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Bronchoalveolar Lavage Neutrophils and Natural Killer Cells Provide Additional Discriminatory Information in Patients with a Sarcoidosis-Suggestive BAL Profile

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Abstract

Background

Elevated bronchoalveolar lavage fluid (BALF) CD4/CD8 ratios are widely considered supportive of sarcoidosis; however, their diagnostic accuracy remains limited. We evaluated the diagnostic value of BALF leukocyte populations and lymphocyte subsets to improve discrimination between sarcoidosis and alternative diagnoses in patients with marked BAL lymphocytosis and elevated CD4/CD8 ratio.

Methods

This retrospective, single-centre study included 116 BAL samples from patients with BAL lymphocytosis ≥ 30% and CD4/CD8 ratios ≥ 4. Clinical, radiological, histopathological, and BALF flow cytometry data were reviewed. Logistic regression identified independent predictors of sarcoidosis, and ROC analysis assessed the diagnostic performance of individual and combined BALF cellular markers.

Results

Only 26 patients (22.4%) had sarcoidosis despite fulfilling classical BAL criteria. In multivariable analysis, younger age (OR 0.95, 95% CI 0.91–0.98; p = 0.003), lower BALF neutrophil percentage (OR 0.92, 95% CI 0.84–1.00; p = 0.050), and lower NK-cell percentage (OR 0.66, 95% CI 0.45–0.97; p = 0.034) independently predicted sarcoidosis. BALF cellular profile do not differ according to sarcoidosis stage. The predictive model incorporating age, BALF neutrophil and NK cells percentages and the CD4/CD8 ratio demonstrated significantly higher discriminative ability than the model based on CD4/CD8 alone (AUC 0.802, 95% CI 0.706–0.898 vs 0.604, 95% CI 0.48–0.73; χ^2 = 7.09, p = 0.008)

Conclusions

Elevated BALF lymphocytosis and high CD4/CD8 ratio are not specific to sarcoidosis. A multiparametric approach that includes age, BALF neutrophil and NK-cell percentages improves discrimination between sarcoidosis and alternative diagnoses in patients with suggestive BALF profiles, which may help prevent diagnostic misclassification and optimize clinical decision-making.

INTRODUCTION

Sarcoidosis is a multisystem granulomatous disease capable of affecting the lungs, skin, eyes, heart, and lymphatic system, and patients frequently present initially to internists rather than to a single organ-based specialty. Establishing an early and accurate diagnosis is therefore relevant not only to pulmonologists but to the broader diagnostic process led by internal medicine physicians, who must often decide whether further invasive testing is warranted [1]

Bronchoalveolar lavage (BAL) is a useful complementary tool in the diagnostic evaluation of patients with suspected sarcoidosis and other diffuse parenchymal lung diseases. Although BAL findings are not diagnostic in isolation, the presence of lymphocytosis together with an increased CD4/CD8 ratio may increase the likelihood of sarcoidosis when interpreted in an appropriate clinical and radiological context. However, these findings are not specific and may also occur in other inflammatory, infectious, and interstitial lung diseases (ILD) [1–3].

The BAL fluid (BALF) CD4/CD8 ratio has therefore traditionally been considered one of the most useful cellular parameters in the evaluation of suspected sarcoidosis [4]. A ratio above 3.5 has been associated with relatively high specificity for sarcoidosis [5], while marked BALF lymphocytosis is also regarded as a supportive feature [1]. Nevertheless, the diagnostic performance of the CD4/CD8 ratio is variable, and an elevated ratio does not reliably distinguish sarcoidosis from other conditions presenting with a lymphocyte-predominant BALF profile [5, 6, 7]. This limitation is particularly relevant in patients with interstitial lung disease, connective tissue disease-associated ILD, infectious disease, or drug-related lung disease, in whom overlapping BALF cellular patterns may occur.

Whether additional BALF cellular populations can provide clinically relevant discriminatory information in patients who already display a sarcoidosis-suggestive cellular profile remains insufficiently characterized. In particular, the potential contribution of neutrophils and natural killer (NK) cells has received comparatively limited attention [8, 9]. To our knowledge, no previous study has specifically evaluated the diagnostic contribution of BALF neutrophils and NK cells in patients already exhibiting this combined lymphocytosis/CD4-CD8 profile, although related lymphocyte subsets have been explored in broader ILD cohorts [10]. To specifically investigate this diagnostic uncertainty, we focused on patients meeting a predefined, traditionally sarcoidosis-suggestive BAL profile —marked lymphocytosis (≥ 30%) together with an elevated CD4/CD8 ratio ≥ 4)— used here as an enrolment criterion rather than as a diagnostic standard. We therefore hypothesized that neutrophil and NK-cell populations may provide complementary discriminatory information beyond the CD4/CD8 ratio in this specific setting.

