Eosinophilic Globules in Bronchoalveolar Lavage Fluid of Patients With Systemic Sclerosis–Related Interstitial Lung Disease

A Diagnostically Useful, Previously Unreported Finding in a Retrospective and Prospective Study, Including Differential Diagnosis With Other Idiopathic and Secondary Interstitial Lung Diseases

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Abstract

Bronchoalveolar lavage (BAL) is a minimally invasive method possibly representing a diagnostic tool in the evaluation of interstitial lung diseases (ILDs) of different causes. We first describe herein the morphologic, histochemical, and immunohistochemical features of previously unreported eosinophilic globular deposits of acellular amorphous material of uncertain nature in a relatively large series of 227 BAL samples obtained from patients with various ILDs. Overall, eosinophilic globules were detected in 18 cases (7.9%), 16 of which were in patients with systemic sclerosis (SSc)-related ILD (16/50 [32%]) and in 2 cases of apparently idiopathic usual interstitial pneumonia. Apart from the possible diagnostic information of this finding, in patients with SSc, the globules were significantly related to BAL neutrophilia or eosinophilia and extensive ILD in high-resolution computed tomography ($P < .0001$*). Differential diagnosis with other types of acellular globular materials observed in BAL samples is also discussed.*

Interstitial lung diseases (ILDs) may be idiopathic or sustained by a broad spectrum of causes, such as infectious agents, drug toxicity, fume inhalation, collagen-vascular diseases (CVDs), hypersensitivity pneumonia (HP), sarcoidosis, vasculitides, and neoplasms.[1] Diagnosis of ILD requires integration of clinical, laboratory, radiologic, and histopathologic features. In ILD assessment, bronchoalveolar lavage (BAL) fluid examination is a minimally invasive and routinely performed step.[2-5] BAL is often used to rule out some infections (*Pneumocystis*, mycobacteria, viruses, fungi, *Nocardia*) and cancer or to identify diagnostic acellular components (eg, asbestos bodies, alveolar hemorrhage, and alveolar proteinosis [AP]).[6-9] The cell count on BAL specimens is operator-dependent and usually performed by properly trained cytotechnologists or biologists. Although the differential cell count seems to be a helpful parameter for discriminating usual interstitial pneumonia (UIP) from nonspecific interstitial pneumonia (NSIP),[3-5,10] controversial results have been reported on the prognostic value of the differential cell count in some systemic diseases involving the lungs, such as systemic sclerosis (SSc).[11-15]

In daily practice, we recently observed in BAL samples the presence of round, globular deposits of eosinophilic, homogeneous, amorphous material in a subset of patients with ILD secondary to SSc. To the best of our knowledge, this preliminary observation has not been described in the literature.

We report the morphologic, histochemical, and immunohistochemical features of these unusual BAL deposits and describe our analysis of the possible diagnostic and prognostic value of their presence in a relatively large series of patients with ILDs of various causes.

Materials and Methods

A retrospective and prospective study of BAL features was conducted in patients with idiopathic and secondary ILDs between January 2005 and January 2008 diagnosed at the Section of Pathologic Anatomy, Azienda Policlinico, Modena, Italy. A total of 227 samples from patients undergoing a BAL procedure for ILDs of different causes were consecutively collected for study purposes. Basically, BAL samples were sent to the above-mentioned institution to exclude the presence of infectious agents, by using histochemical or immunohistochemical stains, and cancer cells. In some specific pathologic conditions, pathologists were asked to report the CD4/CD8 lymphocyte ratio (sarcoidosis vs hypersensitivity pneumonitis) and/or the percentage of CD1a+ histiocytes (Langerhans cell histiocytosis) and to highlight asbestos bodies (asbestosis), hemosiderin-laden macrophages (alveolar hemorrhage), and amyloid and proteinaceous deposits. In this study, the differential cell count was simply subdivided as follows: lymphocytosis (lymphocytes >20% of total WBCs), neutrophilia (neutrophils >5%), and eosinophilia (>5%).

