Inflammatory risk factors for hypertriglyceridemia in patients with severe influenza

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Abstract
Objective: Inflammation and viral infections can induce significant changes in lipid metabolism. Hypertriglyceridemia (HTG) often occurs secondary to obesity, which is an independent risk factor for influenza virus infection. However, the inflammatory risk factors contributing to HTG in patients with severe influenza have yet to be elucidated.

Materials and methods: Plasma and bronchoalveolar lavage fluid (BALF) samples were collected from 33 patients with severe influenza (n = 26 control patients with normal serum triglyceride levels and n = 7 HTG patients with serum triglycerides >2.3 mM). Levels of 45 putative inflammatory risk factors were quantitated using a commercial enzyme-linked immunosorbent assay kit.

Results: Plasma levels of interferon (IFN)-γ, interleukin (IL)-18, IL-1 receptor antagonist (IL-1RA), monocyte chemoattractant protein-1, macrophage inflammatory protein-1α, hepatocyte growth factor, stem cell factor, and vascular endothelial growth factor A were significantly higher in HTG patients compared with control patients. BALF samples from HTG patients contained significantly higher levels of IL-1RA and lower levels of IFN-γ-inducible protein-10.

Conclusion: HTG in patients with severe influenza is associated with alterations in several inflammatory risk factors. Our results provide new insights that may enable more effective clinical management of severe influenza combined with HCT.

Keywords
Hypertriglyceridemia, lipid metabolism, severe influenza, inflammation, plasma, bronchoalveolar lavage fluid

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**Introduction**

Influenza virus causes a highly contagious acute respiratory tract infection of influenza virus that imposes a serious worldwide public health burden each year.\(^1,2\) Although influenza pandemics are associated with the most significant mortality burden, seasonal influenza severely affects millions of individuals.\(^3,4\) Influenza viruses are segmented RNA viruses encapsulated by lipid envelopes and can be categorized into different subtypes (e.g., H1N1 and H7N9) based on two surface proteins, hemagglutinin and neuraminidase.\(^3,5\) Between 3,000,000 and 5,000,000 cases of severe influenza occur annually worldwide, which result in 250,000 to 500,000 deaths.\(^2\) The clinical signs and symptoms of influenza include fever, chills, headache, cough, fatigue, nasal congestion and sore throat; these can typically be significantly relieved by anti-viral treatments.\(^6\) However, severe influenza is often associated with acute respiratory distress syndrome (ARDS), which can result in life-threatening respiratory failure.\(^6\) Therefore, the identification of risk factors for severe influenza infection is critical to prevent and control the spread of influenza as well as to reduce influenza-associated mortality.

Populations at high risk of influenza include children, the elderly, pregnant women, and individuals with immunodeficiencies and chronic diseases.\(^7,8\) Obesity has also recently been identified as a risk factor for influenza infection.\(^9,10\) Obesity can contribute to dyslipidemia and is the leading cause of diabetes and its associated diseases.\(^11\) Hypertriglyceridemia (HTG), the condition of elevated triglyceride levels, often occurs secondary to obesity-related insulin resistance.\(^12\) A previous study demonstrated that severe HTG was associated with systemic inflammation and contributed to increased risk of cardiovascular disease.\(^13\) Infection by influenza viruses is also characterized by robust inflammation. In fatal cases of H1N1 influenza virus infection in 2009, levels of inflammatory factors including interferon (IFN)-γ-inducible protein-10 (IP-10), interleukin (IL)-1 receptor antagonist protein (IL-1RA), IL-6, IL-8, tumor necrosis factor (TNF)-α, macrophage inflammatory protein (MIP)-1β, and monocyte chemotractant protein-1 (MCP-1, also called chemokine C-C motif ligand [CCL] 2) were remarkably elevated in lung tissues.\(^14\) Moreover, patients with ARDS had significantly higher plasma levels of inflammatory factors including IP-10 and MCP-1 compared with patients showing mild symptoms.\(^15\) Although previous studies suggested connections between systemic inflammation and HTG, the impact of HTG on inflammatory factors in patients with severe influenza virus infection has not been determined.

In the current study, we systematically evaluated levels of inflammatory factors in plasma and bronchoalveolar lavage fluid (BALF) samples from patients with severe influenza virus infection. Based on blood triglyceride levels, these patients were assigned to a control group and a HTG group. We compared the levels of inflammatory factors between these groups to identify inflammatory factors associated with HTG in the setting of severe influenza virus infection.