Accordingly, this study aimed to determine whether BALF neutrophil and NK-cell proportions improve discrimination between sarcoidosis and alternative diagnoses in patients with a predefined sarcoidosis-suggestive BALF profile. We additionally sought to identify clinical and BALF cellular variables independently associated with sarcoidosis and to assess the incremental discriminatory value of these parameters. From an internal medicine perspective, refining the interpretation of an apparently classical BALF profile may help avoid unnecessary invasive procedures and support more timely, multidisciplinary decision-making in patients with suspected sarcoidosis

PATIENTS AND METHODS

Study design and population

This retrospective study was conducted at the Internal Medicine Department of Hospital Universitario Puerta de Hierro Majadahonda (Madrid, Spain). We included all BAL samples obtained between January 2010 and December 2024 that showed lymphocytosis ≥ 30% and a CD4/CD8 T-cell ratio ≥ 4, as determined by flow cytometry. These thresholds were defined *a priori* based on their established literature use as supportive, highly suggestive—but non-specific—findings in sarcoidosis [11]. Operationally, the 30% lymphocyte cutoff identified a lymphocyte-enriched population, and neither threshold was derived from the present dataset or considered an independent diagnostic criterion.

Demographic and clinical data were acquired from electronic medical records, including age, sex, smoking status, presenting symptoms, pulmonary function test, radiological findings, histopathology, treatment and final diagnosis.

The study was approved by the institutional ethics committee. Surgical intervention procedures were health care services provided to patients and the study method was not designated for research purposes. Given the retrospective nature of the study and the use of data obtained during routine clinical care, additional informed consent for participation was waived. All patients had previously provided informed consent for undergoing BAL as part of standard clinical practice.

Diagnostic criteria

Sarcoidosis was diagnosed based on compatible clinical and radiological findings, supported by histological evidence of non-necrotizing epithelioid granulomas, after reasonably excluding alternative causes of granulomatous disease.

Non-sarcoidosis ILDs, including idiopathic interstitial pneumonias, hypersensitivity pneumonitis, and connective tissue disease-associated ILD, were diagnosed according to established international guidelines and multidisciplinary evaluation when available.

Infectious aetiologies were defined by the identification of a respiratory pathogen in microbiological studies and/or clinical and radiological improvement following targeted antimicrobial therapy.

Neoplastic disease was confirmed by histological demonstration of malignancy in lung tissue specimens.

Bronchoalveolar lavage procedure and sample processing

BAL was performed according to international recommendations. Briefly, sterile saline solution was instilled in the right middle lobe or the most affected pulmonary segment identified on imaging. Samples with recovery ≥ 30% of instilled volume were considered adequate.

BALF was filtered, centrifuged, and processed within 4 hours. Cells obtained from BALF were stained with fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll (PERCP) or

allophycocyanin (APC) conjugated monoclonal antibodies targeting diverse surface antigens to identify the different cellular populations: CD45-APC, CD14-PE, CD15-FITC, CD3-FITC, CD4-PE, CD8-PerCP, CD56-PE and CD19-FITC. Mouse IgG1 irrelevant antibodies were used as a negative control of staining. All monoclonal antibodies were purchased from BD Biosciences (San Jose, CA). BALF cells were incubated with the appropriate combinations of antibodies for 30 min at 4º C and washed twice in phosphate buffered saline (PBS) to remove unbound antibodies. Leukocyte populations and lymphocyte subsets were analyzed by flow cytometry using a FACSort flow cytometer with CellQuest and Paint-a-Gate Pro software (BD Biosciences, San Jose, CA, USA), according to standard light scatter and immunophenotypic characteristics (Fig. 1). The percentages of leukocyte populations were quantified by analyzing the cells labeled with anti-CD15 and anti-CD14 antibodies, together with cell size and cellular granularity criteria, in the CD45 + gated cells. This gating strategy excluded debris and other cellular events unsuitable for analysis that were prensent in BALF. Lymphocyte subpopulations were quantified by analyzing CD3 + CD4+ cells as helper T lymphocytes, CD3 + CD8+ cells as cytotoxic T lymphocytes, CD19 + cells as B lymphocytes, and CD56 + CD3- cells as natural killer (NK) cells. Results were expressed as percentages of total lymphocyte population.

