

## Application of Tongbian Scraping Combined with Suhuang Zhike Capsule in Chronic Obstructive Pulmonary Disease

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### Keywords

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### Abstract

**Objective:** This study investigates the application of Tongbian scraping combined with Suhuang Zhike capsule in chronic obstructive pulmonary disease (COPD). **Methods:** 43 patients with COPD treated with Tongbian scraping combined with Suhuang Zhike capsule from January 2023 to June 2023 in our hospital were allocated into the observation group, and 53 patients with COPD treated with Suhuang Zhike capsule were assigned into the control group during the same period. The clinical efficacy and adverse reactions in the two groups were compared, and the changes of traditional Chinese medicine (TCM) syndrome score, lung and immune functions, and 6-min walking distance were observed before and after treatment. **Results:** The total effective rate of clinical efficacy in the observation group was higher than that in the control group ( $p < 0.05$ ). After treatment, TCM syndrome score, COPD evaluation test score, and CD8<sup>+</sup> level were decreased in both groups ( $p < 0.05$ ), and these in the observation group were lower than those in the control group ( $p < 0.05$ ). The forced expiratory volume in the first second (FEV<sub>1</sub>), the predicted percentage of FEV<sub>1</sub> in FEV, the levels of CD4<sup>+</sup> and CD4<sup>+</sup>/CD8<sup>+</sup>, and 6-min walking distance in both groups were elevated ( $p < 0.05$ ), and these in the observation group were increased when compared with those in the control group ( $p < 0.05$ ). There was no significant difference in nausea, vomiting, constipation, dizziness, and total adverse reactions between the two groups ( $p > 0.05$ ). **Conclusion:** Tongbian scraping combined with Suhuang Zhike capsule has good clinical effect in the treatment of COPD, which can alleviate clinical symptoms, improve lung and immune functions, and enhance the activity tolerance, with good safety.



## 1 Introduction

Chronic obstructive pulmonary disease (COPD) is a progressive inflammatory disease with high morbidity and mortality, which is mainly characterized by persistent respiratory symptoms and airflow limitation, with clinical manifestations such as cough, sputum, and dyspnea [1,2]. A clinical study has shown that airway damage in COPD is usually irreversible, so the current clinical management of the disease is based on the principles of relieving symptoms and reducing the risk of disease progression [3].

Chinese medicine believes that COPD belongs to the category of lung emphysema and gasp syndrome, and it is caused by the phlegm stagnation in the airway and abnormal function of dispersing and descending in lung, so its treatment is mainly based on facilitating the flow of the lung-qi, eliminating pathogenic factors, invigorating qi for consolidating superficial resistance, inducing diuresis, and dissipating dampness [4,5]. Suhuang Zhike capsule is a proprietary Chinese medicine with anti-inflammatory, antitussive and antiasthmatic effects. The study of Gu et al. has demonstrated that Suhuang Zhike capsule is effective in relieving cough and has a low incidence of side effects during the experimental process, providing a high degree of safety [6]. Tongbian scraping is a kind of external treatment method of traditional Chinese medicine (TCM) based on the theory of twelve meridians and acupoints, and it has the effect of activating blood circulation and removing blood stasis, dredging the meridians and channels, and strengthening the body resistance to eliminate pathogenic factors. It has been reported that Tongbian scraping has a good clinical effect in the treatment of rheumatoid arthritis, periartthritis of shoulder, insomnia patients [7-9]. The research of Liu et al. found that Tongbian scraping has a good efficacy in the treatment of COPD [10]. Tongbian scraping combined with Suhuang Zhike capsule is in line with

the idea of internal and external treatment in TCM.

Taking all together, this study applies Tongbian scraping combined with Suhuang Zhike capsule in the treatment of COPD patients, compares the clinical efficacy and adverse reactions, and observes the specific effects on TCM symptoms, lung and immune functions, and activity tolerance, with the purpose of finding more effective methods for the treatment of COPD.

## 2 Methods

### 2.1 General data

43 patients with COPD treated with Tongbian scraping combined with Suhuang Zhike capsule from January 2023 to June 2023 in our hospital were allocated into the observation group, and 53 COPD patients treated with Suhuang Zhike capsule were assigned into the control group during the same period. This study were approved by the Ethical Committee of Shangcheng District People's Hospital of Hangzhou, and all patients signed the informed consent form.