**Materials and methods**

**Patients**

Patients were recruited between December 2017 and March 2018 from the Medical Intensive Care Unit (MICU) of the Department of Respiratory and Critical Care Medicine, China-Japan Friendship Hospital, Beijing, China. Patients were clinically diagnosed with influenza virus infection. Cases were classified as severe according to any one of the following...
criteria: severe respiratory symptoms with complications such as ARDS, shock, multi-organ failure, hypoxemia, MICU admission, or mechanical ventilation. The study protocol was approved by the Human Ethics Committee of China-Japan Friendship Hospital (No. 201715) and written informed consent was obtained from each participant or caregiver.

**Influenza virus detection**

Influenza virus infection was confirmed by reverse-transcription quantitative polymerase chain reaction using serum samples as templates. RNA was isolated from serum samples and viral RNA was quantitated using detection kits for avian influenza H1N1 and H7N9 (catalogue numbers DA-BZ093 and DA-BN472, respectively; DAAN Gene Co., Ltd., Sun Yat-sen University, Zhongshan City, Guangzhou Province, China) following the manufacturer’s instructions. Briefly, RNA samples were reverse transcribed using Moloney Murine Leukemia Virus reverse transcriptase and conserved viral sequences were amplified by quantitative PCR. The presence of influenza virus RNA was judged by comparison with the cycle threshold (Ct) values for positive and negative controls.

**Plasma and BALF sample preparation**

Blood samples (2 mL) were collected from each patient upon admission to the MICU. Blood was drawn into tubes containing either ethylenediaminetetraacetic acid (lavender top) or sodium heparin (yellow top) as anti-coagulants and centrifuged at 500 × g, 4°C for 20 minutes. Plasma in the upper layer was transferred using a clean pipette into a 1.5-mL microcentrifuge tube. Bronchoscopy and BALF collection was performed as previously described. All samples were stored at –80°C until use.

**Clinical information and laboratory tests**

Clinical information, including demographic characteristics, was retrieved from hospital records. The following routine laboratory tests were performed following standard procedures: complete blood cell counts (CBCs), prothrombin time, a metabolic panel, a lipid panel, and lymphocyte ratios.

**Multiplex immunoassay for quantitating soluble inflammatory factors**

Levels of cytokines, chemokines and growth factors in plasma and BALF samples were determined using the Cytokine/Chemokine/Growth Factor 45-Plex Human ProcartaPlex™ Panel 1 kit (Thermo Scientific, Waltham, MA, USA) according to the manufacturer’s instructions. We used this kit to quantitate the following 45 soluble factors: brain-derived neurotrophic factor (BDNF); Eotaxin/CCL11; epidermal growth factor (EGF); fibroblast growth factor (FGF)-2; granulocyte-macrophage colony-stimulating factor (GM-CSF); growth-regulated oncogene (GRO) α/chemokine C-X-C motif ligand (CXCL)1; hepatocyte growth factor (HGF); nerve growth factor (NGF)-β; leukemia inhibitory factor (LIF); IFN-α; IFN-γ; IL-1β; IL-1α; IL-1RA; IL-2; IL-4; IL-5; IL-6; IL-7; IL-8/CXCL8; IL-9; IL-10; IL-12 p70; IL-13; IL-15; IL-17A; IL-18; IL-21; IL-22; IL-23; IL-27; IL-31; IP-10/CXCL10; MCP-1/CCL2; MIP-1α/CCL3; MIP-1β/CCL4; regulated upon activation normal T cell expressed and secreted (RANTES)/CCL5; stromal cell-derived factor (SDF)-1α/CXCL12; TNF-α; TNF-β/lymphotoxin-α; platelet-derived growth factor (PDGF)-BB; placenta growth factor (PIGF); stem cell factor (SCF), also called mast cell growth factor; vascular endothelial growth factor (VEGF)-A; and VEGF-D.
Statistical analysis
Data were analyzed using GraphPad Prism 7.0 (GraphPad Software, Inc., San Diego, CA, USA). Quantitative data were presented as means ± standard errors of the mean. Differences between two or multiple groups were assessed using the Student’s t test and chi-square test, respectively. Values of p value < 0.05 were considered statistically significant.

