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Association of bronchoalveolar lavage cellular analysis and radiological findings in fibrotic interstitial lung diseases

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ABSTRACT

Background and aims Inflammation may play a role in driving interstitial lung diseases (ILD). Radiological ground-glass opacity (GGO) may not reliably distinguish fine intralobular fibrosis from inflammatory processes in fibrotic ILD. We therefore investigated the relationship between GGO, fibrosis and leukocytes in bronchoalveolar lavage (BAL).

Methods We recruited patients with fibrotic ILD at a single centre between May 2014 and February 2018. The extent of GGO and fibrosis was evaluated by two radiologists. Linear regression examined the association between leucocyte numbers in BAL obtained from the right middle lobe and GGO/fibrosis extent in whole lung, adjusting for age, sex and smoking. A Z-test was used to compare the association between BAL and GGO/fibrosis.

Results 316 patients were included. Adjusting analyses for covariates, only BAL eosinophil and eosinophil-to-macrophage ratio were positively associated with GGO involvement (0.23 (95% CI 0.03 to 0.42) p=0.023 and 11.21 (95% CI 1.33 to 21.08) p=0.026). Lymphocyte percentages (fibrosis −0.17 vs GGO −0.02 p=0.046); neutrophil percentages (fibrosis 0.38 vs GGO 0.06 p=0.002); neutrophil-to-lymphocyte ratio (fibrosis 0.63 vs GGO −0.05 p=0.027); neutrophil-to-macrophage ratio (fibrosis 14.08 vs GGO 2.57 p=0.015) and neutrophilia (fibrosis 6.81 vs GGO −0.31 p=0.002) all demonstrated a significantly stronger association with fibrosis than GGO.

Conclusions Lack of relationships between radiological GGO and BAL leucocyte counts in fibrotic lung disease indicates that GGO may not always be inflammatory in nature. Higher levels of neutrophils were associated with more extensive fibrosis.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Radiological patterning of ground-glass opacity (GGO) on CT in the context of interstitial lung disease (ILD) is often considered to represent inflammation and is often treated with immune suppression, despite poor outcomes in idiopathic pulmonary fibrosis and limited evidence that GGO distinguishes inflammation from fibrosis.

WHAT THIS STUDY ADDS

⇒ In 316 people with fibrotic ILD, no association was found between bronchoalveolar lavage (BAL) lymphocyte counts and extent of GGO involvement. BAL cell profiles, particularly neutrophil and eosinophils, were more strongly associated with fibrotic changes than with GGO involvement.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ These results highlight a need for caution when interpreting the radiological pattern of GGO in fibrotic ILD and highlight that the presence of granulocytes may more closely link to fibrotic changes. BAL cell profiles may aid differentiating between inflammatory GGO and fibrotic GGO and potential response to immunosuppressive therapy.

INTRODUCTION

Interstitial lung disease (ILD) encompasses a wide spectrum of diffuse parenchymal conditions characterised by inflammation and fibrosis affecting the pulmonary interstitium. Various aetiologies, such as environmental factors (including toxins, pollutants or occupational hazards), infections, autoimmune conditions, drug toxicities and genetic factors, can lead to ILD, but in many cases, no cause can be attributed.[1–3] The current pathogenic paradigm for fibrotic ILDs (fILDs) proposes that recurrent lung injury generates an inflammatory response, which can result in fibrosis and irreversible lung damage.[4] Therefore, clinical management plans have focused on trying to treat pulmonary inflammation

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prior to the development of fibrosis and irreversible damage.[1]

High-resolution CT (HRCT) of the lung is a non-invasive tool to diagnose and identify disease progression in ILD and has often been used to discriminate between inflammation and fibrosis, but there are limited data defining the nature of inflammation on HRCT. Ground-glass opacity (GGO) is an area of increased lung opacity through which vessels and bronchial structures may still be seen, caused by the partial displacement of air. GGO may be caused by partial airspace filling by fluids, cells and/or thickening of lung interstitium by inflammation or fibrosis.[5] In the context of ILD, GGO may resolve partially or completely, or may progress, and in turn may reflect fine fibrosis or inflammation or a combination of both.[6–9]

Bronchoalveolar lavage (BAL) can provide valuable cellular information about the composition of the distal airway and alveolar space.[10 11] It has previously been shown that BAL lymphocyte proportion is inversely related to extent of fibrosis on CT.[12] Recent work has also shown a lack of relationship between GGO on CT and BAL lymphocyte proportions.[13] In this study, we aimed to further characterise the relationships between BAL leucocyte cell type proportions and CT fibrosis and ground-glass extent to understand whether immune cell differentials reflect the inflammatory or fibrotic processes in ILD.