BAL was performed as routine clinical evaluation. Bronchoscopy was performed, and the BAL sample was processed using 150 to 200 mL (3-4 instillations of 50 mL) of room temperature sterile physiologic saline in the middle or inferior right lobe. Recovered lavage fluid was obtained by gentle mechanical suction. A mean fluid volume of 80 mL was retrieved. Cells were collected by centrifugation, and slides were stained with H&E and May-Grünwald-Giemsa stains. When requested, additional stains (Grocott, periodic acid–Schiff [PAS], PAS-diastase [PAS-D], Congo red, Perls, Ziehl-Neelsen, Gram, and trichrome) and immunostains (for cytomegalovirus, herpes simplex virus, antiadenovirus, CD1a, CD4, and CD8) were performed.

Immunostains with collagen IV (clone CIV-22, Ventana, Tucson, AZ) and surfactant protein (SP)-A (PE-10, Dakopatts, Glostrup, Denmark) were performed in all cases showing eosinophilic globules in BAL samples by using an automated immunostainer (Benchmark, Ventana); 3,3′-diaminobenzidine was used as the chromogen and Harris hematoxylin as the counterstain. BAL interpretation was done in a blinded manner to knowledge of clinical data, imaging features, and lung function parameters.

Overall, the series consisted of 227 cases of ILD undergoing BAL examination **Table 1**, including idiopathic UIP (also known as idiopathic pulmonary fibrosis [IPF]), idiopathic NSIP, cryptogenic organizing pneumonia, HP, Langerhans cell histiocytosis, eosinophilic pneumonia, sarcoidosis, Wegener granulomatosis, systemic lupus erythematosus, alveolar hemorrhage, drug toxicity (from statins, amiodarone, and propylthiouracil), pneumoconiosis, lipoid pneumonia, infection (*Pneumocystis*, cytomegalovirus, *Strongyloides*, *Aspergillus* and mycobacteria), AP, polymyositis or dermatomyositis, rheumatoid arthritis, mixed connectivitis, and SSc.

Table 1
Specific Pulmonary Conditions Analyzed by Bronchoalveolar Lavage in the Present Series

	No. of Cases	No. of Histologically Proven Cases
Idiopathic interstitial lung disease		
Usual interstitial pneumonia	23	15
Nonspecific interstitial pneumonia	12	10
Bronchiolitis-obliterans organizing pneumonia	8	3
Secondary interstitial lung disease		
Hypersensitivity pneumonia	7	3
Langerhans cell histiocytosis	5	0
Eosinophilic pneumonia	9	2
Sarcoidosis	35	12
Wegener granulomatosis	3	3
Systemic lupus erythematosus	2	0
Alveolar hemorrhage	5	2
Drug toxicity	3	3
Pneumoconioses	3	1
Lipoid pneumonia	2	1
Infection		
Pneumocystis pneumonia	12	0
Cytomegalovirus	3	0
Strongyloides	2	0
Aspergillus	20	5
Mycobacteria	9	0
Pulmonary alveolar proteinosis	2	2
Polymyositis/dermatomyositis	4	0
Rheumatoid arthritis	6	2
Mixed connectivitis	2	0
Systemic sclerosis	50	2
Total	227	66

Diagnosis of ILD relied on clinical, laboratory, and imaging studies, and only when clinical, laboratory, and imaging data were insufficiently robust, was histologic examination required.

In all patients with SSc, high-resolution computed-tomography (HRCT) features were evaluated. Cases were then subdivided into 3 main groups, as follows: (1) no pulmonary alterations shown by HRCT, (2) prevalent ground-glass opacities, and (3) reticulonodular and honeycombing changes, suggesting a UIP pattern.

The correlation between different variables was performed by using contingency table methods and tested for significance using the Pearson χ^2 test (SPSS, version 13.0, SPSS, Chicago, IL). A difference with probability (*P*) values less than .05 was considered as significant.

