Serum levels of SIRT3 and other inflammatory factors are associated with clinical outcomes and prognosis in severe community-acquired pneumonia in adults

A prospective study

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Abstract

The aim of this study was to investigate clinical significance of SIRT3 in severe community-acquired pneumonia (CAP) patients.

This prospective observational research enrolled a total of 114 severe CAP patients who went to our hospital during January 2018 to December 2019. Serum SIRT3 and IL-1\(\beta\), IL-6, and tumor necrosis factor (TNF-\(\alpha\)) levels were determined using the enzyme-linked immunosorbent assay (ELISA) method. Demographic data, including age, sex, and body mass index (BMI), as well as clinical symptoms, SOFA and SMART-COP scores were collected. The routine blood test was conducted for all patients and white blood cell (WBC) amount, as well as serum levels of C-reactive protein (CRP), D-Dimer, and procalcitonin (PCT).

Among all patients, 55 cases died during the study period. The serum levels of CRP, PCT, IL-1\(\beta\), and IL-6, as well as SOFA and SMART-COP scores were markedly higher in deceased patients than in the survival patients. The expression of SIRT3 was significantly decreased in severe CAP patients compared with the healthy, especially in the deceased patients. SIRT3 levels were negatively correlated with levels of CRP, PCT, IL-1\(\beta\), and IL-6. Patients with SIRT3 low expression showed remarkably higher expression of CRP, PCT, IL-1\(\beta\), and IL-6, as well as high SMART-COP scores, higher 1-month mortality rate, and shorter survival.

Only SIRT3 and IL-1\(\beta\) were independent risk factors for 1-month mortality in severe CAP patients.

Lower serum SIRT3 level predicts poor clinical outcomes and prognosis in severe CAP patients.

Abbreviations: ATS = American Thoracic Society, BMI = body mass index, BUN = blood urea nitrogen, CAP = community-acquired pneumonia, Cr = creatinine, CRP = C-reactive protein, CRP = C-reactive protein, ELISA = enzyme linked immunosorbent assay, MLR = monocyte to lymphocyte ratio, NLR = lymphocyte ratio, PCT = procalcitonin, PCT = procalcitonin, PLR = platelet to lymphocyte ratio, PSI = pneumonia severity index, SIRT3 = Sirtuin 3, TNF-\(\alpha\) = tumor necrosis factor, WBC = white blood cell.

Keywords: inflammation, prognosis, severe community-acquired pneumonia, SIRT3

1. Introduction

Community-acquired pneumonia (CAP), a kind of infectious pulmonary parenchymal inflammation (including alveolar wall, or pulmonary interstitium in a broad sense) outside the hospital, is a common respiratory disease with high incidence and mortality rates.\(^{1,2}\) It was reported that CAP accounts for more than 3 million deaths annually worldwide.\(^{3,4}\) As CAP, especially severe CAP, often has high mortality rate, biomarkers for diagnosis and prognosis are of great significance.\(^{5-7}\)

Inflammation is deeply associated with CAP development. During CAP, inflammatory response is activated and the secretion of inflammatory factors is increased.\(^{8,9}\) It was reported that cytokines such as NF-\(\kappa\)B, IL-17, TNF-\(\alpha\), IFN-\(\gamma\), and IL-4 were correlated with clinical severity scales such as CURB65 or SOFA in CAP patients.\(^{10}\) And white blood cell (WBC), neutrophil, monocyte, lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), and monocyte to lymphocyte ratio (MLR) levels were all higher in the CAP patients than the healthy individuals.\(^{11}\)

Sirtuin 3 (SIRT3) is a member of Sirtuin family, which play important roles in many diseases. It was found SIRT3 could regulate mitochondrial fatty-acid oxidation by reversible enzyme...
2. Methods and materials

