Article

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Clinical Evaluation of Bacterial Lysate for Immune Regulation and Exacerbation Reduction in Stable Chronic Obstructive Pulmonary Disease

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Background: Chronic obstructive pulmonary disease (COPD) is a leading cause of morbidity and mortality worldwide and is accompanied by systemic immune dysfunction, and recurrent acute exacerbations are the main determinants of disease progression, hospitalisation and poor prognosis. Oral bacterial lysates stimulate mucosa-associated lymphoid tissue and have been reported to reduce respiratory infections and exacerbations, but evidence on their immunomodulatory effect in patients with stable COPD is still limited. This single-centre retrospective cohort study, based on clinical data collected during routine care, was designed to investigate whether bacterial lysate Broncho-Vaxom (OM85-BV) can reduce the frequency of acute exacerbations in patients with stable COPD by regulating immune function.

Methods: 102 patients with stable COPD admitted to outpatient and inpatient departments were included between January 2025 and October 2025. Based on the treatment methods, they were classified into a control group (CG, basic treatment) and an OM85-BV group (basic treatment combined with oral bacterial lysate). Propensity score matching (PSM) was performed at a ratio of 1:1 using the nearest-neighbour method with a caliper of 0.100. In addition, healthy individuals of the same age group who underwent physical examination in The Fourth People's Hospital of Lin'an District during the same period were collected as a healthy control group (HC). HC received no drug treatment, and only admission indicators were collected. HC served solely as a descriptive reference for the distribution of immune indicators in individuals of comparable age and sex without COPD. The primary outcome measures in patients with COPD were the modified Medical Research Council (mMRC) dyspnea questionnaire and the COPD Assessment Test (CAT) at the 6-month follow-up, as well as the frequency of acute exacerbations within 6 months. The secondary outcome measures included albumin (ALB), immunoglobulins IgG, IgA and IgM, secretory immunoglobulin A (sIgA), CD4+ cells, CD8+ cells, CD4+/CD8+, natural killer (NK) cells, interleukin-6 (IL-6), interleukin-11 (IL-11), tumor necrosis factor-α (TNF-α) and safety indicators.

Results: In the descriptive comparison with HC, patients with stable COPD in the OM85-BV group and CG showed significantly decreased CD4+, CD4+/CD8+ and NK cells, as well as significantly increased CD8+ ($p < 0.001$). In repeated-measures analysis of variance (ANOVA), the time × group interaction was significant for CD4+ ($F(2,156) = 6.9$, $p = 0.001$), CD8+ ($F(2,156) = 8.9$, $p < 0.001$), CD4+/CD8+ ($F(2,156) = 18.200$, $p < 0.001$), and NK cells ($F(2,156) = 3.1$, $p = 0.046$). At the 6-month follow-up, the median CAT score in the OM85-BV group was lower than that in the CG [10.00 (4.75, 12.25) points vs 12.50 (7.00, 20.00) points, $p = 0.028$], and the mMRC grade was lower compared to CG [1.00 (0.00, 2.00) vs 2.00 (1.00, 3.00), $p = 0.023$]. The OM85-BV group had fewer acute exacerbations [1.00 (0.00, 1.00) vs 1.00 (1.00, 2.00), $p < 0.001$]. In the negative binomial regression model, the incidence-rate ratio (IRR) of the OM85-BV group relative to CG was 0.630 (95% confidence interval (CI) 0.440 to 0.900, $p = 0.011$). The CD4+, CD4+/CD8+ and NK cells in the OM85-BV group at the 6-month follow-up were higher than those in the CG, whereas the CD8+ was lower (all $p < 0.05$). At the 6-month follow-up, the levels of sIgA, ALB, IgG, IgA and IgM in the OM85-BV group were higher than those in the CG, while IL-11 (T2: median 8.20 vs 11.10, $Z = –4.285$, $p < 0.001$) and TNF-α (T2: median 13.10 vs 15.20, $Z = –2.267$, $p = 0.023$) were lower than those in the CG ($p < 0.05$). Because Shapiro-Wilk tests indicated IL-6, IL-11, and TNF-α at T2 did not meet the normality assumption (Shapiro-Wilk $p < 0.05$), these markers were re-analysed non-parametrically. IL-6 at T2 did not differ significantly between groups ($Z = –1.002$, $p = 0.318$). Adverse events occurred in 4 cases (10.00%) in the CG and 6 cases (15.00%) in the OM85-BV group, including gastrointestinal discomfort and transient skin reactions. The incidence rate of adverse reactions revealed no significant difference between the two groups ($p = 0.48$).

Conclusion: For patients with stable COPD, treatment with bacterial lysate was associated with more favourable changes in cellular immunity (CD4+, CD8+, CD4+/CD8+, NK cells) and humoral immunity (sIgA and immunoglobulins), as well as lower IL-11 and TNF-α levels and significantly reduced acute exacerbations within 6 months. Because the data are observational, these findings are consistent with an immunomodulatory effect of the combination of bacterial lysate and standard treatment but do not establish a causal relationship.

Keywords: stable chronic obstructive pulmonary disease; bacterial lysate; cellular immunity; humoral immunity; acute exacerbations; OM85-BV

Introduction

Chronic obstructive pulmonary disease (COPD) is a common respiratory tract disease. In the process of gradually understanding COPD, it has become increasingly recognized that COPD is not only limited to pulmonary lesions but also a systemic disease that can lead to nutritional depletion, weakened skeletal muscle function, low body weight and immune dysfunction, thus exerting adverse effects on the cardiovascular and nervous systems [1,2]. COPD is a highly debilitating pulmonary disease. As our understanding of its pathogenesis, progression and outcomes has evolved, the concept of COPD has also continued to evolve. The prevention and treatment guidelines [3] proposed by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) jointly established by the World Health Organization and the National Heart, Lung, and Blood Institute (NHLBI) have indicated that COPD is a disease characterized by persistent respiratory tract symptoms and airflow limitation resulting from abnormalities in the airways and/or alveoli caused by exposure to toxic particles or gases. The clinical manifestations of COPD often include dyspnea, cough, expectoration, chest tightness and fatigue after chronic activity; symptoms may vary with weather and seasonal changes; and chronic cough is intermittent and typically precedes the onset of airflow limitation [4]. In 2021, it was estimated that there were 4.43 million new cases of COPD (95% uncertainty interval [UI]: 4.01–4.86) and 50.59 million prevalent cases (95% UI: 44.98–57.12) in China, accounting for nearly one quarter of the global prevalence of COPD. The crude incidence rates and prevalence rates of COPD in China were 311.68 (95% UI: 281.75–341.62) per 100,000 and 3555.69 (95% UI: 3161.20–4014.55) per 100,000, respectively. Approximately 1.29 (95% UI: 1.04–1.54) million people died from COPD, accounting for 10.99% of total deaths in China, with a crude death rate of 90.35 (95% UI: 73.43–108.23) per 100,000 [5].

Immune response plays a crucial role in COPD pathogenesis. A variety of inflammatory cells, such as macrophages, neutrophils and lymphocytes, are present in patients with COPD and are involved in inflammatory responses of the airways and alveoli. In particular, the activation and aggregation of inflammatory cells, as well as the release of inflammatory mediators such as elastase and matrix metalloproteinases, can damage the lung tissue, leading to mucus hypersecretion and emphysema [6,7]. Various immunotherapeutic approaches for COPD are currently being explored in research and clinical practice, including the application of bacterial lysate to enhance immune function and improve clinical symptoms and lung function in COPD patients. Additionally, vaccination is part of immunomodulatory therapy, aimed at reducing the acute exacerbations of COPD by preventing infections. Another study explored the use of monoclonal antibodies targeting specific inflammatory mediators, such as interleukin-5 (IL-5) or its receptor, for the treatment of COPD [8].

The bacterial lysate consists of freeze-dried lysates from eight bacterial species, including *Haemophilus influenzae*, *Streptococcus pneumoniae* (formerly *Diplococcus pneumoniae*), *Klebsiella pneumoniae*, *Klebsiella ozaenae*, *Staphylococcus aureus*, viridans group streptococci, *Streptococcus pyogenes* and *Moraxella catarrhalis* (formerly *Neisseria catarrhalis*). Once introduced into the human body, bacterial lysate activates the immune system by stimulating the mucosal lymphoid tissue, thereby enhancing immune cell activity and immune function and providing protection against infections [9]. Animal pharmacological experiments have shown that bacterial lysate has preventive effects against infections in experimental animals and can elevate cytokine levels and Ig secretion in the respiratory tract mucosa by stimulating the B lymphocytes and macrophages. Clinical trials have demonstrated that bacterial lysate promotes T lymphocyte maturation in COPD patients, enhances the activities of peripheral blood mononuclear cells and macrophages, and increases secretory IgA levels in saliva and IgA levels in bronchoalveolar lavage fluid. Bacterial lysate has been shown to stimulate the immune system, thereby enhancing the host immune defense. Furthermore, bacterial lysate can stimulate the production of IL-6 and IL-11, and IL-6 plays a key role in the differentiation of CD4+ T cells into pro-inflammatory T helper 17 (Th17) cells [10,11]. This study primarily investigates whether the use of bacterial lysate Broncho-Vaxom (OM85-BV) can reduce acute exacerbations in patients with stable COPD by modulating immune function.