MATERIALS AND METHODS

Study population and design

Patients diagnosed with fILD and who underwent BAL cellular analysis at the ILD unit of Royal Brompton Hospital between May 2014 and February 2018 were recruited based on inclusion criteria. Inclusion criteria were adult patients aged ≥18 years, diagnosed with fILD of undetermined aetiology, in whom BAL was performed as part of diagnostic work-up at presentation. This study recruited participants with inclusion criteria of fILD, but with unknown aetiology at time of sampling (online supplemental table S1). The study was performed across a spectrum of fILD in a population of patients previously studied for the assessment of lymphocytes in disease progression of fILD.[12]

BAL cellular analysis

BAL cellular analysis was performed on patients undergoing bronchoscopy which was undertaken in a sitting position, using a flexible bronchoscope under xylocaine anaesthesia and midazolam sedation. BAL was performed in a subsegmental bronchus in the right middle lobe (RML) while in a wedged position. 240 mL of buffered normal saline at room temperature was instilled in 60 mL aliquots. Fluid was aspirated after each fraction by gentle hand suction. Aliquots were mixed and subsequently transported to the laboratory on ice. The BAL samples were centrifuged to separate cells from the supernatant, and cells were resuspended in PBS. The samples were stained using Giemsa dye for subsequent analysis. At least 300 cells were counted from each lavage after having scanned for a representative area. In cases with less than 300 cells, the total number counted was noted, and percentages were still given. Frequencies and percentages of nucleated white blood cells were determined. BAL cellular ratio was calculated by dividing the absolute count of one type of BAL cell by the absolute count of another type of BAL cell. BAL cellular pattern was categorised into three distinct patterns based on the Official American Thoracic Society Clinical Practice Guideline: lymphocytosis, defined as BAL lymphocytes >15%; eosinophilia, defined as BAL eosinophils >1%; and neutrophilia, defined as BAL neutrophils >3%.[10]

Pulmonary function test

Pulmonary function tests (PFTs) were performed as part of the patient's routine clinical care according to American Thoracic Society criteria. Percentage predicted values were determined using GLI equations.

CT scan of the chest and interpretation

Each patient's chest CT, taken with a <1.5 mm inspiratory thin slice and conducted within 120 days of bronchoscopy, was retrospectively reviewed from our institution's electronic database. CT scans were evaluated independently and scored based on the extent of GGO and fibrosis presence by two thoracic radiologists (AN and BR). GGO was defined by an area of increased lung opacity in which vessels and bronchial structures could still be seen without traction bronchiectasis. Fibrosis was identified by the presence of reticulation or traction bronchiectasis within GGO or honeycombing appearance.[14] Chest CTs were independently scored by two experienced radiologists for the extent of GGO and fibrosis on a lobar basis, to the nearest 5% of the lobar volume. A mean lobar score was then calculated to represent the whole lung score. In cases where there was a discrepancy greater than 20% in either GGO or fibrosis scores, or disagreements regarding the presence of fibrosis or honeycombing between the two readers, a third radiologist (AD) reviewed the scans and served as an arbitrator. For all other cases, the final score was determined by averaging the two initial readings.

All patients' data were collected from the medical records at Royal Brompton Hospital, which included population demographics and baseline characteristics (age, sex, smoking history), pulmonary function parameters (forced vital capacity (FVC), diffusing capacity of the lungs for carbon monoxide (DLCO)), chest imaging findings across all lobes and only RML and BAL cells (macrophages, lymphocytes, neutrophils, eosinophils and mast cells).

Statistical analyses

Demographic data and baseline clinical characteristics were reported as mean±SD for normally distributed

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continuous variables, or as median with IQR for non-normally distributed continuous variables, as appropriate based on the distribution of the data. Comparisons between groups were performed using the independent t-test for normally distributed continuous variables and the rank-sum test for non-normally distributed continuous variables. The χ^2 test or Fisher's exact test was used to compare categorical variables, depending on the expected frequencies. Linear regression models were specified to evaluate the association between BAL cells and the extent of GGO or fibrosis throughout the whole lung, with adjustment for covariates of age, sex and smoking. Key assumptions for linear regression were assessed to ensure model validity. Residuals were calculated as the difference between observed and predicted values and examined for normality using histograms with normal curve overlays. The Shapiro-Wilk test was additionally performed to assess residual normality. In all models, residuals were approximately normally distributed, supporting the appropriateness for linear regression analyses. The extent of GGO was measured both in the RML to represent the BAL constituent and in the whole lung, while the extent of fibrosis was measured as the score for the entire lung. Whole lung GGO was used as it was correlated with RML GGO that reflected location of BAL sampling in line with routine clinical practice at the ILD Unit, Royal Brompton Hospital (online supplemental table S2). Z-tests were used to test the equality in coefficients modelled on different outcomes of GGO or fibrosis according to each BAL cellular pattern. P values were derived from Z-tests to determine the equality in coefficients from GGO and fibrosis models. Statistical significance was defined at a $p<0.05$. Statistical analyses were performed using the R Program.