Statistical analysis

Continuous variables were assessed for normality using appropriate tests. Categorical variables were reported as frequencies and percentages while continuous variables were presented as mean and standard deviation. Normally distributed variables were compared using Student's t-test, while non-normally distributed variables were analyzed using Mann–Whitney U or Kruskal–Wallis tests, as appropriate. Correlations between variables were evaluated using Spearman's rank correlation coefficient. A binary logistic regression was performed to determine the association between cellular subsets and sarcoidosis diagnosis. Finally, the discrimination ability was evaluated following an approach based on the area under the curve (AUC)-"receiver operating characteristic"-ROC. DeLong test was used for formal comparison of ROC curves.

A two-sided p-value < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS v25 (IBM Corp., Armonk, NY, USA) and Stata v18 (Stata Corp., Texas, USA) software.

RESULTS

Patient Characteristics

A total of 116 patients met the predefined inclusion criteria of BALF lymphocytosis ≥ 30% and a CD4/CD8 ratio ≥ 4.0. The median age was 63 years (IQR, 51–71), and 50 patients (43%) were men. Despite the presence of this BALF profile traditionally considered suggestive of sarcoidosis, only 26 patients (22.4%) were ultimately diagnosed with sarcoidosis, whereas 90 (77.6%) had alternative diagnoses. These included infections (n = 26, 22.4%), idiopathic interstitial pneumonias (n = 23, 19.8%), connective tissue disease-associated ILD (n = 21, 18.1%), pneumonitis (n = 10, 8.6%; including radiation,

hypersensitivity and drug-induced pneumonitis), neoplasia (n = 3, 2.6%), and other diagnoses (n = 7, 6.0%). These findings demonstrate that the coexistence of BAL lymphocytosis and an increased CD4/CD8 ratio is insufficient to differentiate sarcoidosis from alternative clinical entities in this population.

Table 1 summarizes the main clinical characteristics.

	All patients (n= 116)	Sarcoidosis (n= 26)	Nonsarcoidosis conditions (n= 90)	*p*
Age Median (IQR 25-75)	63 (51-71)	51 (38-66)	64 (56-71)	0.001
Gender (%)				0.823
Male	50 (43)	12 (46)	38 (42)	
Female	66 (57)	14 (54)	52 (58)	
Smoking habit (%)				0.239
Smoker	7 (6)	1 (4)	6 (7)	
Never	55 (47)	16 (61)	39 (43)	
Former	50 (43)	8 (31)	42 (47)	
Unknown	4 (4)	1 (4)	3 (3)	
Pulmonary Function test (PFT) (%)				0.014
Normal	62 (63)	20 (83)	42 (57)	
Restrictive	27 (28)	4 (17)	23 (31)	
Obstructive	9 (9)	0 (0)	9 (12)	
Biopsy (%)				<0.001
Yes	62 (53)	26 (100)	38 (42)	
No	54 (47)	0 (0)	52 (58)	
Treatment received after diagnosis (%)				0.062
Yes	88 (76)	16 (62)	72 (80)	
No	28 (24)	10 (38)	18 (20)	

Table 1. Clinical characteristics of patients according to diagnosis (sarcoidosis vs nonsarcoidosis conditions)

Patients with sarcoidosis were significantly younger than nonsarcoidosis patients (median 51 vs. 64 years, p = 0.001), while sex distribution and smoking history were similar between groups (Table 1).

Diagnostic confirmation was achieved by clinical, radiological, and BALF findings in 47% of cases, while 53% also underwent transbronchial biopsy. Among the 62 biopsies performed, 19 were normal or showed nonspecific changes, 23 showed non-necrotizing epithelioid granulomas, all corresponding to sarcoidosis, while the others 20 revealed nonspecific inflammation, neoplasia, infection, or amyloidosis.

All sarcoidosis patients underwent biopsy; 23 had granulomas, 2 inflammation, and 1 nonspecific changes. The final diagnosis was established by multidisciplinary assessment integrating clinical, radiological and histopathological findings. Histological confirmation of non-necrotizing granulomas was available in 23 of 26 patients; three patients fulfilled accepted clinical-radiological diagnostic criteria in the absence of diagnostic granulomatous histology. Seven of the sarcoidosis patients (27%) had a radiological stage I, 14 (54%) had a stage II, and 5 (19%) had a stage III. Extrapulmonary involvement of sarcoidosis was observed in nearly 35% of the patients (n = 9).

Pulmonary function tests (PFTs) were performed in 98 patients (24 sarcoidosis and 74 nonsarcoidosis). Normal spirometric patterns were significantly more frequent in sarcoidosis (83% vs. 57%), whereas restrictive pattern were more common in nonsarcoidosis conditions (31% vs. 17%; p = 0.014).