Methods

Study Population

This single-centre retrospective cohort study collected clinical data of 102 patients with COPD treated in outpatient and inpatient departments of The Fourth People's Hospital of Lin'an District between January 2025 and October 2025. All data were retrospectively extracted from the hospital electronic medical records. No treatment was administered, and no examinations, follow-up contacts, or specimen collections were carried out for the purpose of this analysis. The variables described below were collected during routine care and were already documented in the medi-

cal record before data extraction started. Patients attended the outpatient clinic at enrolment and were reviewed at 3 months and at 6 months in accordance with the standardized management pathway for stable COPD used at the study centre. The measurements at T0, T1 and T2 correspond to the laboratory tests performed during these three routine visits. The serum and saliva analysed were the residual portions of these routine specimens kept in the hospital sample repository, and the telephone contacts at 3 and 6 months were part of routine chronic-disease management for outpatients with COPD and were documented in the nursing records. Record screening, adjudication of acute exacerbations, calculation of medication adherence and statistical analyses were performed using the extracted data after the follow-up cutoff. Enrolment visits took place between January 2025 and October 2025, and the 6-month follow-up visit for the last enrolled patient was completed in April 2026; Record screening, matching and analysis were performed after April 30, 2026. The number of records at each step is shown in Fig. 1. The final follow-up cutoff was April 30, 2026, ensuring all enrolled patients completed at least 6 months of follow-up. The study was approved by the Medical Ethics Committee of The Fourth People's Hospital of Lin'an District (Approval No. 2026 No.001). Inclusion criteria for COPD patients comprised the following five aspects: (1) Meeting the GOLD2023 COPD diagnostic criteria [12], with a post-bronchodilator forced expiratory volume in 1 s (FEV1)/forced vital capacity (FVC) <0.7 and 30% ≤ FEV1% < 80%; (2) age ≥40 years; (3) body mass index (BMI) between 18 kg/m^2 and 36 kg/m^2; (4) stable clinical symptoms with no changes in basic medications within 1 month; and (5) complete clinical data and follow-up data.

Inclusion criteria were: (1) Age ≥40 years; (2) healthy individuals without a history of COPD or family history of COPD; (3) no history of smoking or cessation of smoking for more than 5 years; and (4) normal pulmonary function (FEV1/FVC ≥0.7). 40 healthy controls were identified from the same hospital during the same period and frequency-matched with COPD patients by age and gender. HC baseline characteristics were as follows: age, 63.85 ± 5.94 years; male sex, 26 (65.00%); BMI, 22.94 ± 2.86 kg/m^2; CD4+, 42.50 ± 5.20%; CD8+, 20.30 ± 3.80%; CD4+/CD8+, 2.15 ± 0.35; and NK cells, 22.80 ± 4.10%. The HC group was frequency-matched for age and sex only, and no matching or adjustment was made for smoking exposure or for any other characteristic. Therefore, the HC group was used solely as a descriptive reference for the distribution of immune indicators in individuals of comparable age and sex without COPD, rather than as a control group for estimating the effect of COPD on immune function.

Exclusion criteria involved the following nine conditions: (1) Age <40 years; (2) incomplete clinical data; (3) concomitant severe cardiovascular, hepatic, renal or hematological disorders; (4) concomitant other primary pulmonary diseases; (5) recent use of immunosuppressants or hormones; (6) suffering from intestinal diseases such as inflammatory bowel disease, intestinal tuberculosis and intestinal tumors that might cause gut microflora dysbiosis; (7) concomitant severe heart disease, malignant tumors, pulmonary interstitial fibrosis, asthma, autoimmune diseases or active tuberculosis; (8) concomitant severe hepatic or renal insufficiency; and (9) acute exacerbation of COPD (AECOPD), defined according to the GOLD 2023 Report as an event characterized by worsening dyspnea and/or cough and sputum over 14 days or less, which may be accompanied by tachypnea and/or tachycardia [12].

Symptom Assessment, Comprehensive Assessment and Treatment Regimens

The symptoms of the patients were graded by the modified Medical Research Council (mMRC) dyspnea questionnaire and COPD Assessment Test (CAT) questionnaire [13,14]. The mMRC scale reflected the severity of dyspnea, ranging from grade 0 to grade 4, with a grade of 2 or above (mMRC ≥2) indicating significant symptoms. CAT score is a comprehensive symptom assessment tool comprising 8 questions across three domains (symptoms, exercise tolerance and self-assessment), with a score range of 0–40 points and a score of 10 points or above (CAT ≥10) denoting significant symptoms. In accordance with the GOLD 2023 report, a history of two or more moderate acute exacerbations, or of one or more acute exacerbations requiring hospitalization, in the past year was classified as a high risk of acute exacerbation, and all other patients were classified as being at low risk. Regarding comprehensive assessment, group A consisted of patients at low risk of acute exacerbation with few symptoms (0 or 1 moderate acute exacerbation not requiring hospitalization in the past year, together with mMRC 0–1 and CAT <10), group B consisted of patients at low risk of acute exacerbation with many symptoms (0 or 1 moderate acute exacerbation not requiring hospitalization in the past year, together with mMRC ≥2 and/or CAT ≥10), and group E consisted of patients at high risk of acute exacerbation (≥2 moderate acute exacerbations or ≥1 acute exacerbation requiring hospitalization in the past year), irrespective of symptom burden. The exacerbation criterion was applied first and the symptom criterion second, and the two symptom instruments were combined with "and/or" exactly as specified in the GOLD 2023 report, so that the thresholds are mutually exclusive and exhaustive (CAT = 10 and mMRC = 2 both belong to the high-symptom category) and every patient could be allocated to one, and only one, of the three groups. Inhaled medications were selected based on the results of a comprehensive assessment. Group A received formoterol (4.5 μg per dose, twice daily, aerosol inhalation). Group B was treated with formoterol plus glycopyrronium bromide [2 sprays per dose (each spray containing 9 μg of glycopyrronium bromide and 4.8 μg of formoterol), twice daily, administered via oral inhalation]. Group E received

initial treatment with formoterol plus glycopyrronium bromide, and was subsequently switched to budesonide plus formoterol plus glycopyrronium bromide [2 sprays per dose (each spray containing 160 µg of budesonide, 9 µg of glycopyrronium bromide and 4.8 µg of formoterol), twice daily (once in the morning and once in the evening), oral inhalation] when blood eosinophil count was ≥300 cells/µL.

In addition to the existing COPD treatment regimen, patients in the OM85-BV group were given oral administration of bacterial lysate Broncho-Vaxom (OM Pharma, Meyrin, Geneva, Switzerland) at a dose of one 7 mg capsule once daily on an empty stomach on the first 10 days of each month, followed by a 20-day interval without the drug, for 3 consecutive months; that is, the drug was taken on days 1–10 of month 1, on days 1–10 of month 2 and on days 1–10 of month 3, giving three 10-day courses and 30 doses in total. Administration of OM85-BV was therefore completed at the end of month 3, and no OM85-BV was taken during months 4 to 6, which constituted an observation period only. Accordingly, T2 denotes the 6-month follow-up after baseline and does not indicate 6 months of continuous OM85-BV administration. Medication adherence was recorded at the routine follow-up visits and telephone contacts, and was calculated retrospectively from these records. If a dose was missed, patients took the next dose at the scheduled time without doubling the dose. Mean adherence in the OM85-BV group was 94.62% ± 6.78%.

Outcome Measures and Data Collection

Outcome Measures

Primary outcome measures comprised CAT score and mMRC grade at the 6-month follow-up, as well as the frequency of acute exacerbations within 6 months. An acute exacerbation was defined as worsening of dyspnea, cough, and/or sputum beyond normal day-to-day variation, requiring systemic corticosteroids/antibiotics, emergency department (ED) visit, or hospitalization [12]. Exacerbation counts were ascertained from outpatient, emergency and hospitalization records, as well as the records of the routine telephone contacts at 3 and 6 months. After data extraction, each candidate event was independently adjudicated on the extracted records by two pulmonologists who were blinded to group allocation; disagreements were resolved by a third senior adjudicator (Cohen's $\kappa = 0.874$).

Secondary outcome measures encompassed four kinds of indicators, namely albumin (ALB) together with immunoglobulins IgG, IgA, IgM and salivary secretory immunoglobulin A (sIgA), T lymphocyte subsets covering CD4+ cells, CD8+ cells, CD4+/CD8+ and NK cells, inflammatory factors covering IL-6, IL-11 and TNF-α, and safety indicators covering the adverse events recorded during the 6-month observation period. T lymphocyte subsets were measured at baseline (T0), at the 3-month follow-up (T1) and at the 6-month follow-up (T2), and ALB, immunoglobulins, sIgA and inflammatory factors were measured at T0 and T2.