Results
Demographic and clinical characteristics of patients with severe influenza infection
Thirty-three patients diagnosed with severe influenza virus infection were categorized into two groups according to their blood triglyceride levels. Seven patients with triglyceride levels >2.3 mmol/L were assigned to the HTG group, while 26 patients with normal triglyceride levels (≤2.3 mmol/L) were assigned to the control group. As shown in Table 1, there were no significant differences in age, male/female gender ratio, height, body weight, and body mass index (BMI) between the HTG group and the control group. The two groups also had similar proportions of patients with a history of smoking and drinking. The prevalence of hypertension (HTN), diabetes mellitus (DM), coronary artery disease (CAD), and chronic kidney disease (CKD) were comparable between the two groups. Around 70% (18/26) of patients in the control group were positive for H1N1 while the

Table 1. Comparison of clinical information for patients with normal triglyceride levels (CON) and hypertriglyceridemia (HTG).

|                        | CON     | HTG     | p     |
|------------------------|---------|---------|-------|
| n                      | 26      | 7       |       |
| Age, years             | 52.0 ± 3.1 | 54.0 ± 4.8 | 0.76  |
| Gender (male/female)   | 13/13 (50%) | 4/3 (42.86%) | 0.99  |
| Height (cm)            | 164.2 ± 1.38 | 167.9 ± 3.88 | 0.28  |
| Weight (kg)            | 67.19 ± 2.03  | 68.14 ± 6.22  | 0.85  |
| BMI                    | 24.87 ± 0.63  | 23.81 ± 1.27  | 0.45  |
| Smoking history (Y/N)  | 8/18 (40.7%) | 2/5 (28.57%) | 0.99  |
| Drinking history (Y/N) | 6/20 (23.08%) | 2/5 (28.57%) | 0.99  |
| HTN (Y/N)              | 9/17 (34.62%) | 1/6 (14.29%) | 0.40  |
| DM (Y/N)               | 12/14 (46.15%) | 3/4 (42.86%) | 0.99  |
| CAD (Y/N)              | 2/24 (7.69%) | 2/5 (28.57%) | 0.19  |
| CKD (Y/N)              | 7/19 (26.92%) | 3/4 (42.86%) | 0.66  |
| H1N1(Y/N)              | 18/8 (69.23%) | 6/1 (85.71%) | 0.64  |
| Outcome (dead/alive)   | 8/16 (30.77%) | 4/3 (57.14%) | 0.38  |
| Combined CMV           | 3/23 (11.54%) | 4/3 (57.14%) | 0.02* |
| Combined aspergillus    | 8/16 (30.77%) | 3/7 (42.86%) | 0.66  |
| SOFA score             | 6.54 ± 0.69  | 6.43 ± 0.61  | 0.94  |
| APACHE II score        | 17.92 ± 1.25 | 15.57 ± 2.22 | 0.38  |
| Oxygenation index      | 156.3 ± 13.15 | 155.8 ± 21.89 | 0.98  |
| ARDS grading           | 2 ± 0.15  | 2 ± 0.32  | 0.99  |
| IPPV (Y/N)             | 21/6 (80.77%) | 5/2 (71.43%) | 0.62  |
| IPPV time              | 24.76 ± 4.83  | 24.8 ± 7.14  | 0.99  |
| ECMO (Y/N)             | 9/17 (34.62%) | 3/4 (42.86%) | 0.69  |

Abbreviations: Y, yes; N, no; BMI, body mass index; HTN, hypertension; DM, diabetes mellitus; CAD, coronary artery disease; CKD, chronic kidney disease; CMV, cytomegalovirus; SOFA, Sequential Organ Failure Assessment; APACHE, Acute Physiology and Chronic Health Evaluation; ARDS, acute respiratory distress syndrome; IPPV, intermittent positive-pressure ventilation; ECMO, extracorporeal membrane oxygenation. *p<0.05.
The majority of patients (6/7) in the HTG group were positive for H1N1. The prevalence of combined aspergillus infection was similar between the two groups. However, the prevalence of combined cytomegalovirus (CMV) infection in the HTG group was significantly higher than that in the control group (Table 1). There were no significant differences in respiratory function and overall health status-associated parameters, including the Sequential Organ Failure Assessment (SOFA) Score, the Acute Physiology and Chronic Health Evaluation (APACHE II), oxygenation index, ARDS grading, use of intermittent positive-pressure ventilation (IPPV), and use of extracorporeal membrane oxygenation (ECMO), between the two groups of patients. Mortality in the HTG group (57%) was higher than that in the control group (30%), but this difference was not statistically significant ($p = 0.38$; Table 1).

We also compared parameters from routine laboratory tests including CBCs, a metabolic panel, a lipid panel, electrolytes, and lymphocyte ratios between the two groups. As shown in Table 2, the control and HTG groups showed no significant differences in most of these parameters. Levels of total lymphocytes, CD3$^+$ T cells, CD3$^+$CD4$^+$ T cells, and other parameters were also compared, but no significant differences were found between the two groups. The detailed laboratory test parameters are shown in Table 2.