Following diagnosis, 88 patients (76%) received treatment—most commonly corticosteroids (64%). All but one sarcoidosis patients were treated with corticosteroids.

Cell Populations in BALF

Leukocyte and lymphocyte subpopulations in BALF performed on the 116 patients are summarized in Table 2.

Table 2
Distribution of BALF cell populations according to sarcoidosis/nonsarcoidosis diagnosis. Data expressed as percentage.

Median (IQR 25–75)	**All patients (n = 116)**	**Sarcoidosis (n = 26)**	**Nonsarcoidosis Conditions (n = 90)**	***p***
Lymphocytes	65.1 (43.7–81.5)	63.2 (41.0-79.4)	65.8 (45.6–82.3)	0.518
Macrophages	18.2 (6.9–38.8)	24.7 (12.4–48.1)	17.0 (5.9–35.6)	0.067
Neutrophils	4.0 (2.2–10.5)	2.9 (1.5–4.3)	4.9 (2.3–12.8)	0.002
Neutrophil Lymphocyte Ratio	0.1 (0.0-0.2)	0.1 (0.0-0.1)	0.1 (0.0-0.2)	0.011
CD4 + T lymphocytes	80.4 (75.5–84.9)	83.8 (78.3–88.1)	79.4 (75.3–84.1)	0.008
CD8 + T lymphocytes	12.0 (8.7–15.2)	11.0 (7.6–13.9)	12.5 (9.2–15.5)	0.190
CD4+/CD8 + lymphocyte ratio	6.7 (4.9–10.0)	7.7 (5.8–11.1)	6.4 (4.8-9.0)	0.306
CD56 + NK cells	1.9 (1.0-3.3)	1.1 (0.7–2.3)	2.1 (1.2–3.3)	0.020
CD19 + B lymphocytes	0.8 (0.4–1.4)	1.0 (0.4–1.4)	0.7 (0.4–1.4)	0.705

BALF lymphocyte percentages and CD4/CD8 ratios did not differ significantly between sarcoidosis and nonsarcoidosis patients. Median lymphocyte percentages were 63.2% and 65.8%, respectively (p = 0.518), while median CD4/CD8 ratios were 7.7 and 6.4, respectively (p = 0.306).

In contrast, several BALF cellular populations differed between groups (Table 2). Patients with sarcoidosis had lower neutrophil percentages than those with alternative diagnoses (median, 2.9% vs. 4.9%; p = 0.002) and lower neutrophil-to-lymphocyte ratios (p = 0.011). Sarcoidosis was also associated with lower CD56 + NK-cell proportions (median, 1.1% vs. 2.1%; p = 0.020) and higher CD4 + T-cell percentages (83.8% vs. 79.4%; p = 0.008). Macrophage, CD8 + T-cell, and CD19 + B-cell proportions did not differ significantly between groups.

Influence of Demographic and Clinical Variables

Significant sex- and age-related differences were observed in BALF composition. Men had higher lymphocyte percentages [Median (IQR 25–75) 69.9 (51.8–85.1) vs. 56.8 (41.6–78.9), p = 0.020)] and NK cell levels [2.3 (1.0-3.9) vs. 1.8 (1.0-2.9), p = 0.044], whereas women exhibited higher macrophage proportions [22.6 (7.1–48.1) vs. 17.0 (6.2–28.9), p = 0.025]. No differences were observed by lung

function pattern or need for post-diagnosis treatment, including corticosteroids. Specifically, BALF neutrophil percentage did not differ significantly according to smoking status [never 4.1 (2.2–10.2) vs former 3.9 (2.1–11.2) vs current smoker 2.2 (1.7–14.3), (p = 0.906)], supporting that the association between neutrophil percentage and diagnostic group was not substantially confounded by smoking habit.

In sarcoidosis patients, the NK cell percentage inversely correlated with age (p = 0.044). Sarcoidosis patients with radiological stage II showed a significantly higher NK cell percentage [2.3 (1.0-3.1)] than patients with stage I [0.7 (0.4–1.4)], but also than stage III [0.9 (0.7–2.3)], (p = 0.038). No other significant associations were found between BALF cell populations and clinical features, which indicates that BALF cellular profiles do not differ according to sarcoidosis stage.

Multivariate and Diagnostic Analysis

In multivariable logistic regression, younger age (OR, 0.95; 95% CI, 0.91–0.98; p = 0.003), lower BALF neutrophil percentage (OR, 0.92; 95% CI, 0.84–1.00; p = 0.050), and lower BALF NK-cell percentage (OR, 0.66; 95% CI, 0.45–0.97; p = 0.034) were independently associated with sarcoidosis.