Sample Collection and Processing

The specimens described in this section were obtained during routine tests at the three follow-up visits, and the residual portions were kept in the hospital sample repository. All immune and inflammatory indicators reported in this study are the results of the tests that were performed on these specimens at the time of the corresponding visit as part of routine clinical care, and these results were retrieved from the hospital laboratory information system for the present analysis. No indicator was re-measured on the residual specimens for the purpose of this study; the residual specimens were retained only so that an original result could be verified if a recorded value was doubtful. A disposable vacuum blood collection system was used to puncture the superficial vein of the upper arm in the early morning after overnight fasting. Depending on specific test items, blood samples were collected and injected into different blood collection tubes. (1) ALB, immunoglobulins and cytokine tests: Approximately 5 mL of blood was collected and injected into the ordinary serum tube without anticoagulant. The already coagulated whole blood was allowed to stand at room temperature (22–25 °C) for 30 minutes, and then centrifuged at 3000 rpm for 15 minutes at 4 °C. After centrifugation, the upper serum was carefully aspirated by a micropipette and aliquoted into 1.5 mL sterile centrifuge tubes, which were stored in a refrigerator at –80 °C for later use. (2) T lymphocyte subset tests: Approximately 3 mL of blood was drawn into an ethylenediaminetetraacetic acid (EDTA) anticoagulant tube and processed within 2 hours of collection. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Paque density gradient centrifugation. After centrifugation at 2000 rpm for 20 minutes at 20 °C, the intermediate white membrane layer (PBMC layer) was aspirated carefully and washed twice with phosphate-buffered saline (PBS) (1500 rpm, 5 minutes). Finally, PBMC was resuspended in RPMI-1640 medium containing 10% fetal bovine serum, and the cell concentration was adjusted to 1×10^6 cells/mL for immediate flow cytometry staining. (3) Saliva sample collection: After rinsing the mouth with water, approximately 2 mL of unstimulated whole saliva was passively collected using a saliva collection tube (Sarstedt AG & Co. KG, Nümbrecht, Germany). After collection, the sample should be immediately stored at 2 °C to 8 °C for temporary preservation. The collected saliva sample was centrifuged at 1000 ×g for 15 minutes at 4 °C. The supernatant was collected, aliquoted and stored at –80 °C for subsequent use. The above sample collection procedures were in accordance with the Chinese standard "GB/T 37864—2019 General Requirements of Quality and Competence for Biobank".

Laboratory Instruments and Reagents

Regarding instruments, CytoFLEX S flow cytometer (Beckman Coulter, Brea, CA, USA) and BS-800 fully automated biochemical analyzer (Mindray, Shenzhen, China) were adopted. ALB was detected by an albumin assay kit (bromophenol blue-based binding method), supplied by LABLEAD (Beijing, China, Catalog No.: HLBB028Hu). IgG, IgA and IgM were measured using an immunoglobulin G/A/M assay kit (immunoturbidimetry assay), provided by Roche Diagnostics [Basel, Switzerland, Catalog No.: 03727874 (IgG), 03727610 (IgA) and 03727726 (IgM)]. sIgA was determined by a human salivary secretory immunoglobulin A enzyme-linked immunosorbent assay (ELISA) kit, obtained from ELK Biotechnology (Wuhan, China, Catalog No.: ELK1844). IL-6, IL-11 and TNF-α were detected by high-sensitivity human interleukin-6 ELISA kit, human interleukin-11 ELISA kit and human tumor necrosis factor-α ELISA kit from R&D Systems [Minnesota, MA, USA, Catalog No.: HS600C (IL-6), D1100 (IL-11) and DTA00D (TNF-α)]. Antibodies for T lymphocyte subsets flow cytometry included Anti-Human CD4 (Beckman Coulter, Brea, CA, USA, Catalog No.: A07752, clone 13B8.2), Anti-Human CD8 (Beckman Coulter, Brea, CA, USA, Catalog No.: A07757, clone B9.11), Anti-Human CD56 (BioLegend, San Diego, CA, USA, Catalog No.: 362504, clone 5.1H11) and Anti-Human CD3 (BioLegend, San Diego, CA, USA, Catalog No.: 300438, clone UCHT1). Within the lymphocyte gate, CD4+ T cells were defined as CD3+CD4+, CD8+ T cells as CD3+CD8+, whereas natural killer (NK) cells were defined as CD3–CD56+.

Quality Control

(1) All researchers were trained in the study protocol, data extraction, and related procedures prior to study initiation. Two researchers independently extracted clinical data from the electronic medical records using a standardized data collection form, and a third researcher cross-checked any discrepancies to ensure accuracy. (2) Data analysts were blinded to treatment group assignments during statistical analysis. (3) As this was a retrospective analysis of clinical data and residual specimens obtained during routine care, with no procedure performed for research purposes, the requirement for informed consent was waived by the ethics committee. Patient information was kept strictly confidential, and all data were anonymized.

Statistical Analysis

The above data were analyzed using IBM SPSS Statistics 27.0 (IBM Corp., Armonk, NY, USA). Shapiro-Wilk normality test was used for continuous variables. Variables conforming to the normal distribution were denoted as mean ± SD, and non-normally distributed variables were represented as [M (Q1, Q3)]. PSM was conducted using a logistic regression model, with group allocation as the dependent variable and age, sex, BMI, current smoking status, pack-years, post-bronchodilator FEV1% predicted, GOLD airflow-limitation grade, baseline CAT score, baseline mMRC grade, exacerbations in the prior year, GOLD 2023 A/B/E group, inhaled therapy category, influenza and pneumococcal vaccination status, ischaemic heart disease, congestive heart failure, diabetes mellitus and hypertension as the covariates. These covariates were specified on clinical grounds before matching and comprise the factors that influence the decision to add an oral bacterial lysate in the outpatient practice of the study centre, together with factors related to symptom burden and the risk of acute exacerbation; no data-driven or stepwise selection was applied, and no covariate was added or removed after examination of the balance results. FEV1% predicted and the GOLD airflow-limitation grade, as well as the CAT score, mMRC grade and the GOLD 2023 A/B/E group, describe overlapping clinical information, so the model contains partially redundant covariates relative to the number of patients available. Matching was performed by the nearest-neighbour method without replacement at a ratio of 1:1 with a caliper of 0.100 of the pooled standard deviation of the logit of the propensity score [15]. Balance was evaluated by the standardized mean difference (SMD), and an SMD below 0.100 was regarded as balanced. Before matching, the two groups were compared as independent samples: normally distributed continuous variables by the independent-samples *t*-test, non-normally distributed continuous variables and ordinal variables by the Mann-Whitney U test, and categorical variables, presented as n (%), by the χ^2 test, with Fisher's exact test used when an expected count was below 5. For sex and for the GOLD airflow-limitation grade, one χ^2 test of the two-category variable was performed, so that the same *p* value applies to both rows of the variable in Table 1; for the GOLD 2023 A/B/E comprehensive assessment group and for the inhaled therapy category, one overall χ^2 test of the three categories was performed and the *p* value is reported once in the row of the variable, whereas the SMD was calculated separately for each category. The SMD of a continuous variable is the difference in means divided by the pooled standard deviation, and the SMD of a category is the difference in proportions divided by the pooled standard deviation of the two proportions, with the OM85-BV group as the first term. Data of the matched pairs were tested by paired methods: normally distributed continuous data were tested by paired-samples *t*-test, non-normally distributed continuous data by Wilcoxon signed-rank test, and categorical data, presented as n (%), by McNemar's test. mMRC grade was expressed as [M (Q1, Q3)] and tested by Wilcoxon signed-rank test. Data at T0 and T2 within the same group were tested by the same paired methods according to the distribution. For the baseline comparison of the immune indicators among the three groups shown in Fig. 3, the HC group was compared with each COPD group by the independent-samples