### Table 2. Comparison of laboratory test parameters for patients with normal triglyceride levels (CON) and hypertriglyceridemia (HTG).

| Parameter          | CON         | HTG         | p  |
|--------------------|-------------|-------------|----|
| WBC ($\times 10^9$/L) | 11.13 ± 1.57 | 8.17 ± 1.58 | 0.35 |
| NEUT %             | 84.22 ± 2.22 | 87.31 ± 2.89 | 0.50 |
| LY ($\times 10^9$/L) | 0.81 ± 0.08  | 0.55 ± 0.13  | 0.13 |
| HGB (g/L)          | 114.1 ± 4.88 | 118.1 ± 9.29 | 0.70 |
| PLT ($\times 10^9$/L) | 178.6 ± 13.63 | 184.7 ± 36.23 | 0.85 |
| ALT (IU/L)         | 83.85 ± 37.38 | 63.57 ± 14.08 | 0.78 |
| AST (IU/L)         | 116 ± 42.39  | 80.71 ± 15.56 | 0.67 |
| TBIL ($\mu$mol/L)  | 12.37 ± 2.34 | 20.9 ± 9.4   | 0.20 |
| DBIL ($\mu$mol/L)  | 7.57 ± 1.67  | 12.81 ± 6.37 | 0.27 |
| Cr ($\mu$mol/L)    | 83.72 ± 14.65 | 105.6 ± 29.86 | 0.50 |
| Urea (mmol/L)      | 7.49 ± 0.97  | 9.77 ± 1.82  | 0.28 |
| GLU (mmol/L)       | 10.94 ± 1.06 | 9.41 ± 1.57  | 0.49 |
| Na (mmol/L)        | 138.3 ± 1.37 | 137.1 ± 2.04 | 0.69 |
| K (mmol/L)         | 3.77 ± 0.07  | 4.11 ± 0.14  | 0.04* |
| Cl (mmol/L)        | 104.4 ± 1.15 | 102.4 ± 1.81 | 0.42 |
| CHO (mmol/L)       | 3.21 ± 0.24  | 3.72 ± 0.40  | 0.32 |
| HDL-C (mmol/L)     | 0.97 ± 0.10  | 0.60 ± 0.12  | 0.09 |
| LDL-C (mmol/L)     | 1.67 ± 0.19  | 1.73 ± 0.45  | 0.91 |
| PT (s)             | 15.29 ± 0.43 | 14.64 ± 0.51 | 0.46 |
| FIB (g/L)          | 9.02 ± 4.01  | 5.73 ± 0.58  | 0.68 |
| D-Dimer (mg/L)     | 7.14 ± 1.33  | 8.37 ± 2.63  | 0.68 |
| PCT (ng/ml)        | 7.16 ± 3.06  | 1.67 ± 0.71  | 0.36 |
| CD3$^+$ (cells/µl) | 624.6 ± 53.52 | 476.7 ± 157.3 | 0.26 |
| CD3$^+$CD4$^+$ (cells/µl) | 385.6 ± 40.18 | 253.4 ± 67.86 | 0.13 |
| CD3$^+$CD8$^+$ (cells/µl) | 221.5 ± 22.73 | 208.3 ± 92.68 | 0.84 |

Abbreviations: WBC, white blood cell; NEUT, neutrophil; LY, lymphocyte; HGB, hemoglobin; PLT, blood platelet; ALT, alanine transaminase; AST, aspartate aminotransferase; TBIL, total bilirubin; DBIL, direct bilirubin; CR, creatinine; GLU, blood glucose; Na, sodium; K, potassium; Cl, chloride; CHO, cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; PT, prothrombin time; FIB, fibrinogen; PCT, procalcitonin. *$p < 0.05$. 

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cells, and CD3⁺CD8⁺ T cells were slightly lower in the HTG group compared with the control group, but these differences were not statistically significant. Similarly, HTG was associated with trends toward reduced levels of high-density lipoprotein cholesterol (HDL-C) and serum procalcitonin (PCT) and increased levels of total bilirubin (TBIL) and urea, but these differences were not statistically significant. Notably, serum potassium levels in the HTG group were significantly higher than those in the control group ($p = 0.04$). However, the mean values for serum potassium in both groups were within the normal range (Table 2).