RESULTS

Patient demographic data

A total of 316 patients were included in the analysis (figure 1). Patients were predominantly older adults, with a mean age of 68.3 years; the majority were male, comprising 64.6% of the cohort. Approximately 57.6% of the patients were ex-smokers. The median baseline FVC was 78.1% (IQR 67.9 to 92.2) and DLCO was 48% (IQR 38.2 to 59). The median interval between CT scan and bronchoscopy was 37 (IQR 2 to 67.5) days (table 1). The median whole lung GGO score was 7.9 (IQR 5.4 to 11.25), and the median whole lung fibrosis score was 17.9 (IQR 11.9 to 25.0).

When comparing baseline demographic data based on BAL cellular patterns, patients with BAL lymphocytosis exhibited preservation of PFT compared with those without (FVC 2.8 L (IQR 2.2 to 3.3) vs. 2.4 L (IQR 1.9 to 3) and DLCO 50.5% (IQR 42.3 to 59.9) vs. 46% (IQR

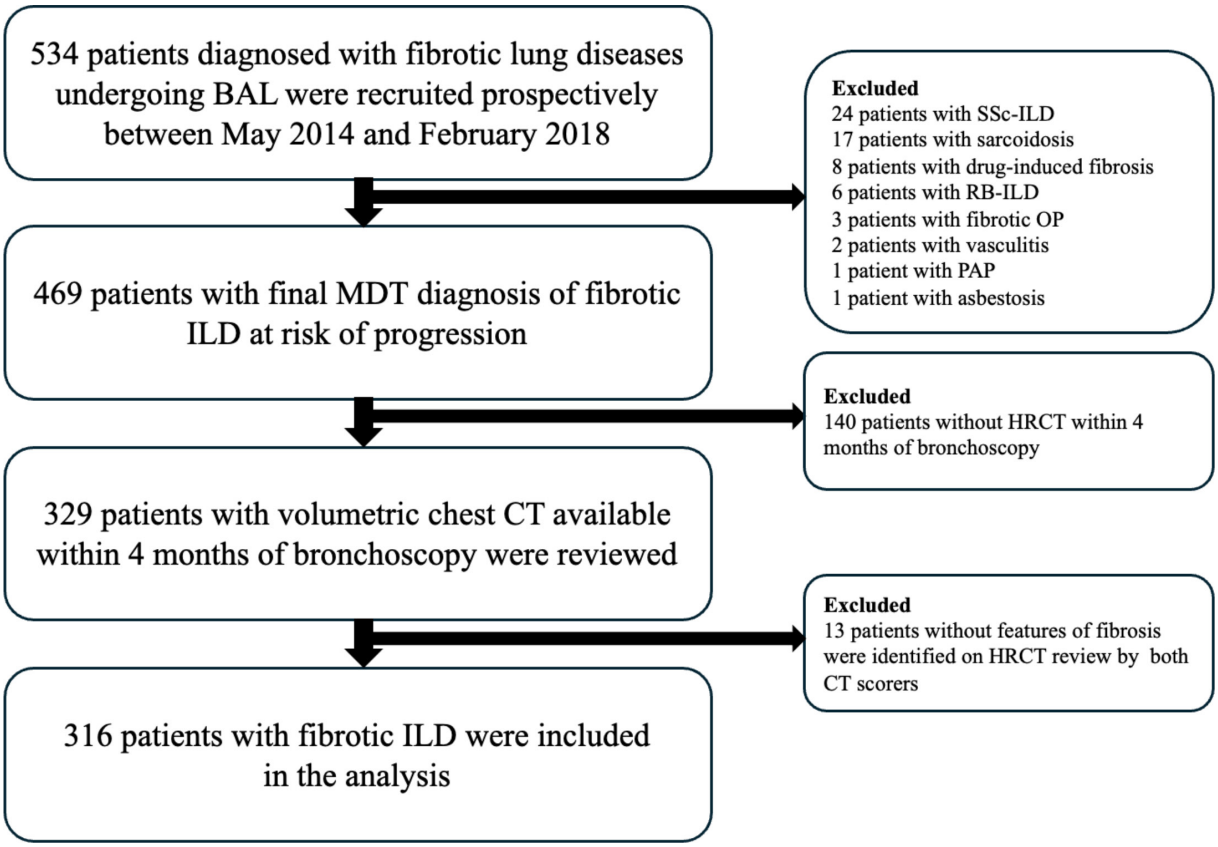


Figure 1 Patient flow diagram. BAL, bronchoalveolar lavage; CTD-ILD, connective tissue disease-associated interstitial lung disease; HRCT, high-resolution CT; MDT, multidisciplinary team; OP, organising pneumonia; PAP, pulmonary alveolar proteinosis; RB-ILD, respiratory bronchiolitis-ILD; SSc-ILD, systemic sclerosis-ILD.

