Am J Transl Res 2022;14(9):6056-6066
www.ajtr.org /ISSN:1943-8141/AJTR0144965

Original Article

Changes of serum P62 and Beclin 1 in patients with pulmonary embolism during treatment and their predictive value for efficacy

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Received June 26, 2022; Accepted July 24, 2022; Epub September 15, 2022; Published September 30, 2022

Abstract: Objective: This study was designed to analyze the changes of serum P62 and Beclin 1 in patients with acute pulmonary embolism (APE) during treatment and their predictive value for efficacy. Methods: In this retrospective study, 84 patients diagnosed as APE in Ningbo First Hospital from January 2020 to January 2022 were enrolled and assigned to a patient group, and 40 healthy individuals who underwent physical examination in Ningbo First Hospital during the same time period were assigned to a control group. Serum P62 and Beclin 1 expressions were compared between the two groups, and receiver operating characteristic (ROC) curves were drawn to analyze the diagnostic value of serum P62 and Beclin 1. The changes of serum P62 and Beclin 1 before and after treatment were evaluated. The correlations of P62 and Beclin 1 levels with the efficacy in patients were analyzed, and corresponding ROC curves were drawn. Additionally, the expressions of serum P62 and Beclin 1 in patients with different disease degrees were determined, and their expressions in relapsed patients were compared before treatment. Results: The patient group showed significantly lower level of serum P62 but higher level of Beclin 1 than the control group ($P<0.05$). The areas of under the curves (AUCs) of P62 and Beclin 1 in diagnosing APE were 0.888 and 0.795 respectively. After treatment, patients showed significantly increased P62 expression ($P<0.05$) and significantly decreased Beclin 1 expression ($P<0.01$). The P62 expression increased with the relief of patient's disease, with a positive correlation with the patient's disease condition, while the Beclin 1 expression decreased with the relief of patient's disease, with a negative correlation with the patient's disease condition ($P<0.05$). In addition, the improved group showed significantly higher serum P62 expression ($P<0.05$) and significantly lower serum Beclin 1 expression than the non-improved group ($P<0.05$), and the AUCs of P62 and Beclin 1 in predicting the efficacy in patients with APE were 0.801 and 0.675, respectively. The relapsed group showed significantly lower serum P62 expression ($P<0.05$) and significantly higher Beclin 1 expression than the non-relapsed group ($P<0.05$), and the AUCs of P62 and Beclin 1 in predicting the recurrence of APE were 0.815 and 0.769, respectively. Conclusion: In patients with APE, serum P62 decreased, but serum Beclin 1 increased. So, both indictors can be applied for diagnosis, efficacy prediction and recurrence prediction of APE.

Keywords: Acute pulmonary embolism, autophagy, P62, Beclin 1, diagnosis, efficacy prediction, recurrence prediction

Introduction

Pulmonary embolism (PE) refers to the clinical and pathophysiological syndrome in which the thrombus in the right heart or venous system blocks the main trunk of pulmonary artery or its branches and causes pulmonary circulation disorder [1]. Acute pulmonary embolism (APE), a worldwide fatal cardiovascular disease, takes 100,000-300,000 lives away each year in the United States alone [2], with characteristics of high incidence, high rate of missed diagnosis and high mortality [3]. APE has become a main health care problem that seriously endangers patients' life. However, the pathogenesis of APE remains unclear. Clarifying its pathogenesis is of profound significance for the diagnosis and treatment of it.

APE often triggers severe hypoxia, lung ischemia and lung injury, and clinically, less than 10% of APE patients suffer from secondary lung infarction [4]. Currently, the research on the mechanism of lung injury mostly focuses on injury and oxidative stress. In the last few years, amid a rise of research on autophagy and apoptosis, some scholars have suggested that both autophagy and apoptosis play an important role in the lung protection against APE [5]. However, there are few studies on the correlation and mechanism of APE with autophagy and apoptosis. Sequestosome 1 (SQSTM1), also known as P62, is an adapter protein related to LC3-II and ubiquitinated-protein aggregates [6]. Its C terminal binds to ubiquitin protein, interacts with LC3-II through N terminal and integrates into phagosome during autophagy, and then degrades in autophagy lysosome [7]. P62 has been shown to be highly expressed in a variety of tumors such as lung and liver cancers and can thus be adopted as a potential target for tumor therapy [8, 9]. Beclin 1 is one of the earliest tumor suppressor genes found in mammals during autophagy. It regulates the activity of Vps-34 by binding to many cofactors, forming Beclinl-Vps34-Vpsl5 complex and inducing the initiation of autophagy [10, 11]. Earlier studies revealed [12, 13] that upregulation of Beclin 1 promoted cardiomyocyte autophagy and thus exerted cardioprotective effects.

This study was designed to analyze the expressions of P62 and Beclin 1 in patients with APE and their predictive value for the efficacy in patients, with the goal of providing potential indicators for APE prognosis.