Results

Morphologic, Histochemical, and Immunohistochemical Features

In this study, we focused our attention on the presence of extracellular, round-to-oval, globular deposits of amorphous,

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acellular, deeply eosinophilic material of different sizes **Image 1A** and **Image 1B**. These deposits had a finely granular appearance, stained for PAS, and were resistant to diastase digestion (PAS-D) **Image 1C** and **Image 1D**. May-Grünwald-Giemsa **Image 1E** and trichrome **Image 1F** stains confirmed the eosinophilic appearance and possible collagen derivation (green staining), respectively. No staining was observed with other stains (Congo red, Grocott, Ziehl-Neelsen, Gram, or Perls).

In immunocytochemical analysis, eosinophilic deposits did not stain with collagen IV and SP-A.

Overall Case Series

We identified the presence of these amorphous eosinophilic deposits in 18 cases (7.9%) of the entire series, but in 16 (32%) of 50 cases of SSc. The 2 additional cases showing the presence of these PAS+ globules had UIP, apparently idiopathic, whereas all other cases did not display these peculiar eosinophilic deposits **Table 2**.

Based on previous sporadic observations of these eosinophilic amorphous deposits in advanced ILD in patients with SSc, a presumptive diagnosis of SSc with lung involvement evident in BAL sample features was made prospectively in 4 cases. In all cases, clinical, laboratory, and imaging studies confirmed the diagnosis of SSc.

Statistical Correlations

As expected, the presence of eosinophilic deposits on BAL was significantly associated with a diagnosis of SSc-related ILD ($P < .001$). Patients with SSc (43 women and 7 men) had no HRCT alterations in 16 cases (32%), ground-glass opacities in 18 cases (36%), and honeycombing in 16 (32%). Eosinophilic deposits were detected in 3 cases with ground-glass opacities and in 13 cases with honeycombing, but not in the HRCT-negative cases ($P < .0001$). In addition, cases with eosinophilic deposits had neutrophilia (8 cases) or neutrophilia and eosinophilia in the BAL samples, while no deposits were observed in the 23 cases with unremarkable BAL cytologic findings ($P < .0001$). Finally, also the presence of neutrophilia (15/16, 7 with honeycombing) and eosinophilia (all 16 cases, 7 with honeycombing) was significantly associated with HRCT-evident alterations ($P < .0001$).

Discussion

BAL examination is a minimally invasive method to analyze diseases involving the peripheral alveolar regions of the lungs.[3-5] In some cases, BAL has true diagnostic usefulness, obviating more invasive procedures. In particular, analysis of BAL samples can be a formidable diagnostic tool to detect infections, malignancies, and some ILDs (eg, Langerhans cell histiocytosis, AP, alveolar hemorrhage, eosinophilic pneumonia, and lipoid pneumonia).[6-9,16-18] In general, the interpretation of BAL results must always be integrated with clinical, laboratory, and imaging data.[3-5,10] In the setting of CVD-related ILDs, the differential cell count may be helpful in correlating inflammatory pattern shown by BAL analysis with various patterns and extent of lung involvement shown by HRCT, as well as in monitoring lung inflammation or excluding complications.[3-5] Welker at al[10] demonstrated that a BAL cell count in ILD can be particularly helpful in sarcoidosis and the HP and UIP patterns, but not in other ILD. In SSc, there are controversies as to whether a differential cell count in BAL samples (ie, for neutrophilia and eosinophilia) is a helpful tool for evaluating alveolitis and in identification of patients at high risk of progression of lung disease or for predicting response to therapy with cyclophosphamide.[11-15]

We observed, in a retrospective (14 cases) and prospective (4 cases) manner, the presence of eosinophilic globular deposits of amorphous material in a subset of BAL fluid samples from patients with ILD. These globular structures resulted in diastase-resistant, PAS+, green-colored trichrome staining and did not react with SP-A and collagen IV. It is interesting that the great majority of patients (16 cases) had SSc, whereas ILD with diffuse and severe reticular and honeycombing changes consisting of the UIP pattern (apparently idiopathic, then IPF) characterized the remaining 2 cases.