Receiver operating characteristic (ROC) curves were constructed to evaluate whether BALF cellular populations independently associated with sarcoidosis improve the discrimination between sarcoidosis and alternative diagnoses. ROC curve analysis evidenced that the predictive model incorporating the age, neutrophil and NK cells percentages to CD4/CD8 ratio demonstrated significantly higher discriminative ability than the model based on CD4/CD8 alone (AUC 0.802, 95% CI 0.706–0.898 vs 0.604, 95% CI 0.48–0.73; χ^2 = 7.09, p = 0.008) (Fig. 2) and therefore, significantly optimizes the BALF-based differential diagnosis between sarcoidosis and nonsarcoidosis conditions. These findings support a potential contribution of clinical information, in addition to BALF cellular profiles, to discrimination between sarcoidosis and alternative diagnoses. Given the limited number of sarcoidosis cases and the absence of independent validation, the performance of the expanded model should be considered exploratory.

DISCUSSION

In the present study, we specifically evaluated patients with marked BALF lymphocytosis (≥ 30%) and elevated CD4/CD8 ratios (≥ 4), representing a population in whom sarcoidosis would commonly be considered on BAL grounds. Notably, despite the simultaneous presence of these two traditionally supportive features, sarcoidosis accounted for only 22.4% of cases, whereas 77.6% of patients were ultimately diagnosed with alternative disorders. Moreover, the CD4/CD8 ratio alone showed limited discriminatory performance (AUC, 0.604). These findings indicate that, even in patients meeting both traditionally supportive BALF criteria, the combination of marked lymphocytosis and an elevated CD4/CD8 ratio does not provide sufficient discriminatory value to reliably distinguish sarcoidosis from alternative diagnoses. This supports the rationale for exploring additional BALF biomarkers to improve diagnostic discrimination in this specific clinical setting.

Current ATS recommendations emphasize that BALF findings should always be interpreted within a multidisciplinary framework, in conjunction with clinical and radiological data, and that no single BALF cellular pattern is diagnostic on its own for any ILD [1]. Similarly, recent reviews have emphasized that elevated CD4/CD8 ratios, although supportive of sarcoidosis, may also occur in other granulomatous, inflammatory, infectious, and neoplastic conditions [2, 3]. Our findings are in line with these observations and further indicate that, within a population already selected for a classically suggestive BALF profile, the CD4/CD8 ratio alone does not reliably distinguish sarcoidosis from its main differential diagnoses.

The diagnostic performance of the BALF CD4/CD8 ratio has long been debated. A recent meta-analysis reported high specificity but only moderate sensitivity for this parameter [12]. Likewise, Zannese et al. demonstrated considerable variability in BALF CD4/CD8 ratios among sarcoidosis patients and suggested that the ratio may be more informative as a marker of disease phenotype and severity than as a standalone diagnostic tool [5]. Our results are consistent with these observations and further suggest that reliance on high CD4/CD8 thresholds in isolation, without integration of additional BALF parameters, may contribute to diagnostic misclassification.

A notable finding of our study was the independent association of lower BALF neutrophil and NK-cell proportions with sarcoidosis in multivariable analysis, together with younger age. Previous studies have primarily associated increased BALF neutrophilia with advanced radiological stages, pulmonary fibrosis, and worse clinical outcomes in sarcoidosis [13], while increased BALF NK-cell proportions have been associated with more severe disease, extrapulmonary involvement, and corticosteroid requirement [8, 9, 14]. Although these studies primarily addressed disease severity and prognosis rather than diagnostic discrimination, our findings raise the possibility that BALF neutrophil and NK-cell proportions may also contain diagnostic information in patients presenting with an otherwise highly suggestive sarcoid BALF profile. Importantly, these findings do not establish neutrophils or NK cells as disease-specific biomarkers, but rather identify potentially complementary cellular features that may improve discrimination between sarcoidosis and alternative diagnoses in this selected population.

Recent studies have increasingly focused on extended BALF immunophenotyping to improve ILD classification. Harada et al. demonstrated that specific BALF lymphocyte subsets contribute to the discrimination of different diffuse ILD subtypes, supporting the concept that the diagnostic information contained in BALF extends beyond conventional differential cell counts and CD4/CD8 ratios [10]. Similarly, d'Alessandro et al. proposed a multiparametric BALF biomarker panel incorporating lymphocyte subsets and KL-6 concentrations to improve differential diagnosis among ILDs [15]. Our results are broadly consistent with this emerging body of evidence and suggest that a combined evaluation of neutrophils, NK cells, and lymphocyte immunophenotyping may provide additional diagnostic information in patients with an otherwise highly suggestive BALF profile.