Methods and data

Clinical data

Retrospective analysis was conducted in patients with APE diagnosed at Ningbo First Hospital from January 2020 to January 2022. Sample size calculation: From a review of the literature, the diagnostic sensitivity of APE was estimated to be 0.74-0.96, with a median of 0.85, and the specificity ranged from 0.71-0.95, with a median of 0.83. With the diagnostic test sample size calculation formula:

$$n = \left(\frac{Z_{1-\alpha/2} \times \sqrt{p \times (1-p)}}{\delta} \right)^2$$

(α was set to 0.05 and the tolerance error δ to 0.08), it was calculated that 84 patients with APE were needed for the patient group in this study, and 44 controls were collected for this study according to the ability to obtain samples on a 2:1 basis. A control sample of 40 cases was eventually collected according to the actual situation. This study was approved by the Medical Ethics Committee of Ningbo First Hospital (2022006A-01).

Inclusion and exclusion criteria

The inclusive criteria of patients: 1) Patients who met the diagnostic criteria in the Consensus of Chinese experts on diagnosis and treatment of APE (2015) according to their pulmonary angiography results [14]; 2) Patients whose interval between onset and treatment was less than 24 hours; 3) Patients with complete clinical data; 4) Patients who were diagnosed with APE for the first time.

The exclusion criteria of patients: 1) Patients with a history of chronic heart failure or hepatic or renal insufficiency; 2) Patients with a history of hemorrhagic diseases; 3) Patients in the acute stage of cardiovascular or cerebrovascular adverse events; 4) Patients with drug allergy; 5) Patients with other contraindications to anticoagulation or thrombolysis; 6) Patients with tumor; 7) Pregnant women.

Therapeutic regimen

Patients were treated with thrombolysis combined with sequential anti-coagulation therapy. Specifically, each patient was given 1.5 million IU urokinase (Chengdu Tongde Pharmaceutical Co., Ltd., State Food and Drug Administration (SFDA) approval number: H51021400) through intravenous drip within 120 minutes, once a day, for 6 consecutive days. The next day after urokinase treatment, 0.1 ml/10 kg low-molecular-weight heparin calcium (Shenzhen SCIPROGEN Bio-Pharmaceutical Co., Ltd., SFDA approval number: H20060191) was injected subcutaneously for 6 consecutive days. On the third day of subcutaneous injection of low-molecular-weight heparin calcium, 2.5 mg warfarin (Beijing Jialin Pharmaceutical Co., Ltd., SFDA approval number: H20103600) was taken orally in the meantime, and on the 6th day, the subcutaneous injection of low-molecular-weight heparin calcium ended. The overlap-

ping treatment time of warfarin and low-molecular-weight heparin calcium was 4 days.

Enzyme-linked immuno-sorbent assay (ELISA)

Serum was collected from the control group, and serum was also collected from the patient group before treatment and after 2 weeks of treatment. The changes of P62 (ab289654) and Beclin 1 (ab254511) levels in the patients' serum were measured through ELISA, with kits from Abcam Company of the United States. The operation procedures were conducted strictly in accordance with the instructions of the kits, and a 680 automatic microplate reader produced by BioRad Company in the United States was used for the measuring.

Outcome measures

Primary outcome measures: Serum P62 and Beclin 1 levels were compared between the two groups, and corresponding receiver operating characteristic (ROC) curves were drawn. The changes of serum P62 and Beclin 1 before and after treatment were evaluated. Patients were grouped according to the clinical efficacy after treatment. The criteria for efficacy evaluation were as follows. It was seen as markedly effective if the patient had disappeared pulmonary embolism, recovered blood perfusion, basically disappeared PTE signs, and basically recovered pulmonary function. It was seen as effective if the patient had reduced lung lesion area by more than 50% and alleviated symptoms and signs. It was seen as ineffective if the patient had no improvement in imaging examination results, symptoms and signs, or showed disease progression or clinical death. Patients with markedly effective treatment outcome were assigned to an improved group, and those with effective or ineffective treatment outcome were assigned to a non-improved group. The serum P62 and Beclin 1 levels before treatment were compared between the two groups , and corresponding ROC curves for prediction were drawn.

Secondary outcome measures: The clinical data of the two groups were compared. The patients were grouped according to the guidelines published by American Heart Association in 2011 [15]. Patients with shock and hypotension were assigned to a high-risk group; patients whose echocardiography showed that the right ventricular motor function was weakened or showed clinical symptoms of cardiac insufficiency were assigned to a medium-risk group; patients with normal blood pressure and no clinical manifestation of right heart failure were assigned to a low-risk group. The expressions of serum P62 and Beclin 1 in each group were determined. The data of outpatient reexamination of patients within 6 months after treatment were inquired. The patients were grouped according to their recurrence, and the serum levels of P62 and Beclin 1 before treatment were compared between relapsed group and non-relapsed group.