2.1. Patients

This prospective observational research enrolled a total of 114 severe CAP patients who went to our hospital during January 2018 to December 2019. The diagnosis of CAP was according to the criteria of the American Thoracic Society (ATS) guidelines for pneumonia. All patients were within 18–90 years-old. The severity of CAP was defined by pneumonia severity index (PSI) score, stage IV–V as severe CAP. The main inclusion criteria were CAP patients who needed to maintain breathing by invasive mechanical ventilation through tracheal intubation; patients with septic shock and blood pressure drop and need to use antihypertensive drugs to maintain blood pressure. The secondary criteria were respiratory rate >30/min; PaO_{2}/FiO_{2} <250; patients with drowsiness, disturbance of consciousness, lethargy, coma, delusion; obvious increase of serum levels of creatinine (Cr) and blood urea nitrogen (BUN) caused by CAP; patchy or patchy infiltrations in multiple lobes with or without pleural effusion by CT or X-ray; WBC <4.0 × 10^{9}/L; PLT <100 × 10^{12}/L; temperature <36°C. Patients meeting one of the main inclusion criteria or 3 or more of the secondary criteria were defined as severe CAP. The following patients were excluded: patients with cancers; patients with severe renal or liver dysfunction before diagnosis of CAP; patients with cardiovascular diseases such coronary heart disease and heart failure; and patients with pulmonary tuberculosis, lung infarction, lung disease caused by autoimmune diseases, lung infection after organ transplantation. In addition, blood samples of 114 healthy individuals who went to physical examination were collected during the same period. All patients signed the informed consent. The present study was approved by the Ethic Committee of Tianyou Hospital Affiliated to Wuhan University of Science and Technology.

2.2. Measurement of serum SIRT3 and inflammatory factors

Fasting cubital venous blood (5 mL) of all patients were collected within 24 hours after admission. The blood samples were collected in tubes containing EDTA and were centrifuged at 2000 g for 15 minutes. The enzyme-linked immunosorbent assay (ELISA) method was used for measurement of serum SIRT3, interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α using commercially available ELISA kits (SIRT3, MBS2022533 MYBio; IL-1β DLB50 R&D Systems; IL-6 MBS175877 MYBio; TNF-α DTA00D R&D Systems).

2.3. Data collection

Demographic data including age, sex, and body mass index (BMI), as well as clinical symptoms, comorbidities, SOFA and SMART-COP scores, duration of ICU stay, and duration of mechanical ventilation were collected. The routine blood test was conducted for all patients and WBC amount, as well as serum levels of C-reactive protein (CRP), D-Dimer, and procalcitonin (PCT). For 1-month survival analysis, all course death for patients was considered and the survival duration was defined from the admission time to the death or the last follow-up.

2.4. Statistical analysis

The distribution of the data was analyzed by Kolmogorov-Smirnov method. The data distributing normally was expressed as mean ± SD. Normally distributed data were expressed by mean ± SD and non-normally distributed data were expressed by median (range). Chi-square test was used to compare the rates. Comparison between 2 groups was analyzed by Student t test and Mann–Whitney U test for normally and non-normally distributed data, respectively. Correlation between SIRT and inflammatory factors was determined using Pearson analysis. Kaplan-Meier curve was performed for survival analysis. Logistic regression analysis was conducted for 1-month mortality using binary regression analysis by a step back method. P < .05 was considered as statistically different. All calculations were made using SPSS 18.0 (SPSS Inc., Chicago, IL).

3. Results

3.1. Characteristics of all patients

The present study included 114 severe CAP patients, with median age 59.5 (45–79) years, male: female 63: 51. No significant difference was found between the health and the CAP patients for age, sex, and BMI. Among all patients, 55 cases died during the study period. The serum levels of CRP, PCT, IL-1β, and IL-6, as well as SOFA and SMART-COP scores were markedly higher in deceased patients compared with the survival patients (P < .05, Table 1).