### 2.2 Inclusion and exclusion criteria

Inclusion criteria: (1) Patients met the diagnostic criteria for stable COPD in the *Guidelines for the Diagnosis and Treatment of Chronic Obstructive Pulmonary Disease (2013 Revision)* [11]; (2) Patients met the diagnostic criteria for TCM in *Diagnostic Criteria for Chinese Medicine in Chronic Obstructive Pulmonary Disease (2011 Edition)* [12]; (3) Patients aged 35 to 90 years.

Exclusion criteria: (1) Patients with broken skin and contact skin disease; (2) Patients with severe primary diseases of the lungs such as bronchial asthma, tuberculosis, etc.; (3) Patients with underlying diseases such as hypertension, diabetes mellitus, etc.; (4) Patients with insufficiencies of vital organs such as heart, liver, and kidney; (5) Patients with coagulation abnormality due to long-term use of anticoagulant

medications; (6) Patients with psychiatric disorders and poor adherence to treatment; (7) Patients who were allergic to the drugs used in this study.

## 2.3 Treatment methods

### 2.3.1 Control group

Patients in control group were orally administrated with Suhuang Zhike capsule (Yangtze River Pharmaceutical Group Beijing Haiyan Pharmaceutical Co., Ltd., National Medical Products Administration (NMPA) Approval No.: Z20103075, Specification: 0.45 g/capsule) 3 times a day for two weeks, with 3 capsules each time.

### 2.3.2 Observation group

Based on the treatment in control group, patients in observation group were given Tongbian scraping at the acupoints of Dazhui, Dazhu, Gaohuang, and Shentang, pericardium meridian of hand Jueyin, heart meridian of hand Shaoyin, lung meridian of hand Taiyin, large intestine meridian of hand Yangming, and triple energizer meridian of hand Shaoyang. Patients were asked to take supine position and expose the scraping site. After Tongbian (a brass scraping plate) was wiped by 75% alcohol and scraping site was rubbed by saline, Tongbian was used to apply the appropriate amount of scraping oil evenly to the scraping site. From top to bottom, and inside to outside, Tongbian was maintained at an angle of 45° to the skin, and scraped in one direction at a rate of 70-80 times/min for 20-30 min until the skin was flushed or flaky and striated plaques were seen on the skin. If patients were not prone to be scraped on the skin, do not force. Scraping was performed every 3 days for a total of 2 weeks.

## 2.4 Observational indicators

### 2.4.1 Clinical efficacy

After 2 weeks of treatment, clinical efficacy was evaluated according to the *Guidelines for the Exploration and Verification Publishing*

*Diagnosis and Treatment of Chronic Obstructive Pulmonary Disease (2013 Revision)* [11]. Criteria were detailed below. Clinical control: normal laboratory indexes and disappearance of pulmonary wheezing sound and clinical symptoms; significantly effective: obvious improvement of laboratory indexes, pulmonary wheezing sound and clinical symptoms; improvement: mild improvement of laboratory indexes, pulmonary wheezing sound and clinical symptoms; ineffective: no improvement or even aggravation of laboratory indexes, pulmonary wheezing sound and clinical symptoms. Total effective rate = the number of (clinical control + significantly effective + improvement) cases / total number of cases × 100%.

### 2.4.2 TCM symptom score

Before and after 2 weeks of treatment, TCM symptom score was assessed according to the patients' main symptoms (cough, expectoration, wheezing, chest tightness, fever, and pulmonary murmur) by referring to *Diagnostic Criteria for Chinese Medicine in Chronic Obstructive Pulmonary Disease (2011 Edition)* [12]. The absence, mildness, moderateness, and severity of patients' main symptoms were recorded as 0, 2, 4, and 6 points, respectively, with the higher score indicating the more severe symptoms.