t-test or the Mann-Whitney U test as appropriate, whereas the propensity score-matched CG and OM85-BV groups were compared with each other by the paired-samples *t*-test or the Wilcoxon signed-rank test as appropriate; the *p* values of these three-group comparisons were all corrected by the Holm method. The frequency of acute exacerbations was analysed using a negative binomial regression model, and the results were expressed as incidence-rate ratio (IRR) with 95% confidence interval (CI). This model included the group as the only explanatory variable, used a log link and the logarithm of the observation time as an offset, and did not include the matched-pair identifier as a cluster or stratification variable. Sensitivity analyses were carried out in the full unmatched cohort of 44 patients in the CG and 58 patients in the OM85-BV group. CAT score at the 6-month follow-up was analysed by multivariable linear regression, and the frequency of acute exacerbations within 6 months was analysed by negative binomial regression. Because the mMRC grade is an ordinal variable, it was compared by rank-based tests only, with age, sex, BMI, current smoking status, pack-years, FEV1% predicted, baseline CAT score, baseline mMRC grade, exacerbations in the prior year and inhaled therapy category as covariates. Collinearity among the covariates was examined by the variance inflation factor (VIF), and overdispersion of the count model was examined by the ratio of the deviance to the residual degrees of freedom. The results were expressed as adjusted differences or IRR with 95% CI. This study implemented repeated measures analysis of variance (ANOVA) for selected variables, conducted paired comparisons across time points based on estimated marginal means, and applied Bonferroni correction. This model was specified with time (T0, T1 and T2) as the within-subject factor, group as the between-subject factor, and the group × time interaction as the effect of interest, with the individual patient as the unit of analysis and without the matched-pair identifier as a stratification factor. Mauchly's test of sphericity was initially conducted. The uncorrected F-value was used if the test was not significant, whereas the degrees of freedom and corresponding *p*-value after Greenhouse-Geisser correction were used if the test was significant. Because matching was carried out without replacement, every patient contributes one observation to the repeated measures ANOVA and to the negative binomial regression, and both models therefore treat patients as independent. In a propensity-score matched sample, analysing the data as independent samples rather than as matched pairs produces wider confidence intervals and more conservative inference than the corresponding paired analysis. Therefore, the *p* values from these two models are conservative rather than anti-conservative; the paired structure was retained for the comparisons within matched pairs reported in Table 1 and Table 3. The significance level was set at a two-sided $\alpha = 0.05$ [16].

Results

Baseline Data Characteristics

This study included 44 cases in the CG and 58 cases in the OM85-BV group, with the screening flow chart presented in Fig. 1. After PSM, both the CG and OM85-BV groups consisted of 40 cases, and an additional 40 cases were included in the HC. The probability density diagrams before and after PSM were displayed in Fig. 2. The baseline data of the two groups before and after matching were listed in Table 1, and the two groups showed standardized mean differences below 0.110 for all measured covariates after matching, with the A and B categories of the GOLD 2023 A/B/E group above 0.100 (–0.109 and 0.102) and all remaining covariates below 0.100. Post-matching, current smokers were 24 (60.00%) in the CG group and 25 (62.50%) in the OM85-BV group (SMD = 0.051), pack-years were 32.45 ± 14.28 vs 33.10 ± 13.85 (SMD = 0.046), FEV1% predicted was 55.42 ± 12.35 vs 54.87 ± 12.86 (SMD = –0.044), GOLD grade 2 was 28 (70.00%) vs 27 (67.50%). According to Fig. 3, the baseline CD4+, CD4+/CD8+ and NK cells of patients with stable COPD in the OM85-BV and CG groups were significantly decreased, while CD8+ was increased compared to HC ($p < 0.001$). The comparison with HC is descriptive, because HC was frequency-matched for age and sex only.

Comparison of Primary Outcome Measures

Primary outcome measures referred to CAT score and mMRC at the 6-month follow-up and the frequency of acute exacerbations within 6 months. At the 6-month follow-up, the OM85-BV group showed a lower median CAT score than CG [10.00 (4.75, 12.25) points vs 12.50 (7.00, 20.00) points, $p = 0.028$], and also had a lower mMRC grade compared to CG [1.00 (0.00, 2.00) vs 2.00 (1.00, 3.00), $p = 0.023$]. The frequency of acute exacerbations was lower in the OM85-BV group [1.00 (0.00, 1.00) vs 1.00 (1.00, 2.00), $p < 0.001$]. In the negative binomial regression model, the IRR of the OM85-BV group relative to CG was 0.630 (95% CI 0.440 to 0.900, $p = 0.011$). The distributions of the primary outcome measures are shown in Fig. 4.

Comparison of Secondary Outcomes

Cellular Immunity

The results of Mauchly's test of sphericity for the four indicators were not significant, and all met the sphericity assumption. ANOVA results showed that the interaction effect in CD4+ between the two groups was statistically significant ($F(2,156) = 6.9$, $p = 0.001$), and the difference in time effect was significant ($F(2,156) = 21.9$, $p < 0.001$), but no significant difference was observed in the between-group effect ($F(1,78) = 1.1$, $p = 0.290$), with a 95% CI of difference ranging from –1.299 to 0.392. The interaction effect in CD8+ was statistically significant between the two groups ($F(2,156) = 8.9$, $p < 0.001$), while

Table 1. Comparison of data of COPD patients before and after matching.

Variable	CG (n = 44) before	OM85-BV (n = 58) before	*p* before	SMD before	CG (n = 40) after matching	OM85-BV (n = 40) after matching	*p* after matching	SMD after matching
Age, years	64.10 ± 6.70	64.20 ± 6.40	0.939	0.015	64.17 ± 6.62	64.33 ± 5.72	0.914	0.026
BMI, kg/m^2	22.80 ± 3.40	22.50 ± 3.50	0.665	–0.087	22.75 ± 3.17	22.61 ± 3.23	0.840	–0.044
CAT, points	16.00 (8.00, 25.00)	18.00 (8.00, 26.00)	0.444	0.080	16.50 (8.00, 26.00)	19.50 (7.75, 26.50)	0.441	0.083
mMRC	2.00 (1.00, 3.00)	2.50 (1.00, 3.00)	0.390	0.090	2.50 (1.00, 3.00)	3.00 (1.00, 4.00)	0.389	0.091
Exacerbations in prior year	1.00 (1.00, 1.00)	1.00 (1.00, 2.00)	0.444	0.080	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)	1.000	0.000
Female, n (%)	14 (31.82)	18 (31.03)	0.933	–0.017	13 (32.50)	13 (32.50)	1.000	0.000
Male, n (%)	30 (68.18)	40 (68.97)	0.933	0.017	27 (67.50)	27 (67.50)	1.000	0.000
Current smokers, n (%)	26 (59.09)	41 (70.69)	0.222	0.245	24 (60.00)	25 (62.50)	0.820	0.051
Pack-years	32.50 ± 14.20	33.00 ± 13.90	0.859	0.036	32.45 ± 14.28	33.10 ± 13.85	0.837	0.046
FEV1% predicted	55.76 ± 11.34	50.87 ± 9.72	0.021	–0.463	55.42 ± 12.35	54.87 ± 12.86	0.846	–0.044
GOLD grade 2, n (%)	30 (68.18)	42 (72.41)	0.642	0.093	28 (70.00)	27 (67.50)	0.809	–0.054
GOLD grade 3, n (%)	14 (31.82)	16 (27.59)	0.642	–0.093	12 (30.00)	13 (32.50)	0.809	0.054
Comprehensive assessment			0.085				0.943	
A	16 (36.36)	13 (22.41)		–0.310	13 (32.50)	11 (27.50)		–0.109
B	24 (54.55)	31 (53.45)		–0.022	23 (57.50)	25 (62.50)		0.102
E	4 (9.09)	14 (24.14)		0.413	4 (10.00)	4 (10.00)		0.000
Inhaled therapy			0.825				0.955	
LABA or LAMA single	5 (11.36)	9 (15.52)		0.122	5 (12.50)	5 (12.50)		0.000
LABA + LAMA dual	33 (75.00)	42 (72.41)		–0.059	30 (75.00)	29 (72.50)		–0.057
ICS + LABA + LAMA triple	6 (13.64)	7 (12.07)		–0.047	5 (12.50)	6 (15.00)		0.073
Influenza vaccination, n (%)	5 (11.36)	17 (29.31)	0.029	0.457	5 (12.50)	6 (15.00)	0.744	0.073
Pneumococcal vaccination, n (%)	5 (11.36)	9 (15.52)	0.546	0.122	3 (7.50)	4 (10.00)	0.692	0.089
Hypertension, n (%)	22 (50.00)	28 (48.28)	0.863	–0.034	18 (45.00)	19 (47.50)	0.823	0.050
Ischaemic heart disease, n (%)	12 (27.27)	16 (27.59)	0.972	0.007	8 (20.00)	9 (22.50)	0.785	0.061
Congestive heart failure, n (%)	10 (22.73)	11 (18.97)	0.642	–0.093	5 (12.50)	4 (10.00)	0.723	–0.079
Diabetes mellitus, n (%)	12 (27.27)	13 (22.41)	0.572	–0.113	9 (22.50)	8 (20.00)	0.785	–0.061

A, B and E represent the Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2023 comprehensive assessment (ABE) groups; SMD, standardized mean difference; LABA, long-acting β2-agonist; LAMA, long-acting muscarinic antagonist; ICS, inhaled corticosteroid. Data before matching refer to 44 patients in the CG and 58 patients in the OM85-BV group, and data after matching refer to 40 patients in each group. The statistical methods used before and after matching, the way in which the *p* value and the SMD are reported for two-category and three-category variables, and the definition of the SMD are described in the Statistical Analysis subsection.

