) and died on days 17 and 15 after disease onset. Perplexingly, neither had serious underlying disease and both had undergone the process of virus positive-negative transformation. Most of the survivors showed nearly complete resolution of thoracic CT abnormalities within 30 days. However, several continued to have shortness of breath and there were persistent radiologic images of ground glass opacities and reticular opacities.

Measurement of Plasma Cytokines

The variable trends of plasma cytokines in the 16 patients are illustrated in Figures 1A and 1B.

TNF-α, IL-6, IL-8, and IFN-γ Levels

As shown in Figure 1A, there were no significant increases in TNF-α, IL-6, IL-8, or IFN-γ levels in any of the patients. The serum levels of these 4 cytokines varied more greatly than those of TGF-ß1 in both healthy controls and patients. In
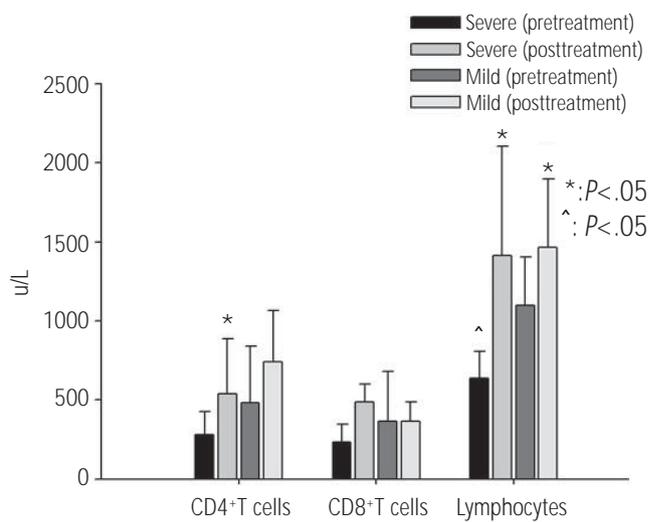

Figure 2. Analysis of T cell subsets and lymphocytes. Using 2 independent *t* tests, pretreatment lymphocyte counts were seen to be much lower in the severe disease group (PaO$_2$ >60 mm Hg) than in the mild disease group (PaO$_2$ <60 mm Hg) (P=.002). Using the paired-samples t test, the levels were seen to increase significantly with treatment in both the severe group (*P=.014) and the mild group (P=.02). CD4+ T cell counts also increased significantly in the severe group after treatment (*P=.034, paired-samples *t* test).

A

B

C
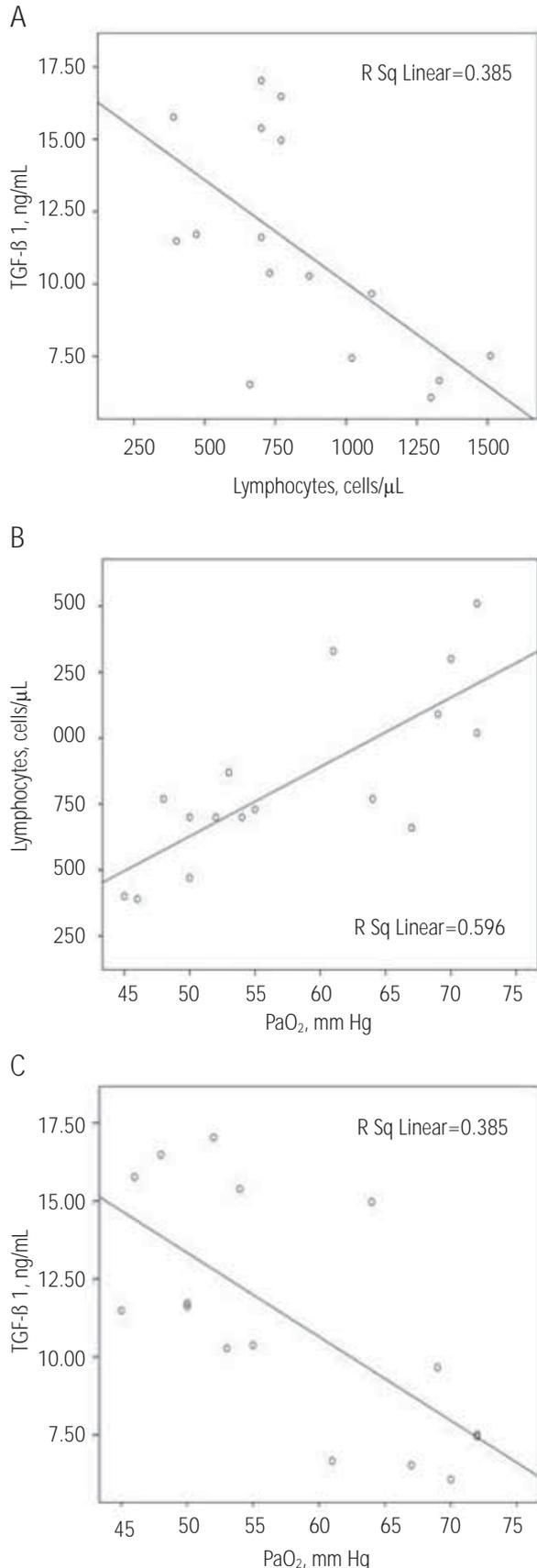
contrast to TGF-ß1, plasma concentrations of the 4 cytokines were lower than or within the normal range. Pretreatment IL-6 and TNF-α levels were slightly lower in the mild disease group than in the control group (P=.083 and P=.094, respectively). Furthermore, IL-6 levels were seen to increase after treatment (P=.085). In general, the levels of the 4 cytokines remained highly stable throughout the observation period.