### 2.4.3 Lung and immune functions

Before and after 2 weeks of treatment, COPD Assessment Test (CAT) score [13] was used to assess the severity of patients' condition in two groups, which included 8 items of cough, expectoration, chest tightness, sleep, energy, mood, activity tolerance, and daily exercise, with a total score of 40 points (0-10 points: mild, 11-20 points: moderate, 21-30 points: severe, 31-40 points: extremely severe). The COSMED lung function instrument purchased from Shanghai Hanfei Medical Equipment Co. Ltd. was used to detect the levels of forced expiratory volume in the first second (FEV<sub>1</sub>) and forced expiratory volume (FEV)

in the two groups of patients, and the FEV<sub>1</sub>/FEV level was calculated. Levels of CD4<sup>+</sup> and CD8<sup>+</sup> were detected by FACSCalibu flow cytometer purchased from Becton Dickinson (USA), based on which CD4<sup>+</sup>/CD8<sup>+</sup> level was calculated.

**2.4.4 6-min walking distance**

Before and after 2 weeks of treatment, activity tolerance was assessed in both groups by 6-min walk test [14], and 6-min walking distance was recorded in both groups. The distance was positively correlated with patients' activity tolerance.

**2.4.5 Adverse reaction**

The occurrence of nausea, vomiting, constipation, and dizziness in both groups during the treatment was recorded.

**2.5 Statistical methods**

Statistical analysis was performed using SPSS 20.0. Count data were expressed as cases (%), comparisons between the two groups were carried out using  $\chi^2$  test, and measurement data were described as mean  $\pm$  standard deviation. Independent samples *t*-test was used for the comparison between the two groups, and paired samples *t*-test was used for the comparison at different time points in the same group. Differences

were considered to be statistically significant at  $p < 0.05$ .

**3 Results**

**3.1 Comparison of general data between the two groups**

There was no statistically significant difference in terms of gender, age, duration of the disease, and smoking history between the two groups ( $p > 0.05$ ), as shown in Table 1.

**3.2 Comparison of clinical efficacy between the two groups**

The total effective rate of clinical efficacy in the observation group was higher than that in the control group ( $p < 0.05$ ), as seen in Table 2.

**3.3 Comparison of TCM symptom score before and after treatment between the two groups**

Before treatment, there was no statistically significant difference in the comparison of TCM symptom score in the two groups ( $p > 0.05$ ). After treatment, TCM symptom score in the two groups was reduced ( $p < 0.05$ ), and the score in the observation group was lower than that in the control group ( $p < 0.05$ ). The results were displayed in Table 3.

**Table 1** Comparison of the general data in two groups.

Groups	Cases	Gender (cases)		Age (years old)	Duration of the disease (years)	Smoking history (cases)
		Male	Female			
Observation group	43	25	18	62.54 $\pm$ 7.46	7.34 $\pm$ 2.12	25
Control group	53	30	23	63.47 $\pm$ 7.68	7.06 $\pm$ 1.94	34
		$\chi^2/t$		0.023	0.600	0.675
		$p$		0.880	0.552	0.502

**Table 2** Comparison of clinical efficacy between the two groups [case (%)].

Groups	Cases	Clinical control	Significantly effective	Effective	Ineffective	Total effective rate
Observation group	43	21 (48.84)	15 (34.88)	5 (11.63)	2 (4.65)	41 (95.35)
Control group	53	14 (26.42)	17 (32.08)	10 (18.86)	12 (22.64)	41 (77.36)
		$\chi^2$				6.168
		$p$				0.013

**Table 3** Comparison of TCM symptom score before and after treatment between the two groups (mean ± standard deviation, point).

Groups	Cases	Cough		Expectoration		Wheezing	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Observation group	43	4.35 ± 1.32	0.68 ± 1.23 *	3.46 ± 2.31	1.07 ± 1.44 *	4.35 ± 1.58	1.38 ± 1.67 *
Control group	53	4.16 ± 1.41	1.48 ± 1.46 *	3.52 ± 1.67	1.94 ± 1.68 *	4.13 ± 1.55	2.59 ± 1.89 *
<i>t</i>		0.676	2.862	0.148	2.687	0.686	3.284
<i>ρ</i>		0.501	0.005	0.883	0.009	0.495	0.001

  

Groups	Cases	Chest tightness		Fever		Pulmonary murmur	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Observation group	43	4.23 ± 1.44	1.62 ± 1.20 *	1.77 ± 1.03	0.12 ± 0.44 *	3.66 ± 2.12	1.32 ± 1.56 *
Control group	53	4.11 ± 1.64	2.23 ± 1.55 *	1.75 ± 1.13	0.52 ± 0.67 *	3.74 ± 1.54	2.21 ± 1.87 *
<i>t</i>		0.376	2.116	0.090	3.368	0.214	2.495
<i>ρ</i>		0.708	0.037	0.929	0.001	0.831	0.014

Note: Comparison with before treatment: \*  $\rho < 0.05$ .