Statistical analyses

In the present study, SPSS 20.0 software was adopted for statistical analyses of the collected data. Measurement data (mean ± sd) were compared between groups using the independent-sample t test, and compared within groups using the paired t test, and expressed as t. Counting data (%) were analyzed using the Chi-square test. ROC curves were drawn to analyze the diagnostic and predictive value of P62 and Beclin 1 for APE. The One-way ANOVA was adopted for multi-group comparisons, and the LSD-t test was used for post hoc test. The Spearman test was adopted to analyze the correlations of P62 and Beclin 1 with the severity of the patients' disease. $P<0.05$ was considered statistically significant.

Results

Comparison of clinical data

According to comparison of clinical data, there was no significant difference between the control group and the patient group in age, sex, blood pressure, past medical history, smoking history and alcoholism history ($P>0.05$, **Table 1**).

Expressions of P62 and Beclin 1 in patients with APE

The serum P62 and Beclin 1 in the control group and the patient group were quantified via ELISA. According to the results, the patient group showed significantly lower serum P62 expression and significantly higher Beclin 1

Table 1. Comparison of baseline data between the two groups

Items	control group (n=40)	Patient group (n=84)	*P*-value
Age			0.259
≥65 years old	20	51	
<65 years old	20	33	
Sex			0.751
Male	25	50	
Female	15	34	
Blood pressure			
Diastolic blood pressure	81.25±5.56	80.73±4.89	0.603
Systolic blood pressure	131.45±8.14	128.23±8.90	0.056
Past medical history			
Hypertension	18	30	0.321
Diabetes mellitus	12	20	0.461
Smoking history			0.746
Yes	25	55	
No	15	29	
Alcoholism history			0.774
Yes	8	15	
No	32	69	

expression than the control group (P<0.05, **Figure 1**).

Diagnostic value of P62 and Beclin1 in patients with APE

According to ROC curves of P62 and Beclin 1 in the diagnostic value for patients with APE, the area under the curve (AUC) of P62 in diagnosing APE was 0.888, while that of Beclin 1 in diagnosing APE was 0.795 (**Figure 2**; **Table 2**), suggesting that both P62 and Beclin 1 had certain value in diagnosing APE.

Changes of P62 and Beclin 1 in patients with APE before and after treatment

The changes of P62 and Beclin 1 in patients with APE before and after treatment were compared. According to the results, after treatment, the P62 expression in the patients increased significantly (P<0.05), while Beclin 1 expression in the patients decreased significantly (P<0.01, **Figure 3**).

Association of P62 and Beclin 1 with patients' disease condition

The patients were grouped according to the severity of their disease at admission, including 10 patients in the high-risk group, 38 patients in the medium-risk group and 36 patients in the low-risk group. Then the levels of P62 and Beclin 1 among the three groups were compared. According to analysis, the serum P62 expression of the high-risk group was significantly lower than that of the medium-risk and low-risk groups, and the P62 expression of the medium-risk group was significantly lower than that of the low-risk group (P<0.05, **Figure 4**). In addition, the high-risk group showed significantly higher serum Beclin 1 expression than the other two groups, and the medium-risk group showed significantly higher serum Beclin 1 expression than the low-risk group. According to the Spearman test, the P62 expression increased with the relief of the disease, showing a positive correlation with the disease condition, while the Beclin 1 expression decreased with the relief of the disease, showing a negative correlation with the disease condition (P<0.05, **Figure 4**).

Association of P62 and Beclin 1 with the efficacy in patients

Patients were grouped according to the clinical efficacy after treatment, including 60 patients in the improved group and 24 patients in the non-improved group. The comparison of P62 and Beclin 1 levels between these two groups showed that the pretreatment serum P62 expression of the improved group was significantly higher than that of the non-improved group (P<0.05), and the pretreatment serum Beclin 1 expression of the improved group was significantly lower than that of the non-improved group (P<0.05, **Figure 5**).

The value of P62 and Beclin 1 in predicting the efficacy on patients

ROC curves of P62 and Beclin 1 in predicting the efficacy in patients with APE were drawn. According to the results, the AUCs of P62 and Beclin 1 in predicting the efficacy were 0.801 and 0.675, respectively (**Figure 6**; **Table 3**), suggesting that both P62 and Beclin 1 had certain value in predicting the efficacy in patients with APE.