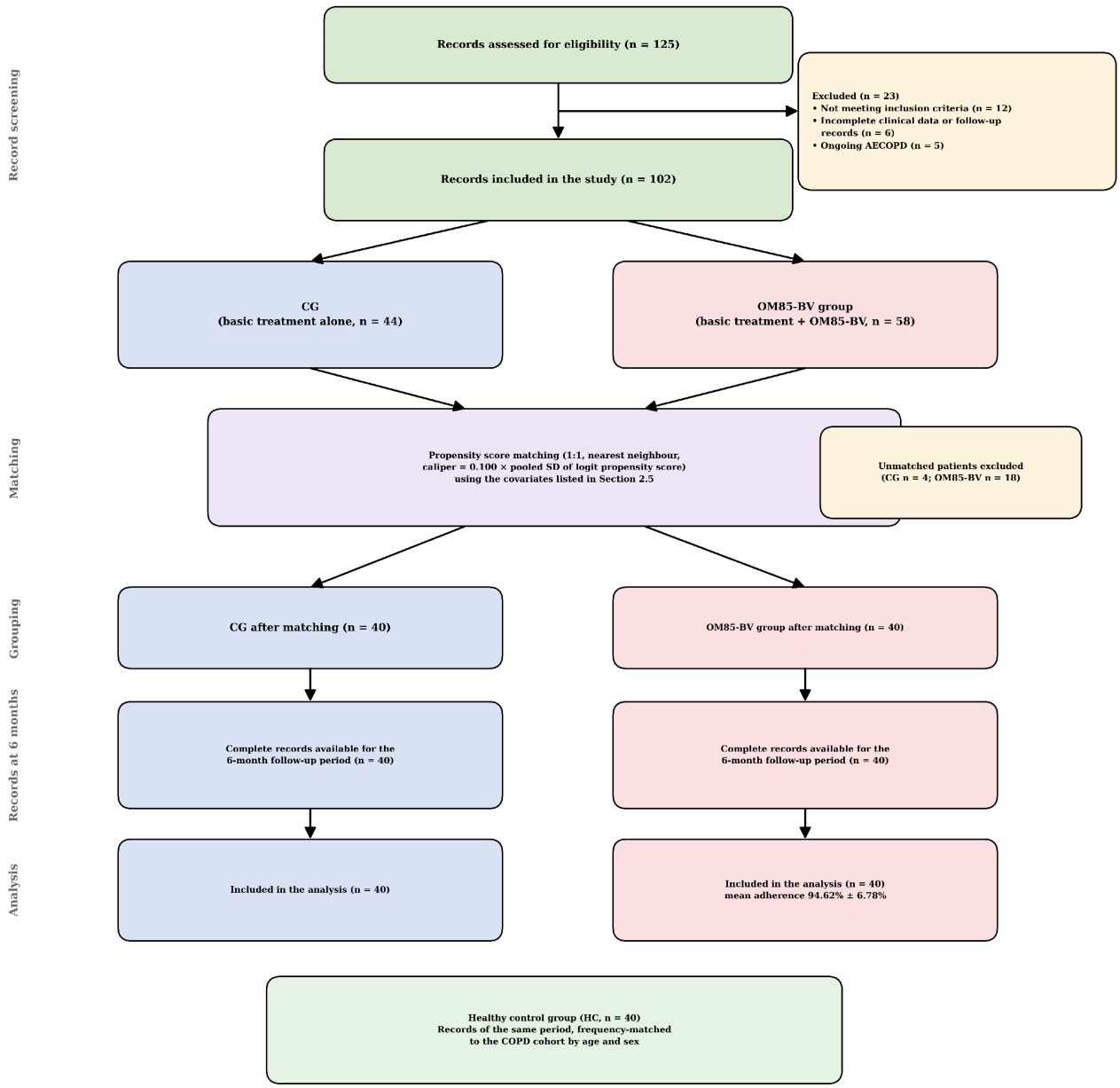

Fig. 1. Flow chart of record screening, grouping and analysis in this retrospective cohort. Patients were grouped according to the treatment actually received during routine care and were not allocated by the investigators; the covariates used for propensity score matching are those listed in the Statistical Analysis subsection. This figure was created by the authors using Microsoft PowerPoint 2019 (Microsoft Corporation, Redmond, WA, USA).

the main time effect across both groups was not significant ($F(2,156) = 1.7$, $p = 0.176$), and the difference was significant in the between-group effect ($F(1,78) = 38.6$, $p < 0.001$), with a 95% CI of the difference ranging from 1.519 to 2.928. There were significant group × time interaction ($F(2,156) = 18.2$, $p < 0.001$), time ($F(2,156) = 18.0$, $p < 0.001$), and between-group effects ($F(1,78) = 30.6$, $p < 0.001$) for CD4+/CD8+, with a 95% CI of the difference ranging from –0.235 to –0.111. There were significant group × time interaction ($F(2,156) = 3.1$, $p = 0.046$), time ($F(2,156) = 11.3$, $p < 0.001$), and between-group effects ($F(1,78) = 33.0$, $p < 0.001$) for NK cells, with a 95% CI of the difference ranging from –2.592 to –1.268. The results of paired comparisons based on estimated marginal means are described in Table 2, and the change trends of cellular immune indicators are shown in Fig. 5. The OM85-BV group had higher levels of CD4+, CD4+/CD8+ and NK cells, as well as lower CD8+ levels than CG at the 6-month follow-up (all $p < 0.05$).

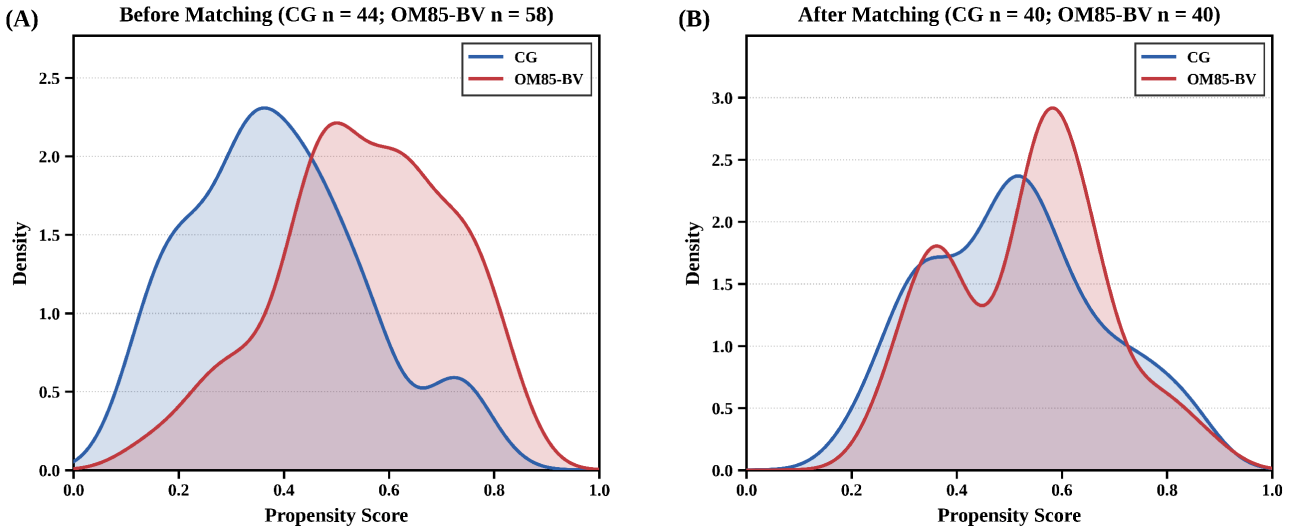

Fig. 2. Probability density diagrams before and after PSM. (A) Before matching; (B) after matching. PSM, propensity score matching.

Table 2. Comparison of cellular immune indicators.

Variable	Time	CG (n = 40)	OM85-BV (n = 40)	*p* (within CG, vs T0)	*p* (within OM85-BV, vs T0)	*p* (between groups)
CD4+, %	T0	30.85 ± 4.18	29.53 ± 4.25	—	—	0.166
	T1	31.90 ± 3.31	32.02 ± 3.32	0.024	<0.001	0.872
	T2	32.39 ± 3.34	34.95 ± 3.54	0.019	<0.001	0.002
CD8+, %	T0	22.91 ± 2.72	22.48 ± 2.75	—	—	0.480
	T1	23.45 ± 2.76	21.34 ± 2.80	0.386	0.068	0.001
	T2	23.95 ± 2.80	19.82 ± 2.79	0.096	<0.001	<0.001
CD4+/CD8+	T0	1.37 ± 0.22	1.33 ± 0.21	—	—	0.410
	T1	1.38 ± 0.22	1.52 ± 0.26	0.874	<0.001	0.010
	T2	1.37 ± 0.22	1.79 ± 0.31	0.977	<0.001	<0.001
NK cells, %	T0	16.40 ± 3.01	17.43 ± 3.07	—	—	0.132
	T1	16.84 ± 2.98	18.55 ± 2.09	0.482	0.086	0.004
	T2	17.34 ± 3.02	20.39 ± 2.21	0.164	<0.001	<0.001

T0, T1 and T2 represent baseline, the 3-month follow-up and the 6-month follow-up, respectively. Adjusted *p* values represent Bonferroni-corrected pairwise comparisons against T0 within each group, based on estimated marginal means. Repeated-measures ANOVA (group × time) effects were as follows — CD4+: interaction F(2,156) = 6.9, $p = 0.001$; time effect F(2,156) = 21.9, $p < 0.001$; between-group effect F(1,78) = 1.1, $p = 0.290$ (95% CI –1.299 to 0.392). CD8+: interaction F(2,156) = 8.9, $p < 0.001$; between-group effect F(1,78) = 38.6, $p < 0.001$ (95% CI 1.519 to 2.928). CD4+/CD8+: interaction F(2,156) = 18.2, time F(2,156) = 18.0, between-group F(1,78) = 30.6, all $p < 0.001$ (95% CI –0.235 to –0.111). NK cells: interaction F(2,156) = 3.1, $p = 0.046$; time F(2,156) = 11.3 and between-group F(1,78) = 33.0, both $p < 0.001$ (95% CI –2.592 to –1.268).