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Table 1 Baseline demographic data according to BAL cellular pattern

Characteristics	Lymphocytosis n=126	Non-lymphocytosis n=190	Total n=316	P value
(A) Lymphocytosis pattern				
Age (years), mean±SD	67.5±8.2	68.7±8.5	68.3±8.4	0.215*
Male (%)	85 (67.5)	119 (62.6)	204 (64.6)	0.448†
Ever smoker (%)	65 (51.6)	117 (61.6)	182 (57.6)	0.1†
Pulmonary function parameters				
FVC, L (IQR)	2.8 (2.2,3.3)	2.4 (1.9,3)	2.6 (2,3.1)	0.026‡
FVC, %predicted (IQR)	81.3 (68.7,95.3)	76.5 (66.9,88.2)	78.1 (67.9,92.2)	0.031‡
DLCO, mL/min/mm Hg (IQR)	3.9 (3.1,4.7)	3.4 (2.6,4.4)	3.6 (2.8,4.6)	0.022‡
DLCO, %predicted (IQR)	50.5 (42.3,59.9)	46 (36.5,58.1)	48 (38.2,59)	0.006‡
Duration between CT scan and bronchoscopy (days) (IQR)	39.0 (4,75)	36.5 (2,62.8)	37.0 (2,67.5)	0.4‡
Characteristics	**Neutrophilia n=268**	**Non-neutrophilia n=48**	**Total n=316**	**P value**
(B) Neutrophilia pattern				
Age (years), mean±SD	68.3±8.5	68.1±8.2	68.3±8.4	0.865*
Male (%)	173 (64.6)	31 (64.6)	204 (64.6)	1†
Ever smoker (%)	162 (60.4)	20 (41.7)	182 (57.6)	0.023†
Pulmonary function parameters				
FVC, L (IQR)	2.6 (2,3)	2.9 (2.1,3.8)	2.6 (2,3.1)	0.122‡
FVC, %predicted (IQR)	77.7 (67.5,91)	87.4 (70.3,100.8)	78.1 (67.9,92.2)	0.085‡
DLCO, mL/min/mm Hg (IQR)	3.6 (2.8,4.5)	3.9 (3.1,5.3)	2.6 (2,3.1)	0.106‡
DLCO, %predicted (IQR)	47.4 (37.9,58.1)	50.3 (42.9,65.7)	48 (38.2,59)	0.114‡
Duration between CT scan and bronchoscopy (days) (IQR)	37.0 (2,67)	37.5 (8,79.2)	37.0 (2,67.5)	0.551‡
Characteristics	**Eosinophilia n=282**	**Non-eosinophilia n=34**	**Total n=316**	**P value**
(C) Eosinophilia pattern				
Age (years), mean±SD	68.1±8.5	69.6±7.3	68.3±8.4	0.317*
Male (%)	185 (65.6)	19 (55.9)	204 (64.6)	0.353†
Ever smoker (%)	170 (60.3)	12 (35.3)	182 (57.6)	0.009†
Pulmonary function parameters				
FVC, L (IQR)	2.6 (2,3.1)	2.6 (1.8,3.4)	2.6 (2,3.1)	0.578‡
FVC, %predicted (IQR)	77.7 (68,91)	84.9 (60.2,104.3)	78.1 (67.9,92.2)	0.144‡
DLCO, mL/min/mm Hg (IQR)	3.6 (2.8,4.6)	3.7 (3,5.3)	3.6 (2.8,4.6)	0.514‡
DLCO, %predicted (IQR)	47.2 (38.1,58.5)	52.1 (42.6,65.4)	48 (38.2,59)	0.214‡
Duration between CT scan and bronchoscopy (days) (IQR)	36.0 (1,67)	51.5 (14.8,72.2)	37.0 (2,67.5)	0.091‡

*t-test.
†χ^2 test.
‡Ranksum test.
§Fisher's exact test.
BAL, bronchoalveolar lavage; DLCO, diffusing capacity of the lungs for carbon monoxide; FVC, forced vital capacity.

36.5 to 58.1), respectively) (table 1A). A history of cigarette smoke exposure was associated with BAL neutrophilia and eosinophilia (60.4% vs 41.7%) (table 1B) and (60.3% vs 35.3%) (table 1C), respectively.

