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Short communication

Association of TNF-α, TGF-β1, amphiregulin, IL-2, and EGFR WITH pulmonary fibrosis in COVID-19

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ABSTRACT

Pulmonary fibrosis is a well-recognized sequela associated with coronavirus disease 2019 (COVID-19), however the mechanism is yet to be clearly understood. The study was designed to evaluate the association of TNF-α, TGF- β1, amphiregulin, IL-2, and EGFR with pulmonary fibrosis after COVID-19 pneumonia. Non-severe, severe, and critical COVID-19 pneumonia patients were included in this study after the patients agreed and gave written informed consent. Blood samples were analyzed with the ELISA method for cytokine examination. The non-contrast chest CT scan was performed after patients were discharged from hospital. Seventy-nine patients with a mean age of 54 years (57 % men, 43 % women) were fully evaluated. Pulmonary fibrosis was found in 74 patients (93.7 %). Serum levels of TGF-β1 60.55 pg/mL (11.42–2001.16), TNF-α 13.31 pg/mL (3.54–200.32), EGFR 14.9 pg/mL(6.4–53.6), IL-2 12.41 pg/mL(11–14.13), amphiregulin 156.5 pg/mL (21.7–1234). Serum levels of TNF-α increase according to the severity of clinical classification. A significant association between serum levels of TGF-β1, TNF- α, and pulmonary fibrosis with rs-0.247, p = 0.027; rs 0.259, p = 0.046 was found. According to this study, TNF-α and TGF-β1 potentially participate in the process of pulmonary fibrosis in COVID-19.

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Introduction

Pulmonary fibrosis after coronavirus disease 2019 (COVID-19) infection is one of the most recorded sequelae. The prevalence of post-COVID-19 pulmonary fibrosis is up to 83.3 % [1,2]. Fibrosis is characterized by the exaggerated build-up of the extracellular matrix (ECM), the outcome of ECM synthesis rise or degradation decline, or the combination of them. A close relationship between tissue repair, fibrosis, and various mediators are seen during the fibrosis formation process [3].

Tumor Necrosis Factor (TNF) is a pleiotropic cytokine found in two forms, transmembrane and soluble form. TNF function is noticeable when it binds to receptors TNFR1 and TNFR2. TNF and TNFR1 binding promote cell death and inflammation [4]. TNF is expressed by various cells in response to infection. Soluble form plays a role in TGF-β1 expression and fibrotic lesion formation [5]. The serum level of TNF-α, which is one of the cytokines involved in immune response towards COVID-19, increases during illness and decreases during recovery. Higher TNF- α levels were seen in severe COVID-19 patients who require intensive-care in the intensive care unit (ICU) [6].

Activation of type 1 and 2 receptors by TGF-β1 will activate the Smad-signaling pathway and the Smad-independent pathway depending on the cell type and microenvironment. Activation of the TGF-β1 signaling pathway will ultimately result in the increased production of profibrotic mediators and ECM proteins [7]. In the study of TGF-β1 with mice, Zhou et al. reported that pulmonary overexpression of TGF-β1 substantially promotes amphiregulin, an Epidermal Growth Factor Receptor (EGFR) ligand, and amphiregulin which stimulates EGFR signaling is essential for Smad and non-Smad TGF-β signaling [8]. Amphiregulin stimulates fibroblast proliferation via EGFR signaling by triggering phosphatidylinositol 3-kinase (PI3K) and Mitogen-Activated Protein Kinase (MAPK)/Erk [7]. This study aims to evaluate the association of TGF-β1, amphiregulin, EGFR, TNF-α, and pulmonary fibrosis after COVID-19 pneumonia.

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Materials and methods

Patients and study design

This cross-sectional study was conducted in the COVID-19 high care unit (HCU) and intensive care unit (ICU) from June 2020 to February 2021. Blood samples were taken after the patient agreed and gave written informed consent. Non-contrast thorax CT scan was performed on improving and discharged patients (after two negative reverse transcription-polymerase chain reaction (RT-PCR) swabs of SARS-CoV-2).

Study subjects were male and female adult patients aged 18 years old or older who were diagnosed as COVID-19 pneumonia based on positive RT-PCR results of SARS-CoV-2 nasal and oropharyngeal swabs (including non-severe, severe, and critical COVID-19 pneumonia). Patients with the conditions such as pregnancy, chronic obstructive pulmonary disease (COPD), pulmonary tuberculosis, autoimmune disease, lung cancer, and hypertensive heart disease with amiodarone therapy were excluded. The classification of COVID-19 severity was determined based on the World Health Organization (WHO) 2020 criteria [9].

Cytokine testing

Serum levels of TGF-β1, TNF-α, EGFR-, IL-2, and amphiregulin were analyzed by Enzyme-Linked Immunosorbent Assay (ELISA) using the BD CBA Flex set kit (BD Biosciences Pharmingen, USA) according to manufacturer's protocol.