### 3.4 Comparison of lung function before and after treatment between the two groups

Before treatment, there was no statistically significant difference between CAT score and levels of FEV<sub>1</sub> and FEV<sub>1</sub>/FVC in the two groups ( $\rho > 0.05$ ). After treatment, CAT score in the two groups was decreased significantly ( $\rho < 0.05$ ), and the score in observation group was lower than that in the control group ( $\rho < 0.05$ ). After treatment, FEV<sub>1</sub> and FEV<sub>1</sub>/FVC levels in the two groups were increased ( $\rho < 0.05$ ), and the levels in the observation group were higher than those in the control group ( $\rho < 0.05$ ). The results were exhibited in Table 4.

**Table 4** Comparison of lung function before and after treatment between the two groups (mean ± standard deviation).

Groups	Cases	CAT (point)		FEV <sub>1</sub> (L)		FEV <sub>1</sub> /FVC (%)	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Observation group	43	22.67 ± 5.98	15.16 ± 3.87 *	1.17 ± 0.41	1.76 ± 0.44 *	51.46 ± 8.10	60.45 ± 6.54 *
Control group	53	23.41 ± 5.13	19.64 ± 5.41 *	1.23 ± 0.32	1.51 ± 0.38 *	52.15 ± 6.54	56.36 ± 5.64 *
<i>t</i>		0.653	4.563	0.805	2.986	0.462	3.289
<i>ρ</i>		0.516	<0.001	0.423	0.004	0.645	0.001

Note: Comparison with before treatment: \*  $\rho < 0.05$ .

### 3.5 Comparison of T-lymphocyte subsets between the two groups before and after treatment

Before treatment, there was no statistically significant difference in the comparison of CD4<sup>+</sup>, CD8<sup>+</sup>, and CD4<sup>+</sup>/CD8<sup>+</sup> levels between the two groups ( $\rho > 0.05$ ). After treatment, the levels of CD4<sup>+</sup> and CD4<sup>+</sup>/CD8<sup>+</sup> were increased in the two groups ( $\rho < 0.05$ ) and the levels in the observation group were higher than those in the control group ( $\rho < 0.05$ ). Level of CD8<sup>+</sup> was decreased in the two groups ( $\rho < 0.05$ ) and this level in the observation group was lower than that in the control group ( $\rho < 0.05$ ). The results were shown in Table 5.

**Table 5** Comparison of T-lymphocyte subsets between the two groups before and after treatment (mean ± standard deviation).

Groups	Cases	CD4 <sup>+</sup> (%)		CD8 <sup>+</sup> (%)		CD4 <sup>+</sup> /CD8 <sup>+</sup>	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Observation group	43	27.45 ± 2.67	36.45 ± 3.14 *	38.17 ± 4.56	26.27 ± 3.47 *	0.75 ± 0.24	1.34 ± 0.37 *
Control group	53	27.36 ± 2.74	33.46 ± 3.27 *	38.45 ± 4.27	30.66 ± 3.54 *	0.77 ± 0.22	1.12 ± 0.31 *
<i>t</i>		0.162	4.535	0.310	6.096	0.425	3.170
<i>ρ</i>		0.872	<0.001	0.757	<0.001	0.672	0.002

Note: Comparison with before treatment: \*  $\rho < 0.05$ .

### 3.6 Comparison of 6-min walking distance before and after treatment in both groups

Before treatment, there was no statistically significant difference in the comparison of 6-min walking distance between the two groups ( $\rho > 0.05$ ). After treatment, 6-min walking distance in the two groups was elevated ( $\rho < 0.05$ ), and this distance in the observation group was higher than that in the control group ( $\rho < 0.05$ ).

The results were shown in [Table 6](#).

**Table 6** Comparison of 6-min walking distance before and after treatment in both groups (mean ± standard deviation, m).