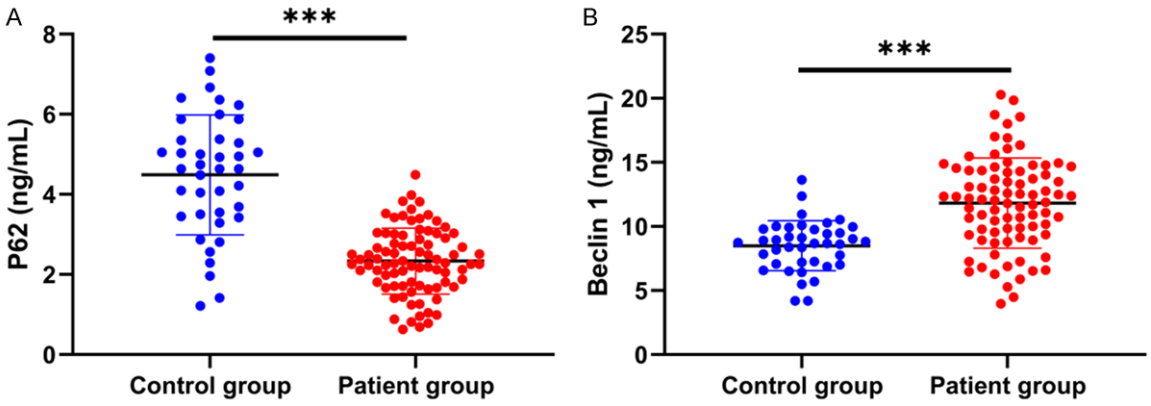

Figure 1. Expression of serum P62 and Beclin 1 in the patient group and the control group. A. Expression of serum P62 in the patient group and the control group measured by ELISA. B. Expression of serum Beclin 1 in the patient group and the control group measured by ELISA. Note: ELISA: Enzyme-linked immuno-sorbent assay. ***P<0.001.

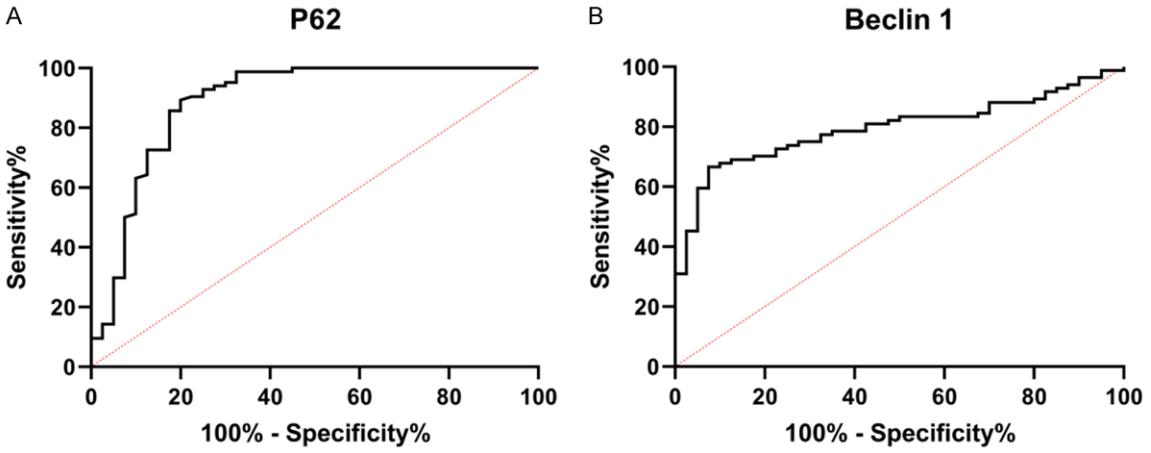

Figure 2. ROC of P62 and Beclin 1 for APE. A. Area of P62 under the ROC curves for APE. B. Area of Beclin 1 under the ROC curve for APE. Note: Acute pulmonary embolism (APE), Receiver operating characteristic (ROC).

Table 2. ROC parameters of P62 and Beclin 1 for APE

Prediction variable	Area under the curve (AUC)	Confidence interval (CI)	Cut-off value	Sensitivity	Specificity	Youden index
P62	0.889	0.814-0.964	3.41	89.30	80.00	69.30
Beclin 1	0.795	0.722-0.877	10.60	66.70	92.50	59.20

Note: Acute pulmonary embolism (APE), Receiver operating characteristic (ROC).

Expressions of P62 and Beclin 1 in patients with recurrence

According to the electronic medical records of the patients in the outpatient department within 6 months, among the 84 patients, there were 6 cases of death, 78 survived cases, and 10 relapsed cases. The patients were grouped groups according to their relapses. Further comparison revealed that the relapsed group showed significantly lower serum P62 expression and significantly higher Beclin 1 expression than the non-relapsed group (P<0.05, **Figure 7**).

Predictive value of P62 and Beclin 1 in patients with recurrence

ROC curves of P62 and Beclin 1 in predicting the recurrence of APE were drawn. According to the results, the AUCs of P62 and Beclin 1 in