SSc is a multisystemic autoimmune disease of unknown etiology characterized by microangiopathy and excessive accumulation of extracellular collagen deposition in the skin and several internal organs.[19] In about 70% of patients, pulmonary involvement develops, characterized by ILD with HRCT features closely resembling, if not indistinguishable from, those observed in idiopathic ILD and by vasculopathy eventually leading to pulmonary hypertension.[20] In addition, ILD is the main cause of death in SSc.[21] Of note, a not insignificant rate of patients with apparently idiopathic ILD (particularly with an NSIP pattern) had instead an underlying unknown CVD, with pulmonary manifestations preceding the systemic symptoms.[22] ILD occurs in more than 80% of patients with SSc sine-scleroderma and may likely be confused with IPF.[23]

Previous ultrastructural studies by Harrison et al[24,25] on lung biopsy specimens from patients with SSc demonstrated important pulmonary damage involving epithelial and endothelial structures leading to interstitial edema and excessive collagen deposition in interstitium and alveoli. More recently, Andersen et al[26] found significantly higher levels of total and pro-matrix metalloproteinase-9 (MMP-9), a collagenase, in BAL fluid samples from patients with SSc-related ILD than in samples from patients with SSc without ILD and from healthy subjects. The authors then suggested that alveolar MMP-9 may have a role in lung remodeling,

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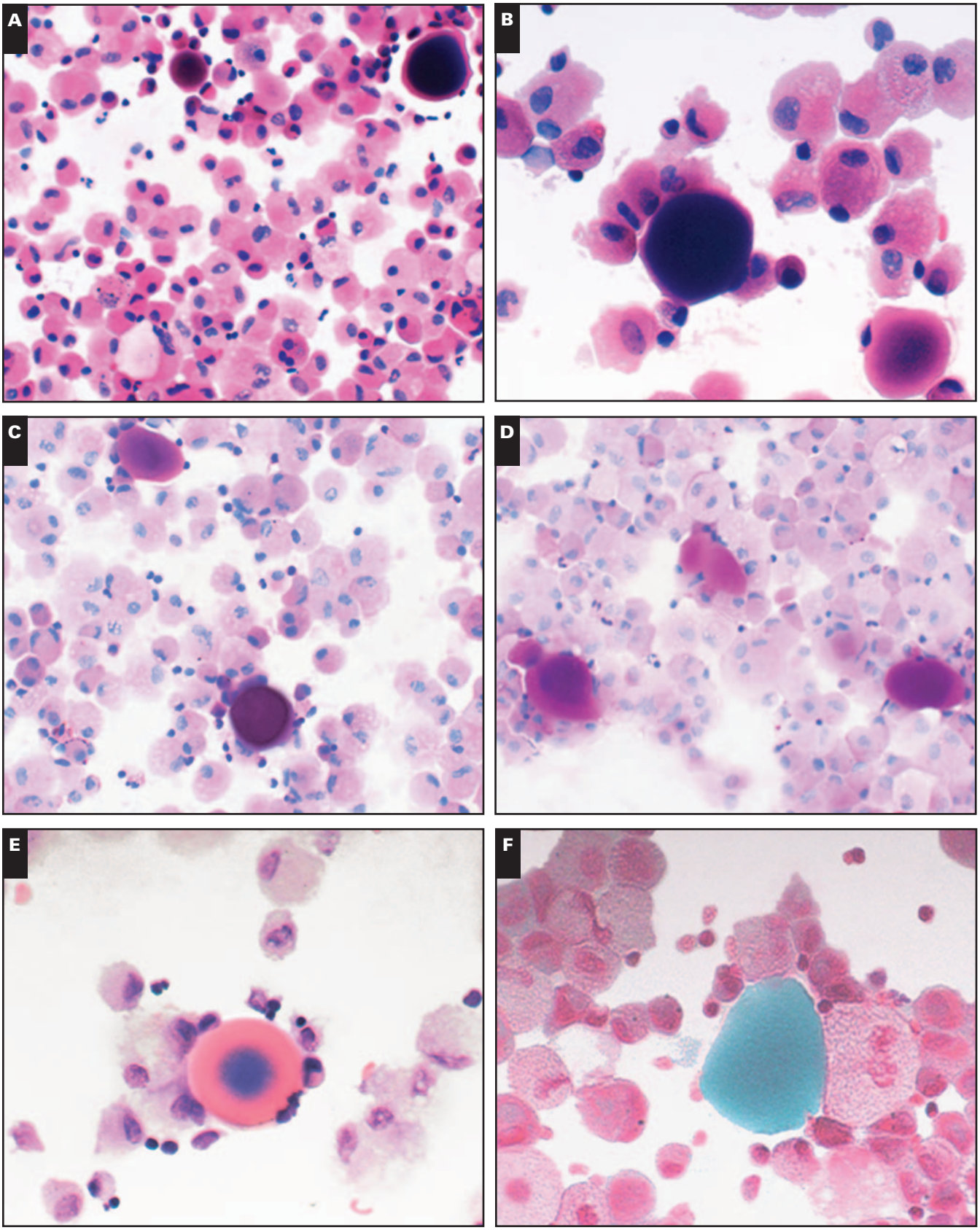