The discriminatory performance of neutrophils and NK cells was only moderate when considered individually. However, the combination of the age, BALF neutrophil and NK-cell proportions with the CD4/CD8 ratio showed significantly greater discriminatory performance than the CD4/CD8 ratio alone

(AUC 0.802 vs. 0.604; DeLong test, p = 0.008). This finding is clinically relevant because the conventional CD4/CD8 ratio showed limited discriminatory performance in this selected population, whereas the addition of neutrophil and NK-cell proportions provided additional discriminatory information. A multiparametric approach to BALF interpretation may therefore be particularly useful in patients with apparently typical sarcoid BALF profiles, in whom diagnostic uncertainty frequently persists and invasive procedures may otherwise be considered.

The association of younger age with sarcoidosis also deserves consideration. Sarcoidosis patients were substantially younger than those with alternative diagnoses, and age remained independently associated with sarcoidosis in multivariable analysis. No significant association was found between BALF cellular composition and sarcoidosis phenotype—including radiological stage, fibrosis, extrapulmonary involvement, or treatment status—which should be considered. These findings suggests that the diagnostic information provided by BALF cellular populations should be interpreted in conjunction with clinical characteristics. The complementary analysis incorporating age further supports the potential value of combining cellular and clinical information, although these findings should be considered exploratory because the present study was not designed to develop or validate a clinical prediction model.

Importantly, the observed improvement in discrimination should be interpreted cautiously. Only 26 patients had sarcoidosis, the combined analysis was derived and evaluated in the same cohort, and no independent validation was performed. Consequently, the observed AUC may be optimistic and should be considered hypothesis-generating rather than evidence of an established diagnostic model. External validation in larger, independent cohorts will be required before these findings can support the development of a diagnostic algorithm.

Several limitations should be acknowledged. First, the retrospective, single-center design may limit the generalizability of the findings and introduces the possibility of selection and information bias. Second, our inclusion criteria deliberately selected patients with BALF lymphocytosis ≥ 30% and a CD4/CD8 ratio ≥ 4. Accordingly, the findings cannot be directly extrapolated to patients with suspected sarcoidosis who have lower BALF lymphocyte percentages or lower CD4/CD8 ratios, in whom the diagnostic value of these cellular parameters remains to be established. Third, the non-sarcoidosis group was heterogeneous and included infectious, inflammatory, autoimmune, neoplastic, and interstitial lung diseases with potentially distinct BALF cellular profiles. This heterogeneity reflects the diagnostic complexity of real-world practice but may also have contributed to the observed associations and limits disease-specific interpretation. Fourth, sarcoidosis patients were younger than those with alternative diagnoses, raising the possibility of residual confounding despite adjustment for age in the multivariable analysis. Finally, the number of sarcoidosis cases was limited to 26, which increases the risk of model overfitting given the number of candidate predictors evaluated. Accordingly, the ROC estimates may be optimistic, as the combined analysis was derived and evaluated in the same cohort and no internal or external validation was performed.

CONCLUSION

In patients selected for a BALF profile traditionally considered suggestive of sarcoidosis, lymphocytosis with an elevated CD4/CD8 ratio was not specific: only 22.4% of those meeting both criteria had sarcoidosis. The CD4/CD8 ratio alone showed limited discriminatory performance, whereas adding BALF neutrophil and NK-cell proportions improved discrimination in this cohort. These findings support a multiparametric interpretation of BALF cellular profiles rather than reliance on the CD4/CD8 ratio alone, but they are exploratory given the small number of sarcoidosis cases, the retrospective single-center design, and the lack of validation. Prospective, multicenter studies with detailed clinical and radiological phenotyping are needed to confirm whether extended BALF profiling adds reproducible diagnostic value.

Declarations

DECLARATION OF INTEREST

The authors declare that they have no conflict of interest.

INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Research Ethics Committee of Hospital Universitario Puerta de Hierro Majadahonda (protocol code PI 207 – 19).

INFORMED CONSENT STATEMENT

All patients had previously provided written informed consent to undergo bronchoalveolar lavage as part of routine clinical care and to allow their clinical data to be recorded in a database for future research purposes.

This retrospective study used data collected during routine clinical practice. No additional procedures were performed for research purposes. The requirement for study-specific informed consent was waived by the Research Ethics Committee due to the retrospective nature of the study.