Humoral Immunity, Inflammatory Factors and Safety Indicators

The changes in humoral immunity and inflammatory factors are listed in Table 3. This study found that at the 6-month follow-up, the levels of sIgA, ALB and immunoglobulins IgG, IgA and IgM were increased in the OM85-BV group and were all higher compared to CG, whereas the levels of inflammatory factors IL-11 and TNF-α were decreased and were lower compared with CG ($p < 0.05$). The IL-6 level was not significantly different between the two groups at the 6-month follow-up ($p = 0.318$). 3 cases of drug-related gastrointestinal discomfort and 1 case of skin reaction were reported in the CG, while 4 cases of drug-related gastrointestinal discomfort and 2 cases of skin reaction occurred in the OM85-BV group. All adverse reactions were transient, and no special treatment was required. There was no obvious difference in the incidence rate of adverse events between groups ($p = 0.480$).

Sensitivity Analysis

In the full unmatched cohort of 44 patients in the CG and 58 patients in the OM85-BV group, the adjusted difference in CAT score at the 6-month follow-up between the OM85-BV group and the CG was –2.850 [95% CI –4.732 to –0.968, $p = 0.003$]. The IRR for the frequency of acute

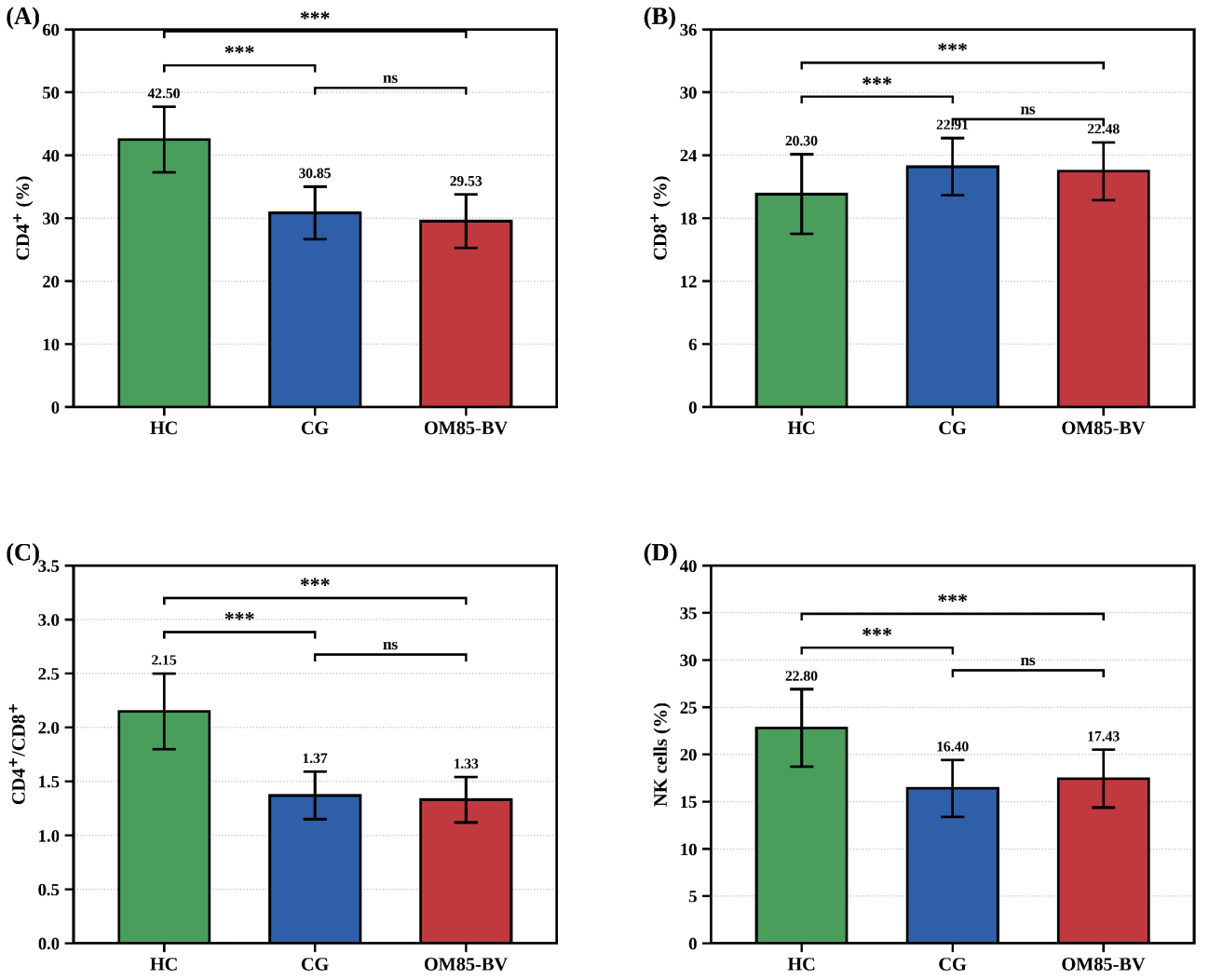

Fig. 3. Comparison of baseline CD4+, CD8+, CD4+/CD8+ and NK cells among the three groups. (A) CD4+; (B) CD8+; (C) CD4+/CD8+; (D) NK cells. *** $p < 0.001$ versus the healthy control (HC) group. ns, not significant. The HC group was compared with each COPD group by the independent-samples *t*-test or the Mann-Whitney U test as appropriate, and the propensity score-matched CG and OM85-BV groups were compared with each other by the paired-samples *t*-test or the Wilcoxon signed-rank test as appropriate; the *p* values of the comparisons among the three groups were all corrected by the Holm method. The comparison with the HC group is a descriptive reference; HC was frequency-matched for age and sex only.

exacerbations within 6 months was 0.615 [95% CI 0.430 to 0.881, $p = 0.008$]. The variance inflation factors of all covariates entered into the three models ranged from 1.067 to 4.213, and the ratio of the deviance to the residual degrees of freedom of the negative binomial model was 1.024, so no multicollinearity or overdispersion was detected. The estimates obtained in the full unmatched cohort are consistent in direction with those obtained in the matched cohort.

Discussion

Impaired cellular immune function has been reported in patients with COPD [17,18]. In this study, comparison between the case groups and the healthy control group revealed decreased CD4+ and CD4+/CD8+, as well as elevated CD8+ in patients with stable COPD, which is consistent with the impaired cellular immune function reported in COPD. Because the healthy control group was frequency-matched for age and sex only, this comparison is a descriptive reference and does not by itself establish that COPD leads to immune dysfunction. The decreases in CD4+ and CD4+/CD8+ are associated with reduced pulmonary surfactant, increased alveolar tension, increased inflammatory factors and decreased complement activity caused by long-term abnormal inflammatory responses in COPD patients, thereby impairing the lung's defense mechanisms and causing recurrent infections. However, recurrent infections lead to immunosuppression, and because most COPD patients are older adults, they often suffer from malnutrition, increased consumption, lymphatic atrophy and im-