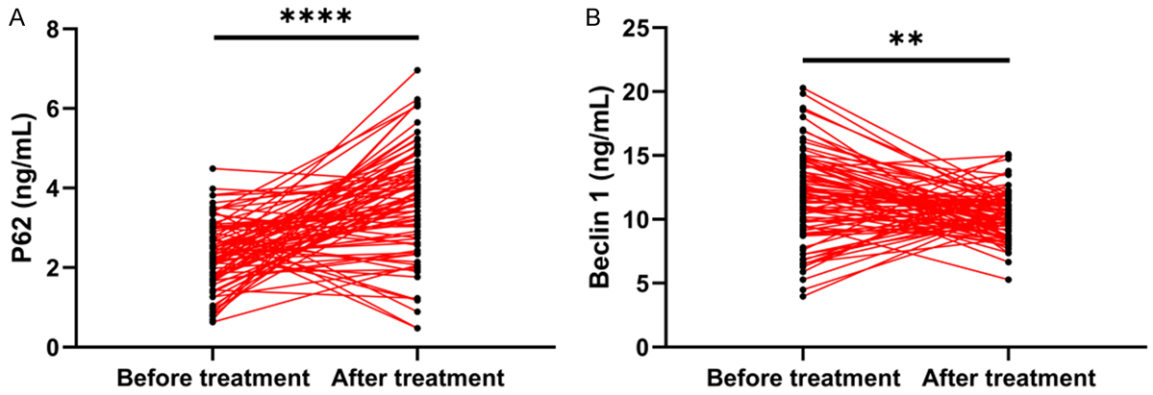

Figure 3. Changes of P62 and Beclin 1 in patients before and after treatment. A. Changes of P62 in patients with APE before and after treatment. B. Changes of Beclin 1 in patients with APE before and after treatment. **P<0.01, ****P<0.0001.

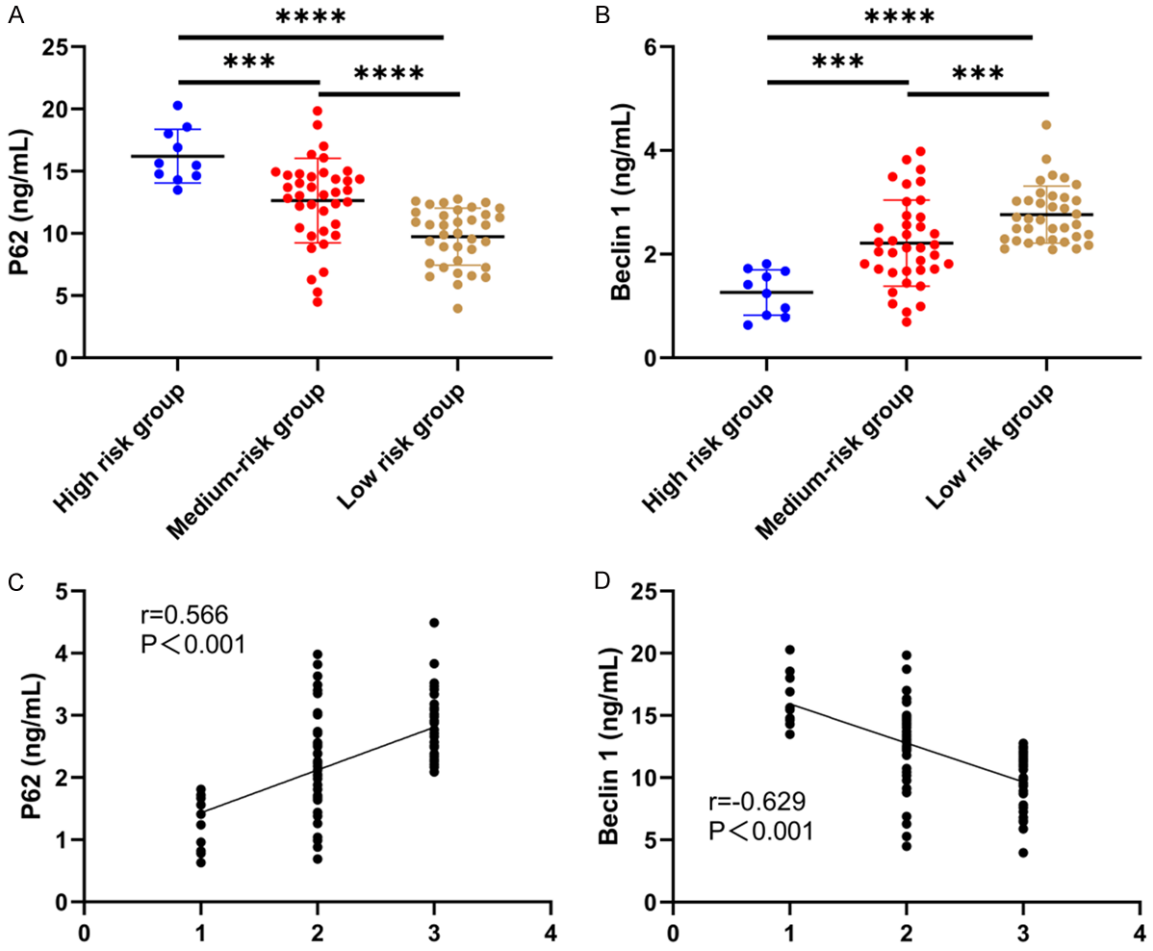

Figure 4. Association of the severity of disease with P62 and Beclin 1. (A) Association between the P62 level and the disease before treatment. (B) Association between the Beclin 1 level and the disease before treatment. (C) Association between the P62 level and the disease according to the Spearman test. (D) Association between the Beclin 1 level and the disease according to the Spearman test. Notes: *** means P<0.001; **** means P<0.0001. In (C, D), 1 means the high-risk group; 2 means the medium-risk group and 3 means the low-risk group.

predicting the recurrence were 0.815 and 0.769, respectively (**Figure 8**; **Table 4**), suggesting that both P62 and Beclin 1 had certain value in predicting the recurrence of APE.