Author contributions

Conceptualization: R. Castejon, P. Tutor-Ureta, R. Laporta, S. Rosado and S. Mellor-Pita. Methodology: R. Castejon, C. Martin, R. Laporta, S. Rosado, L.Rubio, B. Garcia. Formal analysis and investigation: R. Castejon, P. Tutor-Ureta, C. Martín, R. Laporta, S. Rosado and S. Mellor-Pita. Writing - original draft preparation: R. Castejon, C. Martin and S. Mellor-Pita. Writing - review and editing: R. Castejon, P. Tutor-

Ureta, S. Rosado and S. Mellor-Pita. Supervision: R. Castejon and S. Mellor-Pita. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

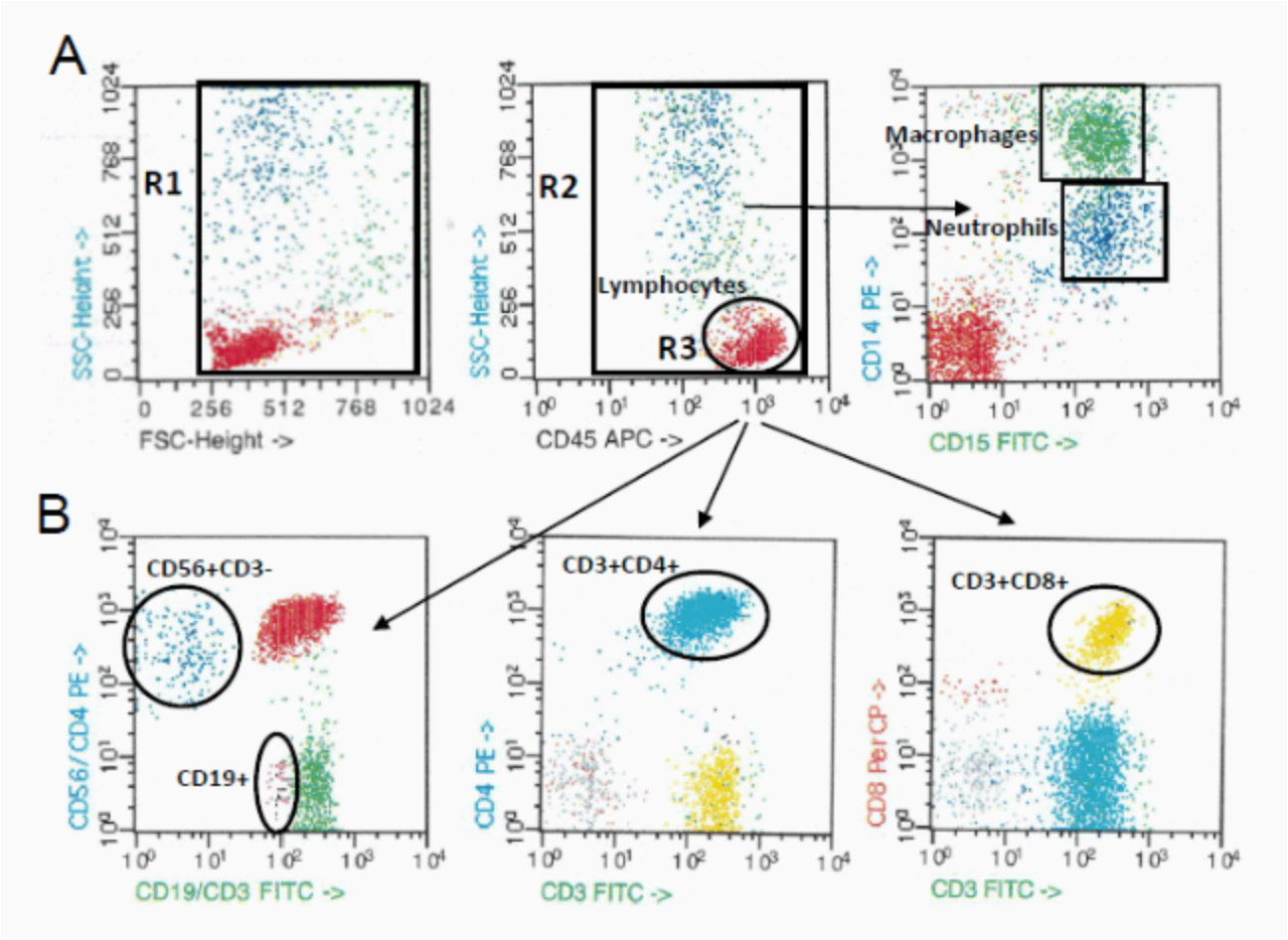


Figure 1

Flow cytometric analysis of BALF cell populations. Panel A shows the distribution of BALF cell populations based on cell size and granularity, excluding cellular debris (R1). Leukocyte populations were analyzed among CD45+ cells (R2) according to CD15 and CD14 expression, allowing the identification and quantification of lymphocytes (red), macrophages (green), and neutrophils (blue). Lymphocyte subpopulations (Panel B) were expressed as percentages of the total lymphocyte population (R3), including CD3+CD4+ T cells, CD3+CD8+ T cells, CD19+ B cells, and CD56+CD3− natural killer (NK) cells.