TGF-ß1 Levels

As summarized in Figure 1B, TGF-ß1 levels were significantly higher in the severe group than in both the control group (P=.027) and the mild group (P=.001); the differences between the control group and the mild group were insignificant (Figure 1B). After 1 week of treatment, the levels increased in the mild group (P=.088) but remained high in the severe group. However, there were no significant differences in TGF-ß1 levels between the 2 patients who died and the survivors from the severe group.

Depletion of Lymphocytes

As shown in Figure 2, lymphocyte levels determined by routine blood testing were lower than the lower limit of normal (approximately 800 cells/µL) in 62.5% (10/16) of the patients. Levels were much lower in the severe group than in the mild group before treatment (P=.002) but not after treatment (P>.05). Nonetheless, they increased significantly after treatment in both the severe group (P=.014) and the mild group (P=.02). Pretreatment and posttreatment CD4^{+} and CD8^{+} T cell counts, measured by flow cytometry, did not differ significantly between the groups (P>.05). Analysis by the paired-samples t test showed a significant increase in posttreatment CD4^{+} T cells in the severe group only (P=.034). CD8^{+} T cell levels also increased with treatment, albeit insignificantly (P=.053). Lymphocyte, CD4^{+}, and CD8^{+} T cell counts in the 2 patients who died decreased after treatment.

CRP Levels

CRP levels were higher than 2mg/dL in 75% (12/16) of the patients, with higher levels detected in the severe group than in the mild group (P=.08). Furthermore, levels showed no significant decrease after treatment in the severe group (P>.05) and they actually increased in the 2 patients who died. In the mild disease group, the levels returned rapidly to normal levels with treatment (P=.045).

Correlation Analysis

Only pretreatment lymphocyte count was correlated negatively with TGF-ß1 levels. Both pretreatment and posttreatment levels were correlated with PaO$_2$ levels (r=-0.621, P=.010; r=0.771, P<.001; and r=-0.688, P=.003, respectively) (Figure 3). No correlation was found between

Figure 3. Correlation analysis. Only pretreatment lymphocyte counts were correlated negatively with TGF-ß1 concentrations (P=.010, r=-0.621), (Figure 3A). Both pretreatment and posttreatment counts were correlated with PaO$_2$ (P=.000, r=0.771, Figure 3B; P=.003, r=-0.688, Figure 3C).

TGF-ß1 levels and the time of antiviral therapy initiation, length of hospital stay, or CRP levels (*P*>.05).

Discussion

We report on a series of 16 hospitalized patients with pneumonia caused by 2009 H1N1 influenza infection in Shenyang, China, from November to December 2009. In total, 2 patients (12.5%) were admitted to the ICU and both died, findings which are similar to the majority of reports from other countries [5-7]. Obesity and preexisting chronic diseases have also been found to be associated with death in such cases [8-10]. In our study, 50% of the patients were obese and 43.75% patients had an underlying medical condition. However, these factors were not predictive of either ICU admission or death, possibly due to the small sample size. Serum samples were not collected at the onset of disease. Before blood collection, immunomodulators such as steroids had been used. The plasma samples were collected on days 7 and 14 after symptom onset. The collection time of the second serum samples was close to the remission phase in the mild group, but approached the peak stage in the severe group.