Image 1 **A**, Globular eosinophilic deposits with mixed granulocytic infiltrate in a bronchoalveolar lavage specimen from a patient with systemic sclerosis (H&E, ×100). **B**, Note the rounded, well-defined margins and finely granular and homogeneous structure of the globular eosinophilic deposits (H&E, ×200). **C** and **D**, Positive staining with periodic acid–Schiff (PAS) (**C**, ×100) and PAS-diastase (**D**, ×100). **E** and **F**, Eosinophilic appearance with May-Grünwald-Giemsa stain (**E**, ×200) and green coloration with trichrome (**F**, ×200) of globular materials.

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promoting lung fibrosis possibly through MMP-9 production by neutrophils.[26]

In our study, we identified eosinophilic globular structures of uncertain nature (but possibly consisting of collagen material as highlighted by the trichrome stain) in 16 of 50 BAL fluid samples from a subgroup of patients with SSc-related ILD. The presence of these globules was also observed in 2 patients with an apparently idiopathic UIP pattern shown by HRCT, but not in several other ILDs of different causes. In addition, eosinophilic globules in SSc were significantly associated with BAL neutrophilia and eosinophilia and with more severe ILD (honeycombing) shown by HRCT; both conditions characterize a clinicoradiologic subset of patients with extensive ILD in SSc.[12-15]

In some cases, the acellular material in the background of BAL fluid may be the key diagnostic component for a specific disease, as in pulmonary AP, diffuse alveolar hemorrhage, asbestosis, *Pneumocystis* infection, pulmonary alveolar microlithiasis (PAM), and amyloidosis. However, eosinophilic globular deposits described in this report need to be mainly differentiated from globular amorphous materials usually observed in AP **Image 2A** and **Image 2B**, *Pneumocystis* infection **Image 2C** and **Image 2D**, PAM, and pulmonary amyloidosis.[27-32]

AP is a rare lung disease characterized by excessive accumulation of surfactant-derived phospholipids and proteins into the alveoli.[3,5,17,18,27-29] An autoimmune pathogenesis related to the presence of anti–granulocyte-macrophage colony-stimulating factor with consequent impaired surfactant phagocytic function of the alveolar macrophages has been suggested. The disease may be congenital, idiopathic, or secondary to several conditions (ie, dust inhalation, infections, and malignancies), frequently has a characteristic "crazy-paving" pattern shown by HRCT, and BAL fluid derived from patients with AP generally appears turbid and milky.[3,5,28]