Comparison of inflammatory factors levels in plasma and BALF samples from patients with and without HTG

To explore HTG-associated changes in soluble inflammatory factors in patients with severe influenza, we used ProcartaPlex immunoassays to quantitate proteins in plasma and BALF samples. Using Luminex xMAP technology, the Human Cytokine & Chemokine & Growth Factor 45-plex ProcartaPlex Panel 1 kit allowed simultaneous detection of 45 soluble factors. Plasma levels of 10 factors were identified as increased in patients with HTG ($p \leq 0.05$) (Table 3). Compared with control patients, patients with HTG had significantly elevated plasma levels of cytokines including IFN-γ, IL-18, IL-1RA, MCP-1 (CCL2), MIP-1α (CCL3), MIP-1β (CCL4), and SDF-1α (CXCL12) and of growth factors including HGF, SCF, and VEGF-A (Table 3 and Figure 1).

We subsequently focused on these 10 inflammatory factors and compared their levels in BALF samples. Most of these factors showed no significant differences between control and HTG patients (Table 4). Levels of IFN-γ, MIP-1α, and MIP-1β in BALF samples from the HTG group were much lower on average than those in the control group. However, these differences were not statistically significant, probably because of the small sample size of the HTG group. Nevertheless, a significant reduction in IL-1RA levels and a slight increase in IP-10 (CXCL10) levels were observed in BALF samples from HTG patients (Figure 2). No significant differences in levels of other soluble factors in BALF were observed between the two groups (Table 4).

Discussion

Obesity contributes to elevated risk of influenza virus infection for nearly 500 million obese individuals worldwide. HTG is a common lipid abnormality associated with visceral obesity, metabolic syndrome, and type 2 diabetes. Obesity was suggested to impair the immune response to influenza and vaccination through changes in cellular immunity. Inflammation and viral infections induce a variety of alterations in lipid metabolism such as decreases in serum HDL and increases in triglycerides.

In the present study, we identified multiple inflammatory factors associated with HTG in patients with severe influenza virus infection. Among the 45 soluble factors we profiled, plasma levels of eight inflammatory factors (IFN-γ, IL-18, IL-1RA, MCP-1, MIP-1α, HGF, SCF, and VEGF-A) were significantly elevated in patients with HTG. Levels of IL-10 in BALF samples from patients with HTG were significantly increased and levels of IL-1RA were markedly decreased compared with levels in patients with normal triglyceride levels.

Obesity, type 2 diabetes, high blood triglycerides, and atherogenesis are characterized by low-grade underlying inflammation. Consistently, we observed increased plasma concentrations of inflammatory cytokines in patients with HTG, and thus we propose a correlation between these inflammatory factors.
Table 3. Plasma levels of 45 inflammatory factors in patients with normal triglyceride levels (CON) and hypertriglyceridemia (HTG).

|        | CON        | HTG        | p         |
|--------|------------|------------|-----------|
| IFN-γ  | 51.28 ± 5.08 | 107.4 ± 7.68 | 0.01*     |
| IL-2p70| —          | —          | —         |
| IL-13  | —          | —          | —         |
| IL-1β  | 7.24 ± 0.99 | 11.75 ± 5.04 | 0.15      |
| IL-2   | 18.94 ± 1.50 | 17.29 ± 2.86 | 0.61      |
| IL-4   | —          | —          | —         |
| IL-5   | 55.48 ± 5.89 | 71.52 ± 14.09 | 0.30      |
| IL-6   | 277.3 ± 73.47 | 484.9 ± 244.8 | 0.28      |
| TNF-α  | 39.17 ± 4.09 | 33.7 ± 6.40  | 0.52      |
| GM-CSF | —          | —          | —         |
| IL-18  | 75.64 ± 8.39 | 148.5 ± 9.90 | 0.01*     |
| IL-10  | 17.54 ± 8.55 | 17.64 ± 3.52 | 0.99      |
| IL-17A | 10.87 ± 3.83 | 16.04 ± 6.45 | 0.62      |
| IL-21  | —          | —          | —         |
| IL22   | —          | —          | —         |
| IL-23  | —          | —          | —         |
| IL-27  | —          | —          | —         |
| IL-9   | —          | —          | —         |
| IFN-α  | —          | —          | —         |
| IL-31  | —          | —          | —         |
| IL-15  | —          | —          | —         |
| IL-1x  | —          | —          | —         |
| IL-1RA | 2639 ± 587.1 | 8120 ± 3470 | 0.01*     |
| IL-7   | 3.66 ± 0.62  | 6.33 ± 2.93  | 0.16      |
| TNF-β  | —          | —          | —         |
| Eotaxin| 25.09 ± 2.02  | 30.46 ± 7.66  | 0.33      |
| GRO-α  | 23.96 ± 6.22  | 21.41 ± 4.42  | 0.84      |
| IL-8   | 46.58 ± 13.73 | 56.33 ± 15.99 | 0.75      |
| IP-10  | 203.1 ± 33.03 | 233.9 ± 38.91 | 0.65      |
| MCP-1  | 223.5 ± 35.11 | 456.1 ± 136.3 | 0.02*     |
| MIP-1α | 17.42 ± 2.24  | 27.92 ± 4.41  | 0.04*     |
| MIP-1β | 62.38 ± 9.48  | 119.9 ± 43.84 | 0.05      |
| SDF-1α | 190.3 ± 14.28 | 310.6 ± 105.3 | 0.05      |
| RANTES | 108.4 ± 10.41 | 120.7 ± 21.38 | 0.61      |
| NGF-β  | 40.63 ± 5.43  | 50.41 ± 16.23 | 0.47      |
| BDNF   | 62.85 ± 13.83 | 36.44 ± 13.68 | 0.34      |
| EGF    | 14.66 ± 2.14  | 14.27 ± 2.31  | 0.93      |
| FGF-2  | 24.92 ± 4.37  | 41.37 ± 15.94 | 0.17      |
| HGF    | 1125 ± 204.8  | 3542 ± 1041  | 0.01*     |
| LIF    | 19.14 ± 4.15  | 15.53 ± 3.35  | 0.64      |
| PDGF-BB| 667.2 ± 87.31 | 708 ± 180    | 0.83      |
| PIGF-1 | 30.67 ± 11.1  | 24.53 ± 8.82  | 0.77      |
| SCF    | 12.69 ± 1.13  | 29.58 ± 4.89  | 0.01*     |
| VEGF-A | 402.3 ± 58.25 | 763.7 ± 194.9 | 0.02*     |
| VEGF-D | 50.05 ± 9.46  | 44.74 ± 16.51 | 0.79      |