Viral infection can induce lymphopenia. An inverse correlation between lymphocyte count and viral load in both nasopharyngeal and endotracheal specimens has been observed in SARS-COV and H5N1 infection [11,12]. Lymphopenia can result in a decrease in virus-specific Ab response and the production of cytokines. The mechanism of lymphopenia is complicated. IFN-γ and TNF-α via the Fas or TNF-α pathway might induce apoptosis of activated T cells [13-15]. In a molecular pathology study, direct virus infection of T lymphocytes of the lymph node has been implicated in the pathogenesis of lymphopenia in H5N1 infection [16]. In the present study, lymphopenia was also common in patients with severe disease. We concluded that lymphocyte counts determined by routine blood tests were more sensitive and convenient than $CD4^+$ or $CD8^+$ T cell counts from T cell subsets. It is a pity that we did not perform a lung pathology study to check whether regionally intense lymphocytic filtration was also involved in lymphopenia. Although the initiation of oseltamivir treatment was delayed in our series of patients, lymphocyte counts rapidly returned to normal levels in all survivors. Noteworthily, the 2 patients who died had persistent lymphopenia. We can therefore conclude that lymphopenia that did not resolve with treatment was a risk factor for death. Oseltamivir resistance-related death can be excluded considering that all the patients (even the 2 who died from ARDS) underwent the process of nasopharyngeal viral load positive-negative transformation. Our results are not consistent with those from a study in Taiwan which found that an initial lymphocyte count of less than 800 cells/µL and initiation of oseltamivir treatment more than 48 hours after symptom onset were associated with the development of respiratory failure [17]. To et al [18] also observed a slower decline in viral shedding in patients with severe disease than in those with mild disease. We speculate that virus replication is an important fatal factor but not the only one.

Initial CRP levels have been found to be predictive of death in SARS-COV infection [3,4]. In our study, CRP was neither a sensitive nor a specific predictor of disease severity. However, irrespective of initial levels, it rapidly returned to normal levels with treatment in all survivors. In the 2 patients who died, levels increased, possibly due to bacterial coinfection. However, all the patients had negative blood or sputum cultures, but it should be borne in mind that most patients had received antibiotics close to the time of culture collection, possibly reducing diagnostic sensitivity.

Various cells in the lung, such as macrophages, epithelial cells, endothelial cells, pneumocytes, and fibroblasts, are involved in the overproduction of cytokines [19]. Several papers have reported a significant increase in levels of proinflammatory cytokines (eg, TNF-α and IL-6), antiviral cytokines (eg, IFN-γ), and neutrophil chemotactic factors (eg, IL-8) in SARS-CoV infection or ALI/ARDS [20-23]. Nonetheless, there have also been reports of no increase in classic cytokines such as TNF-α during SARS-COV infection [19]. In our study, none of these cytokines were overproduced. We are unable to explain this but it is possibly related to the use of corticosteroids, which may have inhibited the activation of lymphocytes or macrophages and prevented the production of cytokines. Our findings do not support the therapeutic use of these cytokine antibodies for H1N1 infection.

To et al [18] assayed the profiles of 25 cytokines and chemokines in patients with H1N1 infection, but they did not analyze TGF-ß1 [18]. TGF-ß1 plays a pivotal role in pulmonary fibrosis [24]. It increases the production of extracellular matrix proteins, enhances the secretion of protease inhibitors, and reduces the secretion of proteases, leading to the deposition of extracellular matrix proteins. It can also induce pulmonary fibrosis directly through stimulation of fibroblast chemotactic migration and proliferation as well as fibroblast-myofibroblast transition. Several viral proteins have been reported to modulate TGF-ß1 signaling, which could induce the proliferation of fibroblasts [25]. Pang et al [27] found higher serum concentrations of TGF-ß1 in SARS patients compared to controls for all clinical courses, including initial, peak, remission, and recovery stages [26,27]. During short-term follow-up, persistent ground glass opacities, reticular opacities, and pathologic findings of fibrosis have been found in some SARS survivors [28,29]. However, long-term follow-up showed a gradual decrease in ground glass opacities and reticulation. Willis et al [30] pointed out that TGF-ß1 was necessary but not sufficient to induce the formation of pulmonary fibrosis [30]. In our study, TGF-ß1 levels increased significantly and remained high after treatment in the severe group, coinciding with reports of patients with SARS-CoV infection [23,26]. This phenomenon could well be explained by the existence of imminent pulmonary fibrosis. The role of elevated TGF-ß1 levels in H1N1 infection, however, requires further investigation as some survivors have also shown signs of pulmonary fibrosis during short-term follow-up.