Groups	Cases	Before treatment	After treatment
Observation group	43	265.36 ± 15.12	338.67 ± 14.21 *
Control group	53	271.34 ± 15.42	318.68 ± 14.69 *
<i>t</i>		1.906	6.728
<i>ρ</i>		0.060	<0.001

Note: Comparison with before treatment: \*  $\rho < 0.05$ .

**Table 7** Comparison of adverse reactions between the two groups [cases (%)].

Groups	Cases	Nausea and vomiting	Constipation	Dizziness	Total adverse reaction rate
Observation group	43	1 (2.33)	2 (4.66)	1 (2.33)	4 (9.32)
Control group	53	1 (1.89)	1 (1.89)	1 (1.89)	3 (5.67)
$\chi^2$		0.022	0.599	0.022	0.466
<i>ρ</i>		0.881	0.439	0.881	0.495

## 4 Discussion

Considering the definite efficacy of Suhuang Zhike capsule in previous studies, this study compared the efficacy of Suhuang Zhike capsule alone or in

### 3.7 Comparison of adverse reactions between the two groups

There was no significant difference in nausea, vomiting, constipation, dizziness, and total adverse reactions between the two groups ( $\rho > 0.05$ ), as seen in [Table 7](#).

combination with Tongbian scraping in 96 COPD patients to find more effective therapy for COPD. According to the results, combination of the two was more effective in the treatment of COPD.

TCM syndrome score and CAT score are commonly used to clinically assess the severity of COPD patients' condition, the higher scores of the two hinting the more severe condition of patients. FEV<sub>1</sub> and FEV<sub>1</sub>/FVC are commonly used indicators to clinically assess the lung function, the lower of the two implying the worse lung function of patients. The results of this study revealed that Tongbian scraping combined with Suhuang Zhike capsule alleviated the clinical symptoms of patients, improved lung function, and its effect was better than that of Suhuang Zhike capsule alone. Suhuang Zhike capsule is made from a variety of Chinese herbs. Notably, *Ephedra sinica* Stapf, *Peucedanum praeruptorum* Dunn, *Cicadae Periostracum*, and *Arctium lappa* L have the effects of facilitating the flow of gastric qi to relieve asthma and dispelling pathogenic wind. *Perilla frutescens*, *Schisandrae chinensis* Fructus, *Pheretima*, and *Eriobotrya japonica* Thunb have the effects of warming lung to lower qi, expelling phlegm, and arresting coughing. Suhuang Zhike capsule can relieve clinical symptoms such as cough and expectoration in COPD patients and improve lung function [15]. A systematic review and meta-analysis reported that Suhuang zhike capsule adjuvant treatment could improve FVC, FEV<sub>1</sub>, FEV<sub>1</sub>/FVC and other pulmonary function indexes of acute exacerbation COPD patients [16]. At the same time, modern pharmacological research shows that ephedrine in *Ephedra sinica* Stapf can reduce nitric oxide synthase and endothelin-1 levels to repair airway damage, thus exerting its asthma-alleviating effect [17]. In addition, *Ephedra sinica* Stapf reduced airway and pulmonary inflammation by regulating inflammatory cytokines and the TGF-β1/Smad2 pathway in COPD model rat [18]. The procyanidins in *Peucedanum praeruptorum* Dunn can enhance tracheal excretion, suppress cough and eliminate phlegm, and Praeruptorin A can antagonize calcium ion activity and relax tracheal smooth muscle [19]. The stilbene in *Perilla frutescens*

relaxes the airways and has the effect of suppressing cough, eliminating phlegm, and relieving asthma [20]. In *Eriobotrya japonica* Thunb, ursolic acid has strong effects of suppressing cough, and triterpenic acid can achieve antifibrotic effect on the lung through the antilipid peroxidation pathway [21]. Tongbian scraping is a treatment method that makes the Tongbian and human body to produce a strong resonance frequency straight through the internal organs mainly by scraping and wiping meridians and acupoints, thereby achieving the effects of promoting blood circulation to dispel blood stasis and activating qi circulation, so as to alleviate patients' cough, expectoration, and dyspnea, and then improve lung function [22]. Tongbian scraping can promote the recovery of lung function by effectively stimulating the acupoints related to heart and lung function [23]. Accordingly, Tongbian scraping combined with Suhuang Zhike capsule has a certain effect in relieving clinical symptoms and improving lung function.