All inflammatory factors are shown in pg/mL; *p<0.05; –, undetectable.

Abbreviations: brain-derived neurotrophic factor (BDNF); epidermal growth factor (EGF); fibroblast growth factor-2 (FGF-2); granulocyte-macrophage colony-stimulating factor (GM-CSF); growth-regulated oncogene-α (GRO-α); hepatocyte growth factor (HGF); nerve growth factor-β (NGF-β); leukemia inhibitory factor (LIF); interferon (IFN)-α and IFN-γ; interleukin (IL); interleukin-1 receptor antagonist (IL-1RA); interferon-γ-inducible protein-10 (IP-10); monocyte chemoattractant protein-1 (MCP-1); macrophage inflammatory protein (MIP)-1α and MIP-1β; regulated upon activation normal T cell expressed and secreted (RANTES); stromal cell-derived factor-1α (SDF-1α); tumor necrosis factor (TNF)-α and TNF-β; platelet-derived growth factor (PDGF)-BB; placenta growth factor (PIGF); stem cell factor (SCF); and vascular endothelial growth factor (VEGF)-A and VEGF-D.
soluble factors and HTG in the setting of severe influenza virus infection. IFN-γ and IL-18 levels were both increased more than two-fold in plasma samples from HTG patients. However, levels of neither cytokine were elevated in BALF samples from HTG patients. IL-18 controls both food intake and energy homeostasis. In patients with obesity and type 2 diabetes, an increased IL-18 concentration might represent a retro-control mechanism that can compensate for insulin resistance induced by other