3.2. Serum SIRT3 levels were decreased in severe CAP patients and were negatively correlated with inflammatory factors

Then, we determined serum SIRT3 levels in CAP patients and the health control. As shown in Figure 1, the expression of SIRT3 was significantly decreased in severe CAP patients compared with the healthy (P < .05). The deceased patients showed remarkably lower SIRT3 levels compared with the survival cases (P < .05). Pearson analysis showed that SIRT3 levels were negatively correlated with levels of CRP, PCT, IL-1β, and IL-6 (P < .05, Table 2).

3.3. Serum SIRT3 levels were associated with clinical outcomes of severe CAP patients

To further investigate role of SIRT3 in severe CAP severe patients, the patients were divided into low SIRT3 group and high SIRT3 group according to the median value of serum SIRT3 (5.66 ng/mL). As summarized in Table 3, patients with SIRT3 low expression showed remarkably higher expression of CRP, PCT, IL-1β and IL-6, as well as high SMART-COP scores (P < .05). Besides, the SIRT3 low group also showed higher 1-month mortality rate. All these results indicated that SIRT3 was...
associated with clinical outcomes and prognosis of severe CAP patients.

3.4. Serum SIRT3 levels were associated with 1-month mortality of severe CAP patients

Finally, survival analysis was conducted for 1-month mortality of all patients. As the univariate analysis for 1-month mortality has been already summarized in Table 1, the binary regression was performed for the factors which showed significant difference in univariate analysis. It was found patients with higher expression of SIRT3 showed significantly lower 1-month mortality rate and longer survival (Fig. 2). Logistic regression showed only SIRT3 and IL-1β were independent risk factors for 1-moth mortality in severe CAP patients (Table 4).

4. Discussion

Despite the development of medical techniques, CAP is still commonly seen in clinic with a high morbidity rate. Clinical biomarkers for CAP are of great significance for diagnosis and prognosis, and novel biomarkers are always needed. In the present study, we demonstrated that serum SIRT3 levels were decreased in severe CAP patients and were associated with patients’ clinical outcomes and prognosis.

SIRT3 showed its anti-inflammation activity in many studies. It was found that SIRT3 could improve inflammation in endotoxin-induced acute lung injury. The activation of SIRT3 showed anti-inflammation activity in both liver and renal injury. In a recent study, Dikalova et al also found that the deficiency of SIRT3 might be associated with vascular dysfunction, increased vascular inflammation and oxidative stress in hypertension. The role of SIRT3 in inflammation might be partly associated with its

![Figure 1. Serum levels of SIRT3 in severe CAP patients and the healthy control.](image_url)
effects on oxidative stress and energy metabolism. It has been found in cardiovascular diseases, SIRT3 protects heart from metabolic dysfunction by regulating glucose and lipid metabolism and making balance for myocardial ATP.\(^\text{[22]}\) In neurodegenerative disease, SIRT3 also shows its anti-oxidative stress effects on oxidative stress and energy metabolism. It has been found in cardiovascular diseases, SIRT3 protects heart from metabolic dysfunction by regulating glucose and lipid metabolism and making balance for myocardial ATP.\(^\text{[22]}\) In neurodegenerative disease, SIRT3 also shows its anti-oxidative stress effects.

Table 3: Clinical outcomes between SIRT3low/high groups.