Thin-section chest CT scan and pulmonary fibrosis criteria

A non-contrast chest CT scan was performed after the patient was discharged from the hospital (after two negative RT-PCR results). Pulmonary fibrosis is defined when the following features were found: 1) Honeycombing 2) Traction bronchiectasis or bronchiolectasis, 3) Bronchial wall thickening4) Parenchymal bands [2]. Pulmonary fibrosis was divided into 3 groups based on the score, mild 1–7, moderate 8–14, and severe > 14 1 [10]. Images were analyzed by a thoracic radiologist.

Statistical analysis

Categorical variables are expressed in terms of frequency and percentage. The orderly distributed variables were disclosed in means ± standard deviation, while data with abnormal distribution were disclosed as median and interquartile range. The correlation was interpreted with the Spearman's rank test and the p-value < 0.05 was deemed as statistically significant.

Results

Within the 9 months study period, there were 79 patients. More than half of the research subjects were male (57 %), with a mean age of 54.6 years old, and 74.7 % with severe pneumonia and critical/ARDS. Serum levels of TGF-β1 and amphiregulin in severe and critical pneumonia were lower than in non-severe cases. Serum levels of TNF-α increased according to the severity of clinical classification and serum levels of EGFR and IL-2 were almost the same in all clinical classifications (Table 1). The more severe the clinical classification, the higher the pulmonary fibrosis score (Table 1). Chest CT scan were performed 33.70 ± 10.53 days since the onset of symptoms.

The results of the Spearman correlation test showed that there was a significant relationship between TGF-β1 and pulmonary fibrosis ($p < 0.05$) with a negative direction and weak strength (0.2–0.4). Likewise, there was a significant relationship between TNF-α and pulmonary fibrosis ($p < 0.05$) with a positive relationship along with weak strength (0.2–0.4). The results of the Spearman correlation test showed no significant relationship between EGFR-IL-2, amphiregulin, and pulmonary fibrosis (> 0.05). The results of the logistic regression test illustrated that comorbidity had no effect on pulmonary fibrosis after COVID-19 (p=0.732, regression coefficient -0.393, OR (95% CI) 0.675, R^2 0.004.

Discussion

This study found a substantial correlation between serum TGF-β1, TNF-α, and pulmonary fibrosis after COVID-19 pneumonia. TGF-β1 expression in COVID-19 is dynamic, at the beginning of infection (3–10 days from symptom onset), TGF-β1 mRNA expression from upper airway samples is lower than controls [11]. Wang et al. reported that serum levels of TGF-β1 in moderate and severe/critical COVID-19 increased significantly from 0 to 10 days from the beginning of symptoms up to the outcome of the disease progression (41–50 days from onset of symptoms), in convalescence, TGF-β1 levels were similar to the healthy controls. Moderate COVID-19 serum TGF-β1 levels were higher than severe/critical and mild [12]. In this study, serum TGF-β1 levels of 60.55 pg/mL were taken 19 days from symptom onset, and serum levels of TGF-β1 in non-severe COVID-19 were higher than severe and critical.

Various cytokines, including TNF, IL-6, IL-1β, and TGF-β1 were highly associated with fibrosis. Transforming growth factor (TGF-β), tumor necrosis factor-α (TNF-α), and platelet-derived growth factor (PDGF) are secreted when the alveolar epithelial cells are injured. These cytokines, including TNF-α, can facilitate the formation of fibroblasts which in turn can lead to collagen deposition. A study on COVID-19 differentially expressed gene known to be associated with pulmonary fibrosis has identified that the TNF signaling pathway is one of the molecular pathways of pulmonary fibrosis secondary to COVID-19 [13]. Cheng et al. reported an up-regulated TNF- α gene in cells infected with SARS-CoV2 [14]. Serum values of TNF- α and sTNFR1 increased in patients with COVID-19. Serum levels of TNF-α and sTNFR1COVID-19 in ICU and non ICU were significantly higher than in healthy controls [15]. TNF-α values from respiratory samples were significantly greater in symptomatic COVID-19 patients in comparison to patients without symptoms in the initial phases of the condition [16]. This study found that serum levels of TNF-α increased according to the severity of clinical classification (Table 1). TNF-α receptor activation will form complex I, IIa, IIb, IIC. Complex I is formed when there is ubiquitinated RIPK1 and causes activation of NF-κB, JNK, and p38 signaling, which then produces proinflammatory molecules [4]. The expression of TNF-α is increased in various lung fibrotic diseases and the elevation of TNF-α level in many fibrotic diseases is accompanied by an increase in PAI-1 expression. PAI-1 (plasminogen activator inhibitor 1) is an inhibitor of plasminogen activators.