Associations between BAL cellular analysis and CT findings

Ground-glass opacity

After confirming that the whole lung GGO correlated with RML GGO where BAL was performed, we subsequently

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assessed the whole lung GGO as representative of the whole lung. Associations of BAL cellular profiles revealed significant positive associations between BAL eosinophil percentage and eosinophil-to-macrophage ratio with whole lung GGO (beta coefficients (β) 0.23 and 11.21, respectively). No other BAL cellular data showed any significant association with whole lung GGO scores (figures 2 and 3).

Fibrosis

The analysis of associations between adjusted BAL cellular profiles and whole lung fibrosis revealed positive associations between BAL neutrophil percentage, eosinophil percentage and neutrophil-to-lymphocyte ratio with fibrosis (β 0.38, 0.25 and 0.63, respectively), whereas negative associations were observed for lymphocyte percentage and lymphocyte-to-macrophage ratio with fibrosis (β −0.17 and −4.69, respectively) (figures 2 and 3A). Additionally, the neutrophil-to-macrophage ratio exhibited a strong positive association with fibrosis (β 14.08) (see figure 3A). Similarly, BAL lymphocytosis was negatively associated with fibrosis (β −3.44), and both BAL neutrophilia and eosinophilia were positively associated (β 6.81 and β 5.04, respectively) (figure 3B).

Different associations between BAL cellular analysis and GGO/fibrosis by linear coefficients of involvement

To understand whether BAL cell profiles could differentiate between GGO and fibrosis, we compared BAL cell profiles between whole lung GGO and whole lung fibrosis scores. Coefficients represent % increment in GGO or fibrosis for each 1-unit increase in BAL cellular analysis. BAL lymphocyte percentages demonstrated a stronger negative association with fibrosis than GGO (β −0.17 vs β −0.02). Conversely, BAL neutrophil percentages, the neutrophil-to-lymphocyte ratio, neutrophil-to-macrophage ratio and neutrophilia all showed significantly higher positive associations with fibrosis than with GGO. (β 0.38 vs β 0.06, β 0.63 vs β −0.05, β 14.08 vs β 2.57, β 6.81 vs β −0.31, respectively) (see table 2).

DISCUSSION

This study investigated the association between BAL cellular profiles and radiological presence of GGO and/or fibrosis in ILD. GGO across the whole lung showed a weak positive association with BAL eosinophil percentage and a strong positive association with the eosinophil-to-macrophage ratio. Furthermore, the absolute BAL cell counts showed no association with GGO, which does not support the assumption of inflammation if GGO is present. The most notable finding was that BAL neutrophil numbers were associated with fibrosis, rather than GGO, and consistent with this, patients with neutrophilia had greater impairment of lung function and had a more extensive smoking history. Interestingly, BAL lymphocytes were not associated with GGO. These data suggest that GGO in the spectrum of fILD examined in this study is not related to inflammation, and that neutrophils may play a role in fibrogenesis.

In healthy adult non-smokers without lung disease, alveolar macrophages should constitute more than 85% of the cells. However, in the presence of ILD, macrophage proportions decrease, with an increase in the percentage of other cell types.[10] Neutrophils play a critical role in acute inflammatory response of lung tissue, significantly contributing to remodelling and fibrosis.[15] In this cohort, neutrophils were found to be associated with fibrosis but not GGO. Similarly, Wells *et al* reported a significant correlation between the reticular pattern in the lavaged lobe and BAL neutrophils, but not with GGO, in patients with cryptogenic fibrosing alveolitis (CFA) and fibrosing alveolitis of systemic sclerosis (SSc).[16] The differences between their study and ours lie in the population, as our study excluded patients with SSc-associated ILD. Additionally, CFA and SSc-ILD were analysed separately. The relationships between BAL neutrophils and GGO were consistent in both ILD subtypes, with similar neutrophil counts observed for the extent of fibrosis. However, outcomes varied significantly across studies, including those with robust statistical power and adjustments for disease severity. Higher neutrophil counts were noted in CFA due to the greater extent of fibrosis. Our study identified a significant association between BAL neutrophils and fibrosis. However, the impact of BAL neutrophils on clinical outcomes in patients with ILD remains unclear. Goh *et al* reported that BAL neutrophils greater than 4% were associated with early mortality in patients with SSc (HR 8.40, 95% CI 1.91 to 36.95, p=0.005), but there was no association with late mortality, time to pulmonary function deterioration and progression-free survival.[17] He *et al* found that the percentages of BAL neutrophils were higher in acute or subacute interstitial pneumonia compared with chronic interstitial pneumonia, but paradoxically the elevated BAL neutrophils were associated with reduced mortality, but only in univariate Cox regression analysis.[18] Further studies are needed to define the relationship between BAL neutrophils and clinical outcomes.