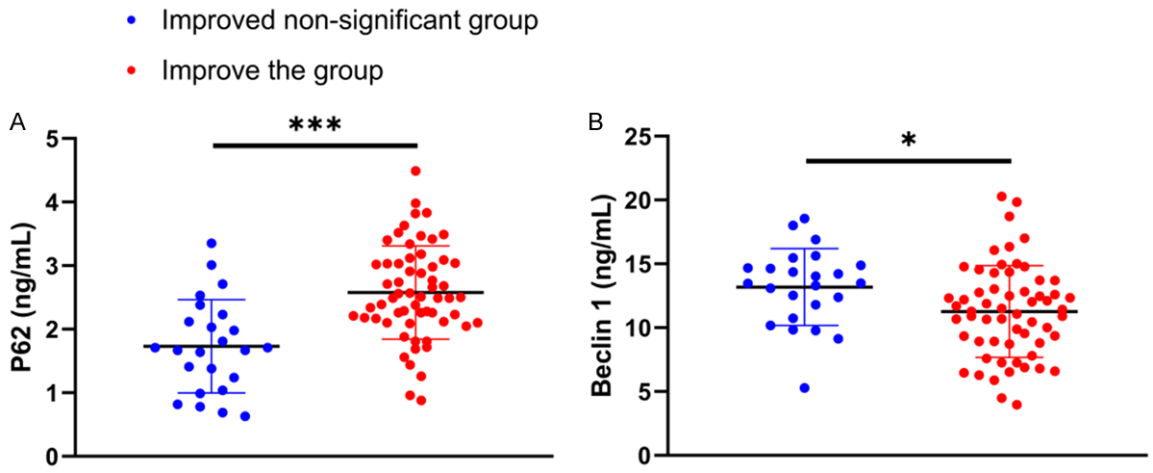

Figure 5. Levels of P62 and Beclin1 in patients with different efficacy. A. Comparison of P62 expression in the improved group and the non-improved group. B. Comparison of Beclin 1 expression in the improved group and the non-improved group. Note: * means P<0.05, *** means P<0.001.

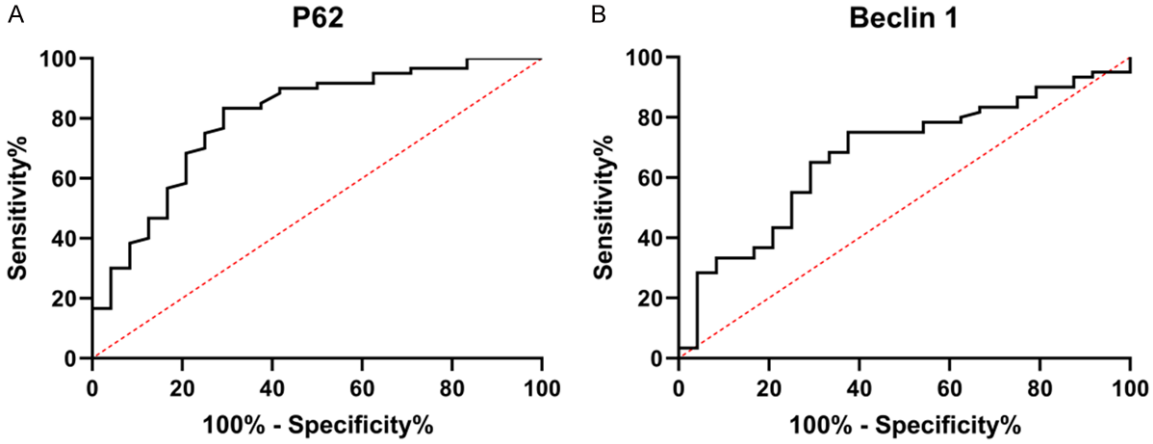

Figure 6. ROC of P62 and Beclin 1 in predicting the efficacy in patients with APE. A. Areas of P62 under the ROC curve in predicting the efficacy in patients with APE. B. Areas of Beclin 1 under the ROC curve in predicting the efficacy in patients with APE. Note: Acute pulmonary embolism (APE), Receiver operating characteristic (ROC).