| Variable                              | SIRT3 low, \(n=57\) | SIRT3 high, \(n=57\) | \(P\) |
|---------------------------------------|----------------------|----------------------|------|
| Mean age, yr                         | 61 (45~79)           | 58 (45~79)           | .401 |
| Sex, male: female                    | 34: 23               | 29: 28               | .212 |
| BMI, kg/m²                            | 23.53 (19.01~26.99)  | 23.31 (19.29~26.83)  | .084 |
| Comorbidities, n (%)                 |                      |                      | .784 |
| Hypertension                         | 33 (57.89)           | 26 (45.61)           |      |
| Diabetes                              | 15 (26.32)           | 13 (22.81)           |      |
| Duration of ICU stay, d              | 14.89 ± 6.33         | 15.19 ± 4.96         | .780 |
| Duration of mechanical ventilation, h| 234.10 ± 121.47      | 196.63 ± 113.97      | .092 |
| Symptoms, n (%)                      |                      |                      | .298 |
| Fever                                | 33 (57.89)           | 23 (40.35)           |      |
| Cough                                | 20 (35.09)           | 23 (40.35)           |      |
| Sputum                               | 18 (31.58)           | 13 (22.81)           |      |
| Shortness of breath                  | 11 (19.30)           | 13 (22.81)           |      |
| Chest pain                           | 10 (17.54)           | 12 (21.05)           |      |
| SOFA                                  | 5 (1~11)             | 5 (1~8)              | .171 |
| SMART-COP                            | 5 (1~3)              | 3 (1~7)              | .001 |
| CRP, mg/L                            | 43.72 (10.88~99.28)  | 33.01 (15.59~93.57)  | .002 |
| PCT, μg/mL                           | 41.51 (10.21~89.43)  | 27.33 (10.74~72.48)  | <.001 |
| D-Dimer, μ/mL                        | 7.95 ± 4.14          | 6.99 ± 3.73          | 1.94 |
| WBC, 10⁹/mL                          | 12.37 (7.48~18.87)   | 14.15 (7.06~18.98)   | .583 |
| IL-1β, pg/mL                         | 57.28 ± 23.05        | 36.48 ± 16.96        | <.001 |
| IL-6, pg/mL                          | 65.91 (12.03~112.47) | 41.17 (11.19~108.09) | <.001 |
| TNF-α, pg/mL                         | 54.58 ± 24.95        | 56.28 ± 21.63        | .698 |
| 1-month mortality, n (%)             | 41 (71.93)           | 4 (7.02)             | <.001 |

Chi-square test was used to compare the rates. Comparison between 2 groups was analyzed by Student t test.

BMI = body mass index, CRP = C-reactive protein, IL = interleukin, PCT = procalcitonin, TNF-α = tumor necrosis factor-α, WBC = white blood cell.

Figure 2: K-M curve for severe CAP patients with low/high SIRT3 expression.

Table 4: Logistic regression for risk factors of 1-month mortality in severe CAP patients.

| Variable | Wald    | Odds ratio | 95% CI         | \(P\) |
|----------|---------|------------|----------------|------|
| SOFA     | 1.699   | 2.026      | (0.700~5.861)  | .192 |
| SMART-COP| 2.930   | 1.952      | (0.907~4.201)  | .067 |
| SIRT3    | 13.317  | 0.207      | (0.089~0.483)  | <.001 |
| CRP      | 1.990   | 1.073      | (0.972~1.184)  | .158 |
| PCT      | 2.846   | 1.289      | (0.959~1.733)  | .002 |
| IL-1β    | 10.582  | 1.104      | (1.040~1.171)  | .001 |
| IL-6     | 3.308   | 1.261      | (0.982~1.620)  | .069 |
| TNF-α    | 2.563   | 0.928      | (0.847~1.016)  | .109 |

BMI = body mass index, CRP = C-reactive protein, IL = interleukin, PCT = procalcitonin, TNF-α = tumor necrosis factor-α, WBC = white blood cell.
SRIT3 in CAP is unclear. All these need more studies to further reveal.

5. Conclusion
This study demonstrated that lower SIRT3 levels predicted poor prognosis and clinical outcomes of severe CAP patients. SIRT3 was negatively correlated with inflammatory factors in severe CAP. SIRT3 has the potential to be used as a prognosis biomarker in CAP.