How neutrophils contribute to fibrogenesis remains to be fully elucidated; however, it may be through the recruitment of other immune cells to the lung, activating inflammatory and fibrotic molecular pathways, and releasing substances that directly promote fibrosis.[15 19] The metabolic activity of neutrophils can be investigated using the uptake of (^{18}F)−2-fluoro-2-deoxy-D-glucose (^{18}FDG) in positron emission tomography. Previous studies have identified the uptake of ^{18}FDG in IPF.[20] Furthermore, increased ^{18}FDG uptake was associated with increasing severity of IPF,[21] supporting a potentially important role for neutrophil activity in the pathogenesis of pulmonary fibrosis.

This study identified a significant association between increasing BAL eosinophils and higher GGO scores. However, when comparing the association of BAL eosinophils with GGO and BAL eosinophil and fibrosis, no

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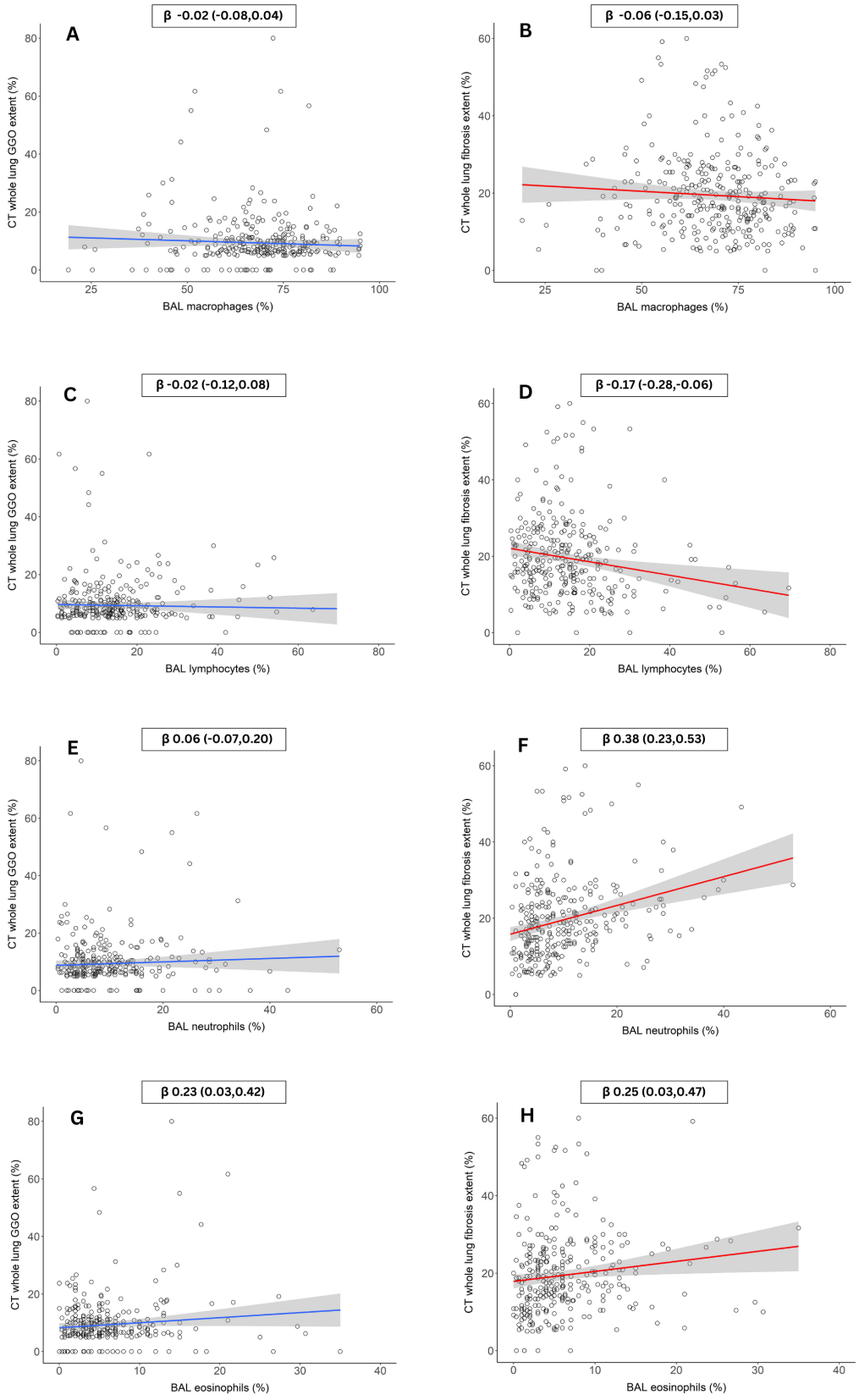


Figure 2 Scatter plot between ground-glass opacity or fibrosis extent with BAL cellular percentages. (A) BAL macrophages (%) and CT whole lung GGO extent. (B) BAL macrophages (%) and CT whole lung fibrosis extent. (C) BAL lymphocytes (%) and CT whole lung GGO extent. (D) BAL lymphocytes (%) and CT whole lung fibrosis extent. (E) BAL neutrophils (%) and CT whole lung GGO extent. (F) BAL neutrophils (%) and CT whole lung fibrosis extent. (G) BAL eosinophils (%) and CT whole lung GGO extent. (H) BAL eosinophils (%) and CT whole lung fibrosis extent. BAL, bronchoalveolar lavage; GGO, ground-glass opacity.