Figure 1. Plasma levels of inflammatory factors in influenza-infected patients with normal triglyceride levels and hypertriglyceridemia. CON, patients with normal triglyceride levels (n=26); HTG, patients with hypertriglyceridemia (n=7). Each dot represents the concentration of the indicated factor in plasma from an individual patient.
| Table 4. BALF levels of 45 inflammatory factors in patients with normal triglyceride levels (CON) and hypertriglyceridemia (HTG). |
|-----------------|----------------|----------------|
|                  | CON            | HTG            | p   |
| IFN-γ           | 39.96 ± 5.43   | 16.81 ± 2.31   | 0.05 |
| IL-12p70        | -              | -              | -   |
| IL-13           | 0.31 ± 0.06    | 0.22 ± 0.05    | 0.39 |
| IL-1β           | 272.1 ± 88.11  | 10.35 ± 3.21   | 0.14 |
| IL-2            | 11.46 ± 0.95   | 13.79 ± 3.00   | 0.36 |
| IL-4            | -              | -              | -   |
| IL-5            | -              | -              | -   |
| IL-6            | 1151 ± 206.5   | 707.2 ± 253.5  | 0.30 |
| TNF-α           | 27.69 ± 3.69   | 26.98 ± 7.29   | 0.93 |
| GM-CSF          | -              | -              | -   |
| IL-18           | 41.94 ± 6.93   | 34.03 ± 6.31   | 0.63 |
| IL-10           | -              | -              | -   |
| IL-17A          | -              | -              | -   |
| IL-21           | -              | -              | -   |
| IL-22           | -              | -              | -   |
| IL-23           | -              | -              | -   |
| IL-27           | -              | -              | -   |
| IL-9            | -              | -              | -   |
| IFN-α           | -              | -              | -   |
| IL-31           | -              | -              | -   |
| IL-15           | -              | -              | -   |
| IL-1α           | -              | -              | -   |
| IL-1RA          | 13005 ± 1540   | 5680 ± 1000    | 0.02*|
| IL-7            | 3.99 ± 0.61    | 6.65 ± 4.27    | 0.26 |
| TNF-β           | -              | -              | -   |
| Eotaxin         | 28.58 ± 4.37   | 20.87 ± 8.53   | 0.42 |
| GRO-α           | 243.4 ± 15.74  | 291.1 ± 14.03  | 0.14 |
| IL-8            | 770.7 ± 124.4  | 600.5 ± 145.8  | 0.51 |
| IP-10           | 402 ± 54.9     | 648.1 ± 65.42  | 0.04*|
| MCP-1           | 806.9 ± 59.68  | 825.7 ± 106.6  | 0.88 |
| MIP-1α          | 142.8 ± 22.71  | 56.02 ± 13.86  | 0.06 |
| MIP-1β          | 1049 ± 211.8   | 302.2 ± 131.2  | 0.08 |
| SDF-1α          | 702.3 ± 74.59  | 705.4 ± 51.06  | 0.98 |
| RANTES           | 28.63 ± 3.53   | 21.85 ± 3.39   | 0.35 |
| NGF-β           | -              | -              | -   |
| BDNF            | -              | -              | -   |
| EGF             | 89.19 ± 14.06  | 92.02 ± 35.25  | 0.93 |
| FGF-2           | 55.52 ± 24.43  | 12.93 ± 4.50   | 0.39 |
| HGF             | 2102 ± 395     | 1854 ± 461.4   | 0.69 |
| LIF             | 100.1 ± 25.06  | 40.41 ± 10.38  | 0.23 |
| PDGF-BB         | 211.8 ± 38.24  | 100.7 ± 28.96  | 0.16 |
| PIGF-1          | 8.38 ± 1.20    | 6.53 ± 0.96    | 0.44 |
| SCF             | 19.8 ± 5.77    | 26.59 ± 6.80   | 0.56 |
| VEGF-A          | 1144 ± 225.9   | 700.4 ± 379.1  | 0.36 |
| VEGF-D          | 15.02 ± 3.79   | 14.1 ± 6.85    | 0.92 |

All inflammatory factors are shown in pg/mL; *p<0.05; -, undetectable.
Abbreviations: brain-derived neurotrophic factor (BDNF); epidermal growth factor (EGF); fibroblast growth factor-2 (FGF-2); granulocyte-macrophage colony-stimulating factor (GM-CSF); growth-regulated oncogene-α (GRO-α); hepatocyte growth factor (HGF); nerve growth factor-β (NGF-β); leukemia inhibitory factor (LIF); interferon (IFN)-α and IFN-γ; interleukin (IL); interleukin-1 receptor antagonist (IL-1RA); interferon-γ-inducible protein-10 (IP-10); monocyte chemoattractant protein-1 (MCP-1); macrophage inflammatory protein (MIP)-1α and MIP-1β; regulated upon activation normal T cell expressed and secreted (RANTES); stromal cell-derived factor-1α (SDF-1α); tumor necrosis factor (TNF)-α and TNF-β; platelet-derived growth factor (PDGF)-BB; placenta growth factor (PIGF); stem cell factor (SCF); and vascular endothelial growth factor (VEGF)-A and VEGF-D.
pro-inflammatory cytokines.\textsuperscript{24,25} In addition, IL-18 is involved in the activation of Th1/Th2 cells, natural killer (NK) cells, IL-17-producing CD\textsubscript{4}+ T cells, and macrophages. IL-18 can also protect the host during the early response against influenza virus infection by enhancing NK cell cytotoxicity and IFN-\gamma production.\textsuperscript{26–28} Interestingly, avian H5N1 and H7N9 influenza viruses encode a PB1-F2 protein which helps suppress IFN-\alpha secretion and activates the NLR Family Pyrin Domain Containing 3 (NLRP3) protein.\textsuperscript{29} Sustained NLRP3 activation can result in massive IL-18 production and lead to a detrimental IFN-\gamma-biased cytokine storm that plays a critical role in the pathogenesis of ARDS.\textsuperscript{30–32}