Author contributions
Data curation: Wei Zhu, Ping Chen, Li Deng, Liangzi Hu.
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References
[1] Prina E, Ranzani OT, Torres A. Community-acquired pneumonia. Lancet 2015;386:1097–108.
[2] Lanks CW, Musani AI, Hsia DW. Community-acquired pneumonia and hospital-acquired pneumonia. Med Clin 2019;103:487–501.
[3] Ferreira-Coimbra J, Sarda C, Rello J. Burden of community-acquired pneumonia and unmet clinical needs. Adv Ther 2020;37:1302–18.
[4] Katz SE, Williams DJ. Pediatric community-acquired pneumonia in the United States: changing epidemiology, diagnostic and therapeutic challenges, and areas for future research. Infect Dis Clin 2018;32:47–63.
[5] Florim TA, Ambroggio L, Brokamp C, et al. Biomarkers and disease severity in children with community-acquired pneumonia. Pediatrics 2020;145:e20193728doi:10.1542/peds.2019-3728.
[6] Curbelo J, Luquero Bueno S, Galván-Román JM, et al. Inflammation biomarkers in blood as mortality predictors in community-acquired pneumonia admitted patients: importance of comparison with neutrophil count percentage or neutrophil-lymphocyte ratio. PLoS One 2017;12: e0173947.
[7] Kim MW, Lim JY, Oh SH. Mortality prediction using serum biomarkers and various clinical risk scales in community-acquired pneumonia. Scand J Clin Lab Invest 2017;77:486–92.
[8] Guertler C, Wirz B, Christ-Crain M, et al. Inflammatory responses predict long-term mortality risk in community-acquired pneumonia. Eur Respir J 2011;37:1439–46.
[9] Ramírez P, Ferrer M, Martí V, et al. Inflammatory biomarkers and prediction for intensive care unit admission in severe community-acquired pneumonia. Crit Care Med 2011;39:2211–7.
[10] Rendon A, Rendon-Ramírez EJ, Rosas-Taraco AG. Relevant cytokines in the management of community-acquired pneumonia. Curr Infect Dis Rep 2016;18:10.
[11] Huang Y, Liu A, Liang L, et al. Diagnostic value of blood parameters for community-acquired pneumonia. Int Immunopharmacol 2018;64:10–5.
[12] Hirschey MD, Shimazu T, Goetzman E, et al. SIRT3 regulates mitochondrial fatty-acid oxidation by reversible enzyme deacetylation. Nature 2010;464:121–5.
[13] Dikalova AE, Itani HA, Nazarewicz RR, et al. Sirt3 impairment and SOD2 hyperacetylation in vascular oxidative stress and hypertension. Circ Res 2017;121:564–74.
[14] Torrens-Mas M, Oliver J, Roca P, Sastre-Serra J. SIRT3: oncogene and tumor suppressor in cancer. Cancers 2017;9:90.
[15] Boniackowski AM, Davis FM, Joshi A, et al. SIRT3 regulates macrophage-mediated inflammation in diabetic wound repair. J Invest Dermatol 2019;139:2528–37. e2.
[16] Sibila O, Meduru GU, Mortensen EM, et al. Improving the 2007 Infectious Disease Society of America/American Thoracic Society severe community-acquired pneumonia criteria to predict intensive care unit admission. J Crit Care 2013;28:284–90.
[17] Kürundkar D, Küründkar AR, Bone NB, et al. SIRT3 diminishes inflammation and mitigates endotoxin-induced acute lung injury. JCI Insight 2019;4.
[18] Wang Y, Li C, Gu J, et al. Celastrol exerts anti-inflammatory effect in liver fibrosis via activation of AMPK-SIRT3 signaling. J Cell Mol Med 2020;24:941–53.
[19] Kim D, Park W, Lee S, et al. Absence of Sirt3 aggravates cisplatin nephrotoxicity via enhanced renal tubular apoptosis and inflammation. Mol Med Rep 2018;18:3665–72.
[20] Dikalova AE, Pandey A, Xiao L, et al. Mitochondrial deacetylase Sirt3 reduces vascular dysfunction and hypertension while Sirt3 depletion in essential hypertension is linked to vascular inflammation and oxidative stress. Circ Res 2020;126:439–52.
[21] Sun W, Liu C, Chen Q, Liu N, Yan Y, Liu B. SIRT3: a new regulator of inflammation, fibrosis via activation of AMPK-SIRT3 signalling. J Cell Mol Med 2018;22:927–36.
[22] Wei Zhu, Ping Chen, Li Deng, Liangzi Hu.