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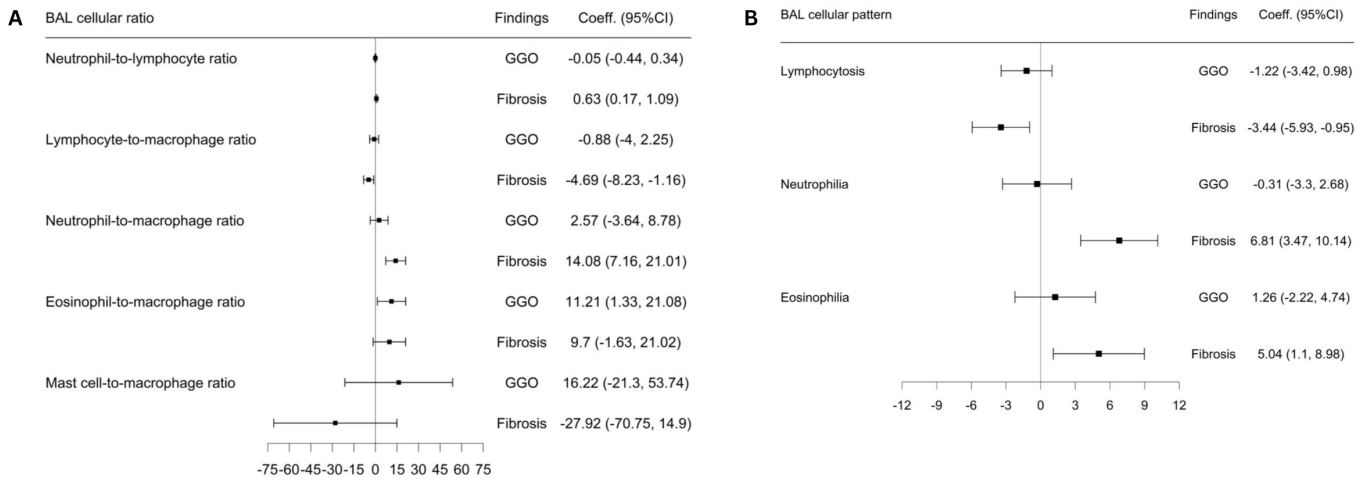


Figure 3 Association of ground-glass opacity or fibrosis with BAL cellular analysis. Forest plot of linear coefficients of involvement between BAL versus right whole lung GGO and BAL versus whole lung fibrosis, adjusted for age, sex and smoking (A) BAL cellular ratio (B) BAL cellular pattern; GGO and fibrosis were defined by the percentages of area involvement; BAL cellular percentage was calculated by dividing the number of each BAL cell type by the total number of BAL cells counted and multiplying by 100; BAL cellular ratio was calculated by dividing the absolute count of one type of BAL cell by the absolute count of another type of BAL cell; BAL cellular pattern was categorised into three distinct patterns: lymphocytosis, defined as BAL lymphocytes >15%; eosinophilia, defined as BAL eosinophil >1%; and neutrophilia, defined as BAL neutrophils >3%. BAL, bronchoalveolar lavage; GGO, ground-glass opacity.

significant difference was observed. Previous studies have correlated BAL eosinophils with GGO on HRCT in CTD-ILD.[22 23] While this cohort did not include patients with CTD-ILD, our finding also suggests that eosinophils may play a role in fILD. Although the role of eosinophils in fILD is not fully understood, they have been implicated in the pathogenesis of fibrosis through the generation of inflammatory cytokines.[24]

BAL lymphocytes play a crucial role in immune responses involved in the pathogenesis of ILD.[25] BAL lymphocytosis is frequently observed in HP, sarcoidosis and drug-induced ILD,[11] but can also be present in other

Table 2 Comparison between linear coefficients of whole lung GGO and whole lung fibrosis associations with BAL cellular analysis