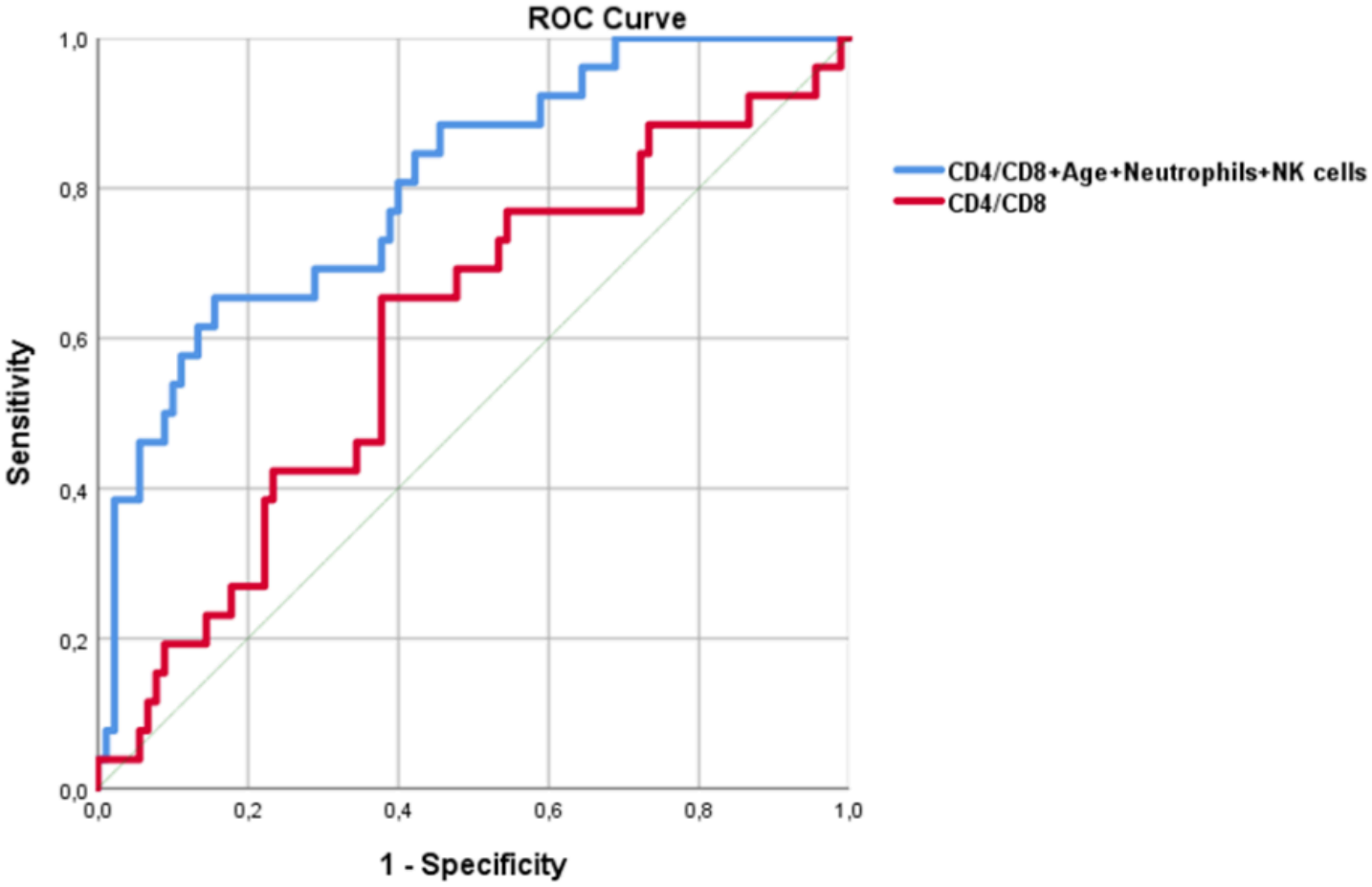


Figure 2

Receiver operating characteristic (ROC) curves comparing diagnostic performance of BALF subpopulations for sarcoidosis identification. DeLong test was applied for statistical comparison of both correlated ROC curves, p= 0.008.