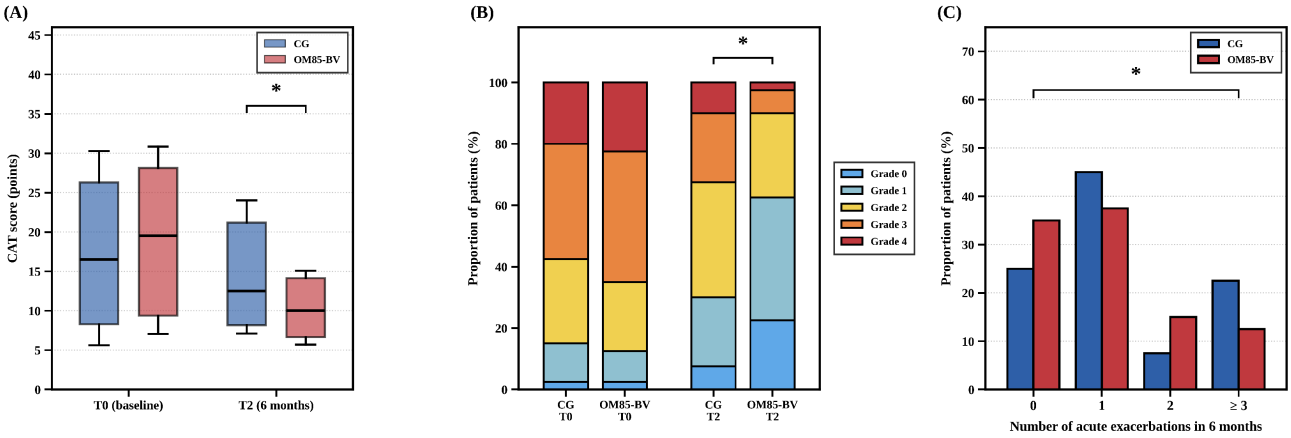

Fig. 4. Comparison of primary outcome measures. (A) CAT score; (B) distribution of mMRC grades; (C) distribution of the frequency of acute exacerbations recorded over the whole 6-month observation period. T0 and T2 represent baseline and the 6-month follow-up, respectively, and apply to panels A and B only; panel C shows counts accumulated over the 6-month observation period and is not related to the T0 and T2 time points. * $p < 0.05$ between the two groups. CAT, COPD Assessment Test; mMRC, modified Medical Research Council.

Table 3. Comparison of humoral immunity, inflammatory factors and safety indicators.

Variable	Time	CG (n = 40)	OM85-BV (n = 40)	Statistic (paired)	*p*
sIgA, mg/L	T0	120.09 ± 16.40	121.43 ± 19.79	$t = -0.329$	0.743
	T2	124.78 ± 16.97*	136.78 ± 18.89*	$t = -3.285$	0.002
ALB, g/L	T0	37.95 ± 3.00	38.40 ± 3.14	$t = -0.658$	0.509
	T2	38.96 ± 3.07*	41.67 ± 3.34*	$t = -4.102$	<0.001
IgG, g/L	T0	9.57 ± 1.84	9.00 ± 2.01	$t = 1.320$	0.192
	T2	10.74 ± 1.97*	12.48 ± 2.33*	$t = -3.926$	<0.001
IgA, g/L	T0	1.77 ± 0.55	1.76 ± 0.54	$t = 0.102$	0.919
	T2	2.08 ± 0.64*	2.70 ± 0.68*	$t = -4.568$	<0.001
IgM, g/L	T0	0.80 ± 0.16	0.81 ± 0.20	$t = -0.247$	0.805
	T2	0.96 ± 0.19*	1.25 ± 0.24*	$t = -6.421$	<0.001
IL-6, pg/mL †	T0	21.40 ± 9.90	20.04 ± 8.03	$t = 0.673$	0.502
	T2	15.20 (9.35, 22.10)*	13.85 (8.90, 20.40)*	$Z = -1.002$	0.318
IL-11, pg/mL †	T0	13.25 ± 2.65	12.54 ± 2.70	$t = 1.185$	0.239
	T2	11.10 (8.85, 13.50)*	8.20 (5.90, 10.85)*	$Z = -4.285$	<0.001
TNF-α, pg/mL †	T0	17.80 ± 3.79	17.41 ± 4.23	$t = 0.435$	0.665
	T2	15.20 (12.10, 18.40)*	13.10 (9.85, 16.20)*	$Z = -2.267$	0.023
Adverse events, n (%)	—	4 (10.00)	6 (15.00)	$\chi^2 = 0.500$	0.480

T0 and T2 represent baseline and the 6-month follow-up. † indicates markers (IL-6, IL-11 and TNF-α) whose values at T2 did not meet the normality assumption and were therefore expressed as median (Q1, Q3); the remaining variables are expressed as mean ± SD. IL-6, IL-11 and TNF-α at T2 did not meet the normality assumption and were tested by Wilcoxon signed-rank test; the other variables were tested by paired-samples *t*-test, and adverse events were compared by McNemar's test. * $p < 0.05$ versus T0 within the same group, indicating a within-group difference at the 6-month follow-up.

paired baseline immune function, thereby further exacerbating the immune dysfunction and creating a vicious cycle [17,18]. The above multiple factors collectively result in reduced production and increased depletion of CD4+ cells and decreased CD4+ cells, leading to decreased CD4+ and CD4+/CD8+. The elevated CD8+ in COPD patients is considered to be linked to two primary mechanisms: relative proportion increase due to decreased CD4+ cells and pre-existing mutual stimulatory relationship between chronic inflammatory response and CD8+ cells in COPD patients. Smoking exposure differed considerably among the groups in this study. After matching, current smokers accounted for 60.00% of CG and for 62.50% of the OM85-BV group, whereas the healthy control group by definition contained only individuals who had never smoked or who had stopped smoking more than 5 years earlier. Smoking

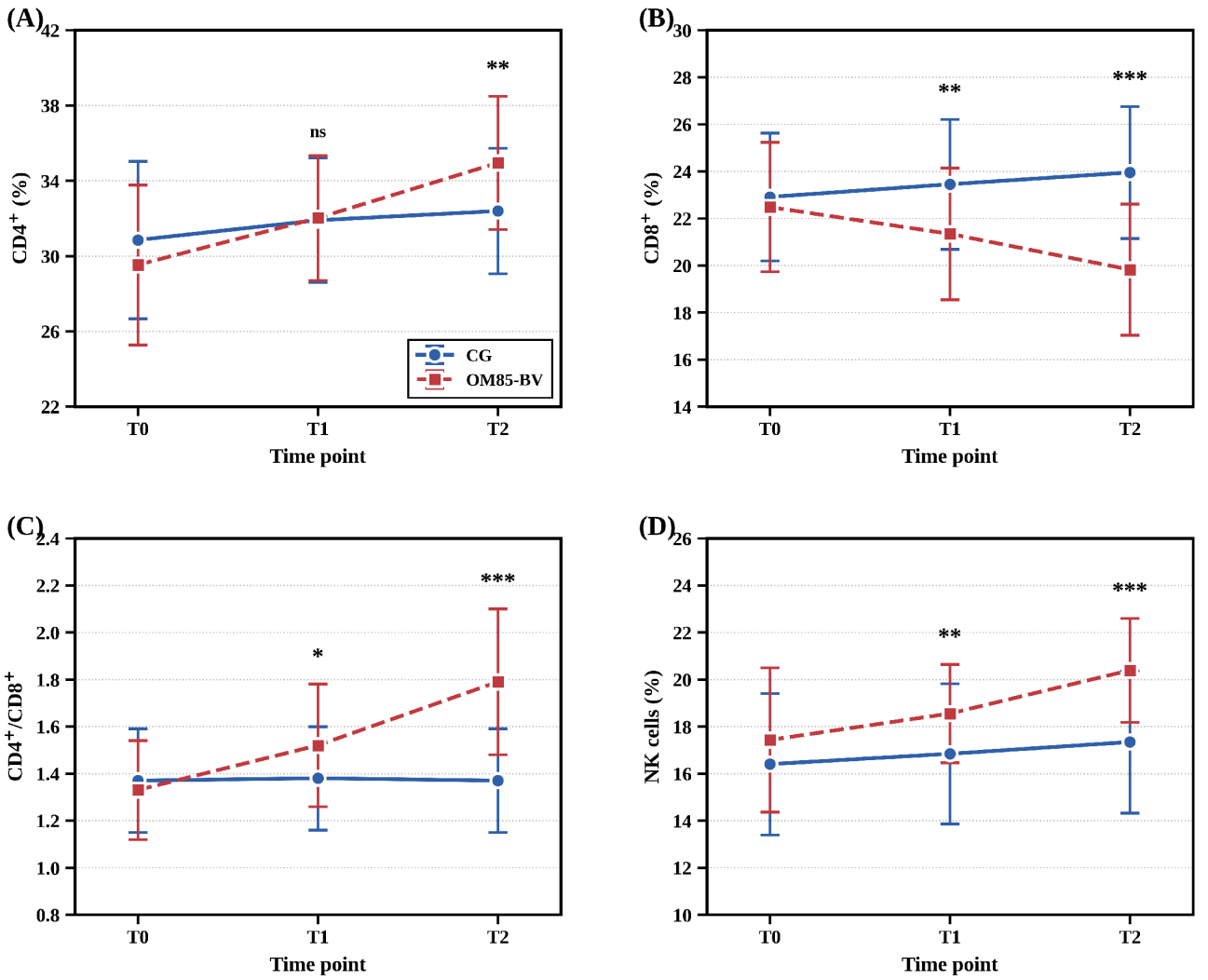

Fig. 5. Comparison of cellular immune indicators. (A) CD4+; (B) CD8+; (C) CD4+/CD8+; (D) NK cells. T0, T1 and T2 represent baseline, the 3-month follow-up and the 6-month follow-up, respectively. ns, not significant; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus CG at the same time point.

itself alters the composition and the function of the peripheral T-lymphocyte subsets and of the NK cells and increases circulating inflammatory factor levels [19]. Therefore, differences in the immune indicators between the two COPD groups and the healthy control group reflect the combined contribution of the disease and differences in smoking exposure, and the present data do not allow these two contributions to be separated. The healthy control group is therefore reported as a descriptive reference only.