BAL	Whole lung GGO coefficients (95% CI)*	Whole lung fibrosis coefficients (95% CI)*	P value†
Macrophage (%)	−0.02 (−0.08 to 0.04)	−0.06 (−0.15 to 0.03)	0.885
Lymphocyte (%)	−0.02 (−0.12 to 0.08)	−0.17 (−0.28 to −0.06)	0.046
Eosinophil (%)	0.23 (0.03 to 0.42)	0.25 (0.03 to 0.47)	0.873
Mast cell (%)	0.74 (−0.38 to 1.86)	−0.40 (−1.68 to 0.89)	0.187
Neutrophil (%)	0.06 (−0.07 to 0.20)	0.38 (0.23 to 0.53)	0.002
Neutrophil-to-lymphocyte ratio	−0.05 (−0.44 to 0.34)	0.63 (0.17 to 1.09)	0.027
Lymphocyte-to-macrophage ratio	−0.88 (−4.00 to 2.25)	−4.69 (−8.23 to −1.16)	0.112
Neutrophil-to-macrophage ratio	2.57 (−3.64 to 8.78)	14.08 (7.16 to 21.01)	0.015
Eosinophil-to-macrophage ratio	11.21 (1.33 to 21.08)	9.70 (−1.63 to 21.02)	0.843
Mast cell-to-macrophage ratio	16.22 (−21.30 to 53.74)	−27.92 (−70.75 to 14.9)	0.127
Lymphocytosis	−1.22 (−3.42 to 0.98)	−3.44 (−5.93 to −0.95)	0.189
Neutrophilia	−0.31 (−3.30 to 2.68)	6.81 (3.47 to 10.14)	0.002
Eosinophilia	1.26 (−2.22 to 4.74)	5.04 (1.10 to 8.98)	0.157

*Coefficients represent % increment in GGO or fibrosis for each 1-unit increase in BAL cellular analysis from separate linear models adjusted for age, sex and smoking status.
†P values were derived from Z-test to determine the equality in coefficients from GGO and fibrosis models, significant p value (p<0.05) represents statistically different coefficient effect sizes between radiological patterns.
BAL, bronchoalveolar lavage; GGO, ground-glass opacity.

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ILDs such as organising pneumonia (OP), nonspecific interstitial pneumonia (NSIP) and lymphoid interstitial pneumonia.[10 11 26–28] A positive correlation between BAL lymphocytes and GGO in ILD has been reported[6]; however, our study did not observe any significant association with GGO. This discrepancy could be due to the differences in the study populations. The previous study included 20% of patients with interstitial pneumonia with autoimmune feature and approximately 13% with idiopathic NSIP, both ILD subtypes that can present with GGO predominance. In contrast, our study included only a minority of patients with these ILD subtypes, and we excluded patients with CTD-ILD. In support of our data, previous studies have observed lower BAL lymphocytes in patients with HP who had UIP pathology,[29 30] as well as in patients with IPF exhibiting UIP CT patterns.[31] We suggest that although BAL lymphocyte counts were low in the fILDs included in this study, increased lymphocytes do not directly correlate with GGO.

This study represents the largest cohort exploring the association between BAL cellular analysis and HRCT patterns. However, there are some limitations. It is a single-centre study, and results may not be generalisable to other centres, and patients with a known diagnosis of CTD-ILD were excluded from this study. Furthermore, the BAL cellular analysis conducted in this study relied on manual counting, which does not precisely subphenotype the leucocyte populations, thereby potentially overlooking important intracellular interactions. Although there was a significant increase in the proportion of smokers who had either neutrophilia or eosinophilia, the number of patients with these conditions was small, which may increase the risk of a chance finding (type I error). There was insufficient data on patients' clinical status at the time of BAL to determine how disease status or treatment response may have influenced BAL cellular profiles. In addition, longitudinal clinical outcome data were not available for this cohort, limiting assessment of the relationship between BAL cellular profiles and disease progression. Finally, the time interval between CT imaging and bronchoscopy could have introduced variability in the assessment of associations between radiological and cellular associations, although these time frames are consistent with decision making in clinical practice.

These findings support the continued use of BAL as a tool in the diagnostic evaluation and management of selected patients with fILD. While GGO on HRCT is often interpreted as a marker of active inflammation, our data demonstrate that GGO does not reliably reflect lymphocytic inflammation. BAL cellular profiles may therefore provide complementary information to imaging. In particular, the presence of prominent GGO in patients without BAL lymphocytosis may support further assessment prior to considering immunosuppressive therapy and its associated risks.

In summary, increased BAL neutrophils were associated with radiological fibrosis but not GGO, and we could not demonstrate any association between BAL lymphocyte numbers and GGO. These data suggest that GGO may not necessarily reflect inflammation. Understanding how these neutrophils are involved in the fibrotic response may help develop novel anti-fibrotic therapies. Further studies will be needed to explore these relationships in greater detail.

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Data availability statement Data are available upon reasonable request. The data supporting this study cannot be publicly available due to ethical concerns.

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