Our study has certain limitations. The retrospective nature of the study yields inherent selection bias; no pregnant woman or children were included; the small sample size might have rendered some valuable indicators insignificant; it would have been desirable to perform long-term follow-up, with chest imaging and pulmonary function tests, of survivors.

Fortunately, the mortality of the current 2009 influenza A (H1N1) pandemic is not as high as that of the avian influenza A (H5N1). Persistent lymphopenia and high CRP levels, which are indicators of immune dysregulation, seem to be correlated with mortality. Virus replication is vital but it is not the only cause of death or of ARDS. Although an exaggerated activation of cytokines has been proposed as a factor of adverse outcome in SARS, only TGF-ß1 was an independent predictor in our study, possibly due to the early use of corticosteroids. Although the impact of corticosteroids on outcomes is controversial, based on our findings, it should be recommended in selected patients. The beneficial effects of immunoregulators are probably the interruption of cytokine storms induced by virus infection. The mechanisms of pulmonary fibrosis are complicated, and therapy targeting TGF-ß1 designed to block lung fibrosis requires further study.

References

1. Smits SL, de Lang A, van den Brand JM, Leijten LM, van Ijcken WF, Eijkemans MJ et al. Exacerbated Innate Host Response to SARS-CoV in Aged Non-Human Primates. PLoS Pathog. 2010; 6(2):e1000756.
2. Peiris JSM, Lai ST, Poon LLM, Guan Y, Yam LYC, Lim W, Nicholls J, Yee WKS et al. Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet. 2003a;361:1319-25.
3. Leong HN, Earnest A, Lim HH, Chin CF, Tan CS, Puhaindran ME, Tan AC, Chen MI, Leo YS. SARS in Singapore—predictors of disease severity. Ann Acad Med Singapore. 2006;35(5):326-31.
4. Wang JT, Sheng WH, Fang CT, Chen YC, Wang JL, Yu CJ et al.. Clinical manifestations, laboratory findings, and treatment outcomes of SARS patients. Emerg Infect Dis. 2004;10(5):818-24.
5. Louie JK, Acosta M, Winter K, Jean C, Gavali S, Schechter R et al; California Pandemic (H1N1) Working Group. Factors associated with death or hospitalization due to pandemic 2009 influenza A(H1N1) infection in California. JAMA. 2009 302(17): 1896-902.
6. Pérez-Padilla R, de la Rosa-Zamboni D, Ponce de Leon S, Hernandez M, Quiñones-Falconi F, Bautista E et al; INER Working Group on Influenza. Pneumonia and Respiratory Failure from Swine-Origin Influenza A (H1N1) in Mexico. N Engl J Med. 2009; 361(7):680-9.
7. Gómez-Gómez A, Magaña-Aquino M, Garcia-Sepúlveda C, Ochoa-Pérez UR, Falcón-Escobedo R, Comas-García A et al. Severe pneumonia associated with pandemic (H1N1) 2009 outbreak. San Luis Potosí, Mexico. Emerg Infect Dis 2010; 16(1):27-34.
8. Chen KF, Gaydos C, Rothman RE. Update on emerging infections: news from the Centers for Disease Control and Prevention. Hospitalized patients with novel influenza A (H1N1) virus infection—California, April-May, 2009. Ann Emerg Med. 2009; 54(5):732-6.
9. Rello J, Rodríguez A, Ibañez P, Socias L, Cebrian J, Marques A et al; H1N1 SEMICYUC Working Group. Intensive care adult patients with severe respiratory failure caused by Influenza A (H1N1)v in Spain. Crit Care. 2009; 13(5):R148.
10. Echevarría-Zuno S, Mejía-Aranguré JM, Mar-Obeso AJ, Grajales-Muñiz C, Robles-Pérez E, González-León M et al. Infection and death from influenza A H1N1 virus in Mexico: a retrospective analysis. Lancet. 2009; 374(9707): 2032-3.
11. Hung IF, Cheng VC, Wu AK, Tang BS, Chan KH, Chu CM et al. Viral loads in clinical specimens and SARS manifestations. Emerg Infect Dis.2004;10(9):1550-7.
12. de Jong MD, Simmons CP, Thanh TT, Hien VM, Smith GJ, Chau TN et al. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. Nat Med. 2006; 12:1203-7.