Similarly, an association between HTG and serum IL-18 levels was reported in human immunodeficiency virus (HIV)-infected patients, suggesting a potential role for IL-18 in the pathogenesis of metabolic disorders among HIV-infected patients.\textsuperscript{33}

IL-1RA, MCP-1, and MIP-1\alpha showed slightly but significantly increased levels in plasma samples from influenza virus-infected patients with HTG. In a mouse HTG model, IL-1RA effectively blocked the increase in serum triglycerides induced by IL-1.\textsuperscript{34} It is worth noting that a significant decrease, not an increase, in IL-1RA levels was observed in BALF samples from HTG patients, which might be attributed to the function of IL-1RA in down-regulating serum triglyceride levels. MCP-1 appears to be the major chemotactic factor produced within the vessel wall and can be identified in areas rich in macrophages. MIP-1\alpha is secreted by endothelial cells, macrophages, and fibroblasts, and functions to recruit monocytes.\textsuperscript{35} Increased levels of MCP-1/CCL2 can predict the risk of developing diabetes independent of clinical, metabolic, and other inflammatory risk factors.\textsuperscript{35} Consistent with our findings, MCP-1 levels has been reported to be associated with HTG, even in influenza virus-uninfected individuals.\textsuperscript{36}

To the best of our knowledge, our study is the first to report increased plasma levels of HGF, SCF, and VEGF-A in patients with influenza virus infection complicated by HTG. HGF was initially identified as a potent hepatotrophic factor responsible for liver regeneration after partial hepatectomy or liver injury.\textsuperscript{37} Moreover, in vascular endothelial cells, HGF potentiates angiogenic activity.\textsuperscript{38} HGF also acts as a paracrine factor to promote morphogenesis, cell growth, and cell motility.\textsuperscript{39} SCF is a hematopoietic growth factor that binds to the receptor tyrosine kinase c-KIT (CD117), exerting its activity during the early stages of hematopoiesis. SCF binding promotes the proliferation of myeloid, erythroid, and lymphoid progenitors in the bone marrow. Therefore, increased SCF levels might reflect enhanced hematopoiesis in...
patients with HTG. Members of the VEGF family (VEGF-A and VEGF-B) are involved in vascular inflammation and remodeling through proinflammatory and angiogenic mechanisms.\textsuperscript{40} Several studies have reported increased levels of circulating VEGF-A were associated with human obesity.\textsuperscript{41,42} Similarly, a significant decrease in VEGF-A levels was observed in patients with dramatic weight loss following gastric bypass surgery, intensive dietetic intervention, or other bariatric surgery procedures.\textsuperscript{42,43} Thus, our study highlights a potential role of the VEGF/VEGF receptor system in the pathogenesis of HTG in the setting of influenza virus infection and suggests that interventions targeting this system may be a valuable strategy for the prevention and treatment of HTG.

Our investigation had several strengths and limitations. Its major strength was its novelty, as this is the first systematic profiling of inflammatory factors in the serum and BALF of patients with severe influenza virus infection and HTG. The major limitation of our study was its small sample size of only 33 patients. Given the inherent biases associated with influenza virus disease and the presence of variables for which we cannot account, the small sample size may have resulted in missed associations between HTG and the 45 candidate inflammatory factors. The patients included in this study were drawn from a single institution, and thus our results may have been affected by referral bias. Although additional investigations are needed to further elucidate the roles of the soluble factors mentioned above, we believe that our data provide useful insights that may enable effective treatment of patients with severe influenza virus infection and HTG.

\textbf{Authors’ contributions}

Conception and design: Tianshu Zhai, Xiaojing Wu, and Qingyuan Zhan; development of methodology: Nannan Zhang; acquisition of data (acquired and managed patients, provided facilities, etc.): Nannan Zhang and Tianshu Zhai; analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Tianshu Zhai, Xiaojing Wu, and Qingyuan Zhan; writing, review, and/or revision of the manuscript: Tianshu Zhai, Xiaojing Wu, and Qingyuan Zhan; administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Qingyuan Zhan; study supervision: Qingyuan Zhan.

\textbf{Declaration of conflicting interest}

The authors declare that there is no conflict of interest.

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