Table 3. ROC parameters of P62 and Beclin 1 in predicting the efficacy in patients with APE

Prediction variable	Area under the curve (AUC)	Confidence interval (CI)	Cut-off value	Sensitivity	Specificity	Youden index
P62	0.801	0.692-0.911	2.040	83.30	70.80	54.20
Beclin 1	0.675	0.550-0.801	13.06	75.00	62.50	37.50

Note: Acute pulmonary embolism (APE), Receiver operating characteristic (ROC).

Discussion

APE refers to the clinical situation that the pulmonary circulation is blocked by endogenous or exogenous emboli in the branch or trunk of the pulmonary artery [4]. Misdiagnosis or missed diagnosis of it will delay the treatment, threatening patients' lives and resulting in a high mortality [16]. Clinical research results show that APE patients face a risk of recurrence within 10 years after treatment, and the recurrence rate is high [17]. Therefore, finding indicators closely related to the recurrence of APE will help to improve the prognosis.

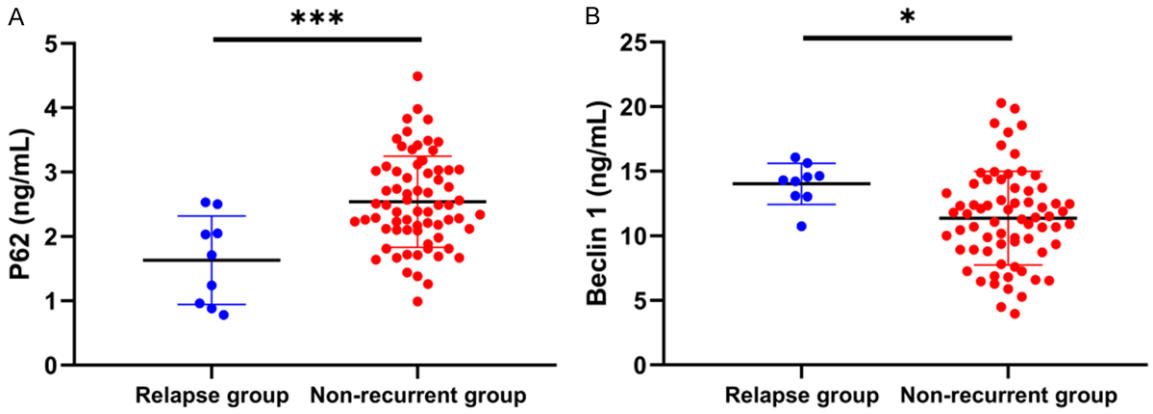

Figure 7. P62 and Beclin 1 expressions in relapsed patients. A. Comparison of P62 in relapsed patients. B. Comparison of Beclin 1 in relapsed patients. Note: * means P<0.05, *** means P<0.001.

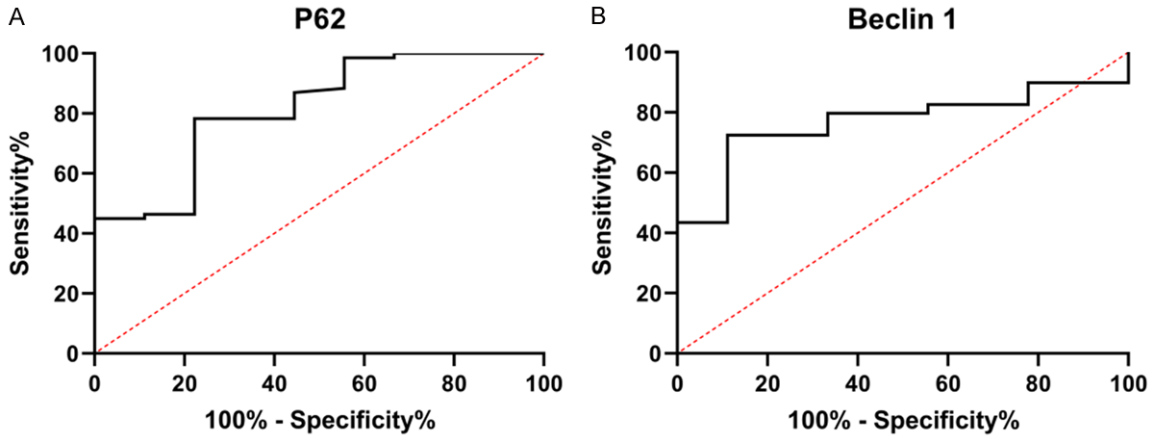

Figure 8. ROC curves of P62 and Beclin 1 in predicting the recurrence of APE. A. Area of P62 under the ROC curve in predicting the recurrence of APE. B. Area of Beclin 1 under the ROC curve in predicting the recurrence of APE. Note: Acute pulmonary embolism (APE), Receiver operating characteristic (ROC).

Table 4. ROC parameters of P62 and Beclin 1 in predicting the recurrence of APE

Prediction variable	Area under the curve (AUC)	Confidence interval (CI)	Cut-off value	Sensitivity	Specificity	Youden index
P62	0.816	0.663-0.968	2.070	78.30	77.80	56.00
Beclin 1	0.769	0.648-0.891	12.890	72.50	88.90	61.40

Note: Acute pulmonary embolism (APE), Receiver operating characteristic (ROC).