13. Bernabei P, Coccia EM, Rigamonti L, Bosticardo M, Forni G, Pestka S et al. Interferon-gamma receptor 2 expression as the deciding factor in human T, B, and myeloid cell proliferation or death. J Leuko Biol. 2001;70: 950-60.
14. Sobek V, Balkow S, Korner H, Simon MM. Antigen-induced cell death of T effector cells in vitro proceeds via the Fas pathway, requires endogenous interferon-gamma and is independent of perforin and granzymes. Eur J Immunol. 2002; 32:2490-99.
15. Jendro MC, Fingerle F, Deutsch T, Liese A, Köhler L, Kuipers JG. Chlamydia trachomatis-infected macrophages induce apoptosis of activated T cells by secretion of tumor necrosis factor-alpha in vitro. Med Microbiol Immunol. 2004;193(1):45-52.
16. Gu J, Xie Z, Gao Z, Liu J, Korteweg C, Ye J. H5N1 infection of the respiratory tract and beyond: a molecular pathology study. Lancet. 2007; 370(9593): 1137-45.
17. Chien YS, Su CP, Tsai HT, Huang AS, Lien CE, Hung MN et al. Predictors and outcomes of respiratory failure among hospitalized pneumonia patients with 2009 H1N1 influenza in Taiwan. J Infect. 2010; 60(2):168-74.
18. To KK, Hung IF, Li IW, Lee KL, Koo CK, Yan WW et al. Delayed Clearance of Viral Load and Marked Cytokine Activation in Severe Cases of Pandemic H1N1 2009 Influenza Virus Infection. Clin Infect Dis. 2010; 50(6):850-9.
19. Zhang Y, Li J, Zhan Y, Wu L, Yu X, Zhang W et al. Analysis of serum cytokines in patients with severe acute respiratory syndrome. Infect Immun. 2004; 72:4410-5.
20. Wong CK, Lam CWK, Wu A KL, Ip WK, Lee NLS, ChanI HS et al. Plasma inflammatory cytokines and chemokines in severe respiratory syndrome. Clin Exp Immunol. 2004; 136: 95-103.
21. Fudala R, Krupa A, Stankowska D, Allen TC, Kurdowska AK. Anti-interleukin-8 autoantibody:interleukin-8 immune complexes in acute lung injury/acute respiratory distress syndrome. Clin Sci (Lond). 2008; 114(6):403-12.
22. Tang NL, Chan PK, Wong CK, To KF, Wu AK, Sung YM et al. Early enhanced expression of interferon-inducible protein-10 (CXCL-10) and other chemokines predicts adverse outcome in severe acute respiratory syndrome. Clin Chem. 2005; 51(12):2333-40.
23. Huang KJ, Su IJ, Theron M, Wu YC, Lai SK, Liu CC. An interferon-gamma-related cytokine storm in SARS patients. J Med Virol. 2005; 75(2):185-94.
24. Roberts AB, Tian F, Byfield S D, Stuelten C, Ooshima A, Saika S et al. Cytokine Growth Factor Rev. 2006; 17:19-27
25. Krein PM, Winston BW. Roles of insulin-like growth factor and transforming growth factor-beta in fibrotic lung disease. Chest 122:289S–293S. J Biomed Sci. 2002; 11:117-26.
26. Zhao X, Nicholls JM, Chen YG. Severe acute respiratory syndrome-associated coronavirus nucleocapsid protein interacts with Smad3 and modulates transforming growth factor-beta signaling. J Biol Chem. 2008; 283(6):3272-80.
27. Pang BS, Wang Z, Zhang LM, Tong ZH, Xu LL, Huang XX et al. Dynamic changes in blood cytokine levels as clinical indicators in severe acute respiratory syndrome. Chin Med J. 2003;116: 1283-7.

28. Nicholls JM, Poon LL, Lee KC, Ng WF, Lai ST, Leung CY et al. Lung pathology of fatal severe acute respiratory syndrome. Lancet. 2003, 61:1773-8

29. Ketai L, Paul NS, Wong KT. Radiology of severe acute respiratory syndrome (SARS): the emerging pathologic-radiologic correlates of an emerging disease. J Thorac Imaging. 2006; 21(4):276-83.

30. Willis BC, Liebler JM, Luby-Phelps K, Nicholson AG, Crandall ED, du Bois RM et al. Induction of epithelial-mesenchymal transition in alveolar epithelial cells by transforming growth factor-beta1: potential role in idiopathic pulmonary fibrosis. Am J Pathol.2005; 166: 1321-2.

▌ **Pei Liu**

Department of Infectious Diseases
The First Affiliated Hospital,
China Medical University,
No. 155 Nanjing north Street, Shenyang 110001
Liaoning Province, China.
E-mail: syliupei2003@yahoo.com.cn