T-lymphocyte subsets are commonly used in clinical assessment of the body's cellular immune function, of which CD4<sup>+</sup> is a T-helper lymphocyte that signals and initiates immune response, while CD8<sup>+</sup> is a T-suppressor lymphocyte that inhibits and fights viruses [24,25]. The main manifestation of impaired immune function is a decrease in the number of CD4<sup>+</sup> and a relative increase in the number of CD8<sup>+</sup>. Therefore, the lower levels of CD4<sup>+</sup> and CD4<sup>+</sup>/CD8<sup>+</sup> and higher level of CD8<sup>+</sup> indicate the worse immune function of patients. According to our results, Tongbian scraping combined with Suhuang Zhike capsule can improve the immune function of patients, and its effect was better compared with the use of Suhuang Zhike capsule alone. Modern pharmacological research has revealed that *Ephedra sinica* Stapf and *Peucedanum praeruptorum* Dunn have anti-inflammatory effects, and *Arctium lappa* L and *Schisandrae chinensis* Fructus can tonify qi, activate blood circulation, and enhance the immune

system [19,20,26,27]. Suhuang Zhike capsule can also improve body's immune function by reducing patients' airway inflammation and allergic reaction, relieving the damage of inflammatory cytokines to the immune system. The study of Jiang et al. has also demonstrated that Suhuang Zhike capsule has the effect of improving patients' immune function, which was in lined with the results of our study [28,29]. Tongbian scraping can congest the skin with blood and bruises through stimulating the regulation of vascular relaxation and contraction and vascular wall permeability. The body will form stimulants during autolytic hemolysis, leading to faster metabolism, which in turn promotes anti-inflammation in the body [30]. Tongbian scraping is performed using Tongbian made by brass that is highly conductive and has a sterilizing and disinfecting effect [31]. Accordingly, Tongbian scraping combined with Suhuang Zhike capsule also has a role in improving the immune function of COPD patients. Tongbian scraping treatment has the effect of qi and blood promotion, in the process of copper plate stimulating the meridians on the body surface, the movement of human qi and blood is mobilized, the pores on the skin begin to evaporate water and excrete toxins, the function of the patient's body gradually recovered, so as to achieve the purpose of treating diseases.

The 6-min walk distance test is a common method for the clinical assessment of patients' activity tolerance, with shorter distance indicating poorer activity tolerance. The results of this study demonstrated that Tongbian scraping combined with Suhuang Zhike capsule enhanced patients' activity tolerance, and its effect was better compared with Suhuang Zhike capsule alone. Tongbian scraping combined with Suhuang Zhike capsule can alleviate the clinical symptoms of patients with COPD, improve lung and immune functions, inhibit the progression of the disease, reduce the severity of the disease, and remove the factors that lead to the gradual decline of

patients' activity tolerance from the root. Thus, Tongbian scraping combined with Suhuang Zhike capsule has a certain role in enhancing patient's activity tolerance.

In addition, the results of this study showed that the incidence of nausea, vomiting, constipation, dizziness, and total adverse reactions were similar in the two groups, indicating that Tongbian scraping combined with Suhuang Zhike capsule had a high level of safety in the treatment of patients with COPD, and it can be promoted in the clinic.

## 5 Conclusion

In conclusion, Tongbian scraping combined with Suhuang Zhike capsule has good clinical effect in the treatment of COPD, which can alleviate clinical symptoms, improve lung and immune functions, and enhance the activity tolerance, with good safety. Due to the limited clinical samples and observation time in this study, there may be some limitations, and it is still necessary to expand the sample size and extend the observation time at a later stage for further research.

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Not applicable.

## Conflicts of Interest

The authors declare no conflicts of interest.

## Author Contributions

Substantial contributions to conception and design: X.H., Z.Y.; Data acquisition, data analysis and interpretation: X.H.; Drafting the article or critically revising it for important intellectual content: Z.Y.; Final approval of the version to be published: All authors. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved: All authors.



### Ethics Approval and Consent to Participate

This study were approved by the Ethical Committee of Shangcheng District People's Hospital of Hangzhou, and all patients signed the informed consent form.

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### Availability of Data and Materials

The data presented in this study are available on request from the corresponding author.

### Supplementary Materials

Not applicable.

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