Autophagy is the process of cell catabolism, in which lysosome mediates the elimination of defects, damaged organelles, denatured protein and pathogens in cytoplasm to benefit the normal function of cells and the maintenance of homeostasis [18]. Under normal physiological conditions, autophagy does not affect the survival and the function of cells. However, insufficient or excessive autophagy *in vivo* can cause abnormal cell function, leading to aggravation of pathological damage of related tissues and cells or even death [19, 20]. Previous research has revealed that autophagy can restore normal cell homeostasis by inhibiting inflammatory cytokines to reduce tissue inflammation-related damage [21]. Additionally, Zhao et al. [22] found that the activation of autophagy can effectively improve the pulmonary inflammatory response. However, APE can result in local blood flow occlusion, circulatory

disturbance, and is prone to induce pulmonary infarction and lung injury [23]. The research results suggest a correlation between APE and autophagy. This study detected the expressions of P62 and Beclin 1 in patients with APE, and revealed a low P62 expression and a high Beclin 1 expression in them, also a high value of P62 and Beclin 1 in diagnosing APE. Autophagy-related protein Beclin1 is a crucial factor that connects autophagy with tumor cell genesis and apoptosis. As an autophagy adapter, p62 acts as a substrate in the process of cell autophagy, and degrades through autophagy, with a wide range of functions [24, 25]. According to the results of the present study, it can be speculated that the changes of lung injury caused by pulmonary embolism in patients lead to the activation of autophagy level. Our further results showed that P62 decreased in patients with insignificant efficacy, while Beclin 1 increased in those patients, suggesting that P62 and Beclin 1 levels were related to the efficacy in patients. ROC analysis showed that P62 had a higher value in predicting the efficacy in patients with APE than Beclin 1.

The present study also analyzed the changes of P62 and Beclin 1 in patients before and after treatment. According to the results, after treatment, the serum expression of P62 in patients increased significantly, while that of Beclin 1 decreased. Furthermore, the levels of P62 and Beclin 1 were found to be linked with the patient's disease condition. These results indicated the involvement of P62 and Beclin 1 in the occurrence of APE. Becattini et al. [26] found that the levels of D-dimer in patients with APE increased with the severity of APE, suggesting that the two were correlated with the severity of APE, and they could be used as outcome measures to reflect the severity of APE. In the present study, a correlation was found between P62 and Beclin 1 through correlation test analysis, and the levels of P62 and Beclin 1 changed with the severity of the disease, suggesting that p62 and beclin 1 can also be used as outcome measures for the severity of APE.

Clinical research has shown that APE is often characterized by thromboembolism, that is, falling deep vein thrombosis will enter the pulmonary artery with blood flow, which lead to a high morbidity and mortality [27]. APE occurs and develops quickly, and can destroy the balance of coagulation system and fibrinolysis system, and increase coagulation factors and fibrin. Although it can be relieved by relatively effective anticoagulation therapy, such as urokinase therapy, it may still recur [28]. Finally, this study analyzed the expression of P62 and Beclin 1 in relapsed patients. The results showed that P62 decreased while Beclin 1 increased in relapsed patients. Araz et al. [29] found that mean platelet volume had a sensitivity of 91% and a specificity of 77% in predicting the recurrence of APE, while Yang et al. [30] showed that the AUC of D-dimer in predicting the recurrence of APE at 12 months after treatment was 0.683 and the AUC in predicting the recurrence of APE at 24 months was 0.730, suggesting that D-dimer could be a predictor of recurrence of APE. In the present study, the AUCs of P62 and Beclin 1 in predicting the recurrence of APE were 0.816 and 0.769, respectively, and the sensitivity and specificity of them were relatively stable. Our results suggested that both P62 and Beclin 1 had a value in predicting the recurrence of APE.

This study has determined the clinical value of P62 and Beclin 1 in patients with APE, but it still has some limitations. First of all, we did not follow up the patients, but just checked the electronic medical records of their outpatient examinations to analyze their survival, with a short spanning time in query, which may compromise our results to a certain extent. Secondly, the sample size is limited in such a retrospective study. Finally, the mechanism of P62 and Beclin 1 in APE remains unclear. In particular, P62 and Beclin 1 are differentially expressed in a variety of diseases, and their specificity for APE needs further validation. Therefore, further investigation is needed in the future to determine the mechanism of P62 and Beclin 1 in APE.

To sum up, serum P62 decreased, but serum Beclin 1 increased in patients with APE, so these two indexes can be adopted for diagnosis, efficacy prediction and recurrence prediction of APE.

Acknowledgements

This work was supported by Zhejiang Provincial Basic Public Welfare Research Project (No. Y19H010009) and Ningbo key technology research and development projects (No. 2021Z018).

Disclosure of conflict of interest

None.

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