Bacterial lysate is a type of immunomodulator used in the treatment of COPD, and refers to freeze-dried lysates derived from various common respiratory tract pathogens. It possesses multiple immune activities and can activate the body's immune system and strengthen immune cell activity by stimulating mucosa-associated lymphoid tissue, thereby providing anti-infective protection to the respiratory tract. A clinical study has demonstrated that bacterial lysate can reduce the frequency of acute exacerbations in COPD patients, shorten the length of hospital stay and duration of antibiotic use, improve clinical symptoms and signs, and improve lung function and immune function [20]. The results of this study showed that the indicators related to cellular immunity (CD4+, CD8+, CD4+/CD8+, NK cells) and humoral immunity (sIgA, immunoglobulins) improved, while IL-11 and TNF-α levels declined, and the frequency of acute exacerbations was significantly decreased within 6 months in patients with stable COPD after treatment with bacterial lysate, indicating that bacterial lysate combined with basic treatment has synergistic immunomodulatory effects.

Bacterial lysate activates the innate and adaptive immunity by providing pathogen-associated molecular patterns (PAMPs), thus promoting immune maturation and enhancing immune defense. The main components of bacterial lysate encompass lysates from Haemophilus influenzae, *Streptococcus pneumoniae* (formerly *Diplococcus pneumo-*

niae) and Klebsiella pneumoniae, and can modulate both innate and adaptive immunity and possess immunomodulatory effects [21]. Bacterial lysate is represented as an immunomodulator composed of inactivated antigens derived from the chemical lysis of several respiratory tract pathogens. Its mechanism has largely been confirmed through animal or *in vitro* studies, primarily depending on Toll-like receptors (TLRs). TLRs are dimeric proteins expressed by dendritic cells (DCs) and monocytes that recognize bacterial PAMPs. On the one hand, TLR2/6 and TLR9 can activate DCs, enabling them to play a vital role in the immune system. DCs present the antigens and stimulate the T cells to differentiate into mature T helper 1 (Th1) and T helper 2 (Th2) cells. Th1 cells activate the CD8+ T cells and NK cells and contribute to the lysis and clearance of the viruses and phagocytosis of the infected cells. Th2 cell polarization can impair immune function, increasing the risk of disease severity. On the other hand, antigen-presenting cells (APCs) secrete a large amount of IL-12 upon activation by pattern recognition receptors, and IL-12 induces NK cells to produce interferon-γ (IFN-γ). Both IL-12 and IFN-γ are key factors in Th1 cell maturation. Bacterial lysate selectively activates DCs through NF-κB- and mitogen-activated protein kinase (MAPK)-dependent pathways [22], modulates interferon-β production and inflammasome activity [23], and enhances the CD4+ Th1 response, thereby promoting the Th1/Th2 balance [24]. De Boer et al. [25] have shown that OM85-BV activates multiple cytokines to attenuate the Th2 response and enhance Th1 function. In the mouse experiment of Navarro et al. [26] involving bacterial lysate, CD4+ and CD8+ cells with Treg gene are present in the intestinal mucosa, and bacterial lysate can promote Treg expression and repositioning. In mouse airways, bacterial lysate treatment induces FoxP3-CD4+T lymphocytes to differentiate into FoxP3+CD4+T lymphocytes, accumulates DCs in the trachea, and is associated with an increased number of Tregs in the trachea. Moreover, Tregs can express the CCR9-specific subset capable of suppressing allergic airway inflammation, whereas the CCR9 phenotype is present only in lymphocytes from mesenteric lymph nodes and Peyer's patches, indirectly demonstrating the localization function of bacterial lysate. OM85-BV stimulates the innate and adaptive immune responses through DC activation, enhanced Th1 response and increased IgA levels. In particular, OM85-BV has the ability to induce the production of interferon-β, a key cytokine with immunomodulatory and antiviral effects, and administration of OM85-BV has been shown to enhance the type I interferon response and to protect against respiratory viral infection in experimental models [27].

The changes in cellular immune indicators over the 6-month follow-up period after the 3-month course of combined therapy are consistent with short-term improvements in cellular immune indicators in COPD patients. The lower frequency of acute exacerbations observed during the 6-month follow-up in the OM85-BV group is consistent with this finding, although the present design does not allow changes in immune indicators to be identified as the cause of the difference in the frequency of acute exacerbations. Due to the small sample size, short observation period and individual differences in this study, a larger sample size and additional research observation indicators are needed to validate the reliability of the findings. In addition, the number of covariates included in the propensity score model was relatively large for the available sample size, which may have led to overfitting and instability of the estimated propensity scores. Therefore, the balance obtained should be interpreted as balance in the measured covariates within this particular matched sample and not as evidence that confounding has been removed; humoral immunity and inflammatory markers were assessed at only two time points (baseline and the 6-month follow-up), which limits inference regarding their temporal trajectories. Furthermore, certain flow cytometry details, such as compensation settings, daily quality-control calibration, the number of acquired events and the analysis software, were not fully documented in the medical records. After PSM, the standardized mean differences for the measured covariates were all below 0.110, but the A and B categories of the GOLD 2023 A/B/E group remained above the conventional threshold of 0.100 (–0.109 and 0.102), indicating that balance was not achieved for every category of every covariate; nevertheless, as an observational study, residual confounding from unmeasured variables cannot be entirely excluded, and the results should be interpreted with caution. The results of the matched-cohort and sensitivity analyses in the full unmatched cohort are generally consistent in direction, although this consistency does not by itself demonstrate that the findings are free from confounding. Inclusion criterion (5) required complete clinical data and follow-up records, so the analysis is restricted to complete cases [28]. Patients with incomplete records were not enrolled, and their characteristics and outcomes may have differed from those of the analysed patients; therefore, selection bias cannot be excluded. The absence of loss to follow-up in the analysed cohort follows from this enrolment rule and should not be read as evidence of a particularly high quality of follow-up. The repeated measures ANOVA and the negative binomial regression were fitted without the matched-pair identifier, so the correlation within the matched pairs was not modelled in either analysis, and the resulting standard errors were conservative. Residual confounding by variables that were not recorded, such as socioeconomic status, exposure to indoor and outdoor air pollution, nutritional status, and the reason for which the treating physician chose to add an oral bacterial lysate, remains possible, so the estimates reported here represent associations observed in a matched observational sample rather than treatment effects. mMRC grade is an ordinal variable with five categories and was compared by rank-based tests throughout, without be-

ing treated as a continuous variable. A covariate-adjusted comparison of mMRC grade would require an ordinal regression model, and the proportional-odds assumption of such a model cannot be evaluated reliably at the present sample size; the covariate-adjusted analysis was therefore restricted to CAT score and the frequency of acute exacerbations, and a covariate-adjusted ordinal analysis of mMRC grade in a larger sample is warranted. mMRC grade and CAT score are the symptom assessment instruments recommended by GOLD for the comprehensive assessment of COPD [12], and the minimal clinically important difference of CAT score has been determined in a prospective analysis [29], which supports the use of both instruments as outcome measures responsive to treatment.

Conclusion

In this retrospective study, the addition of bacterial lysate OM85-BV to standard COPD treatment was associated with more favourable cellular immune indicators (CD4+, CD8+, CD4+/CD8+, NK cells), with higher humoral immune indicators (sIgA, IgG, IgA, IgM), with lower inflammatory factors (IL-11, TNF-α) and with fewer acute exacerbations within 6 months in patients with stable COPD. These associations are consistent with an immunomodulatory effect but do not establish a causal relationship; given the retrospective design, small sample size, number of covariates entered in the propensity score model relative to the sample size, short observation period and the possibility of residual confounding from unmeasured variables, the results should be interpreted with caution. Larger prospective randomized controlled trials are warranted.

Availability of Data and Materials

The data used and analyzed during the current study are available from the corresponding authors on reasonable request.

Author Contributions

STJ, CQX, and QKW contributed to the study conception and design and project supervision. LZ contributed to the collection and organization of the data. CQX contributed to the data analysis and the review and proofreading of the report. STJ contributed to the drafting of the manuscript and all authors revised the manuscript critically for important intellectual content. All authors gave final approval of the version to be published. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki. The study was approved by the Medical Ethics Committee of The Fourth People's Hospital of Lin'an District (Approval No. 2026 No.001). The study was a retrospective analysis of clinical data and residual specimens obtained during routine care. No treatment was administered, and no examinations, follow-up contacts or specimen collections were performed for research purposes; therefore, the ethics committee waived the requirement for informed consent.

Acknowledgment

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Conflict of Interest

The authors declare no conflict of interest.

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