In this study, the incidence of pulmonary fibrosis after COVID-19 was high (93.7 %). The longstanding results of pulmonary COVID-19 sequelae are not completely known. From one case study, pulmonary fibrosis in COVID-19 disappeared after 65 days from symptom onset [17]. The SARS pulmonary lesion evaluated by CT scan showed rapid improvement within one year of recovery and subsequently persisted for up to the following 14 years [18]. In this study pulmonary fibrosis was mediated by TGF-β1 and TNF-α (Fig. 1). Do these different mechanisms affect the resolution of pulmonary fibrosis?

This study has several limitations: 1. This study only measured serum biomarker levels. Whether the levels in the peripheral blood are identical to the local environment of the lung tissue is not known. 2. This study did not have healthy individuals as controls so the biomarkers could not be compared with controls. 3. There is no sequential examination of TGF-β1 and TNF-α. Therefore, the

Table 1
Biomarker and CT scan score.

Variable	Non-severe Pneumonia/ Moderate	Severe Pneumonia	Critical/ ARDS	Total
TGF-β1*(pg/mL)	77.01 (11.42–1869.23)	49.51 (11.93–1014.39)	61.33 (17.78–2001.16)	60.55 (11.42–2001.16)
TNF-α* (pg/mL)	4.97 (3.54–139.07)	11.44 (3.67–77.4)	21.08 (4.53–200.32)	13.31 (3.54–200.32)
EGFR* (pg/mL)	14.95 (10.4–37.5)	13.8 (6.4–33.9)	15.9 (7.7–53.6)	14.9 (6.4–53.6)
IL-2* (pg/mL)	12.6 (11–14.01)	12.23 (11.35–13.16)	12.6 (11.23–14.13)	12.41 (11–14.13)
Amphiregulin* (pg/mL)	179.3 (52.1–1234)	132.6 (28.2–1234)	156.5 (21.7–905.7)	156.5 (21.7–1234)
Chest CT scan score#	4.45 ± 3.22	8.08 ± 4.24	10.76 ± 3.33	8.32 ± 4.38

*median #mean ARDS, acute respiratory distress syndrome; TGF, transforming growth factor; TNF, tumor necrosis factor; EGFR, epidermal growth factor receptor; IL, interleukin.

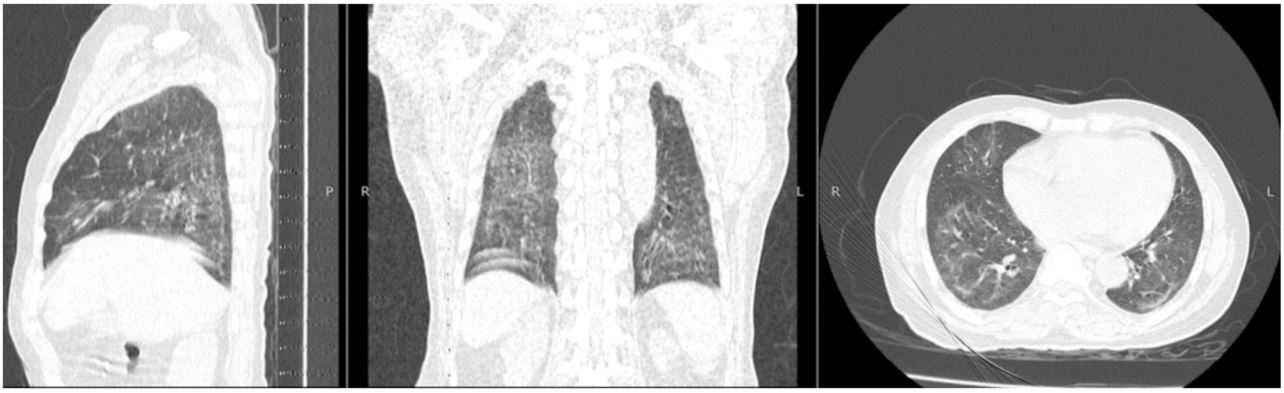

Fig. 1. Chest CT images of a 48-year-old male, forty-four days post covid from symptom onset with a score of 16 (severe), showed a parenchymal band in both lung and traction bronchiectasis and brochiolectasis in the apical segment of the right upper lobe and posterobasal segment of the right lower lobe.

association of TGF-β1, TNF- and fibrosis cannot be explained in detail. 4. Chest CT Scan was done after two negative reverse transcription-polymerase chain reaction (RT-PCR) swabs of SARS-CoV-2 (conversion).

Conclusion

TNF-α and TGF-β1 might play a role in pulmonary fibrosis in COVID-19 after discharge.

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Ethical approval

This study was granted permission from the ethics commission of Dr. Soetomo Academic Hospital (No: 0008/KEPK/V/2020) and followed the Helsinki Declaration.

Credit authorship contribution statement

Conceptualization: DM; Patients and samples: DM, HH, AB; Collection and interpretation of radiological material: AW; Blood sample examination: A; Writing-original draft: DM; The authors had seen and approved the manuscript.

Competing interests

The authors have no conflicts of interest to declare that are relevant to this study.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jiph.2022.08.007.

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