BAL cytologic features are often diagnostic in these cases and show a dirty background with an amorphous PAS+ granular substance, foamy macrophages with intracellular inclusions, and acellular diastase-resistant PAS+ globules.[3,5,17,18,28-30] Apart from the different clinicoradiologic scenario, BAL fluid with eosinophilic globules described herein was grossly limpid, associated with an inflammatory component consisting of granulocytes and eosinophils, without a dirty background. In contrast with globules in AP, those reported here are negative for surfactant proteins.[3,5,17,18,28-30]

Eosinophilic globules did not stain with PE-10, a monoclonal antibody recognizing the SP-A (the most abundant protein in lung surfactant) but not SP-B, SP-C, or SP-D. Although this finding cannot entirely exclude that these globules might be related to surfactant, it seems a very unlikely possibility.

Frothy eosinophilic material with "bubbles" in an inflammatory-to-necrotic background is often observed in *Pneumocystis* infection, a disease occurring in association with the defective immunity in several underlying conditions, from AIDS to immunosuppressive therapies for malignancies, transplantation, or CVD.[9,30] However, isolated or aggregates of round, indented, or helmet-shaped organisms are commonly well evidenced by a methenamine silver stain (ie, Grocott) in the frothy material.[31]

PAM is another rare autosomal recessive heritable pulmonary disorder of uncertain pathogenesis in which mutations involving the *SLC34A2* gene have been recently demonstrated.[32,33] PAM is characterized by formation of several microliths in the alveolar spaces and bilateral infiltrates with a sand-like or snowstorm micronodular appearance on radiographs and HRCT.[33] Several spherical to ovoid concentrically laminated microliths with radial striations, ranging from 250 µm to 1 mm, can also be observed in BAL samples.[32] Microliths are mainly composed of calcium and phosphorus and show positive staining with von Kossa stain.[32,33]

Amorphous eosinophilic materials may occasionally be present in pulmonary amyloidosis. However, Congo red stain highlights apple-green birefringence in polarized light.[34]

Finally, corpora amylacea should also be considered in the differential diagnosis. However, these incidental and clinically insignificant eosinophilic structures had a rounded to spherical form with concentric rings and radiating striations

Table 2
Presence of Eosinophilic Periodic-Acid–Schiff–Diastase–Positive Globular Material and Interstitial Lung Disease

	No. of Cases	No. (%) of Eosinophilic Globules
Idiopathic interstitial lung disease		
Usual interstitial pneumonia	23	2 (9)
Nonspecific interstitial pneumonia	12	
Bronchiolitis-obliterans organizing pneumonia	8	0 (0)
Secondary interstitial lung disease		
Hypersensitivity pneumonia	7	0 (0)
Langerhans cell histiocytosis	5	0 (0)
Eosinophilic pneumonia	9	0 (0)
Sarcoidosis	35	0 (0)
Wegener granulomatosis	3	0 (0)
Systemic lupus erythematosus	2	0 (0)
Alveolar hemorrhage	5	0 (0)
Drug toxicity	3	0 (0)
Pneumoconioses	3	0 (0)
Lipoid pneumonia	2	0 (0)
Infection		
Pneumocystis pneumonia	12	0 (0)
Cytomegalovirus	3	0 (0)
Strongyloides	2	0 (0)
Aspergillus	20	0 (0)
Mycobacteria	9	0 (0)
Pulmonary alveolar proteinosis	2	0 (0)
Polymyositis/dermatomyositis	4	0 (0)
Rheumatoid arthritis	6	0 (0)
Mixed connectivitis	2	0 (0)
Systemic sclerosis	50	16 (32)
Total	227	18 (7.9)

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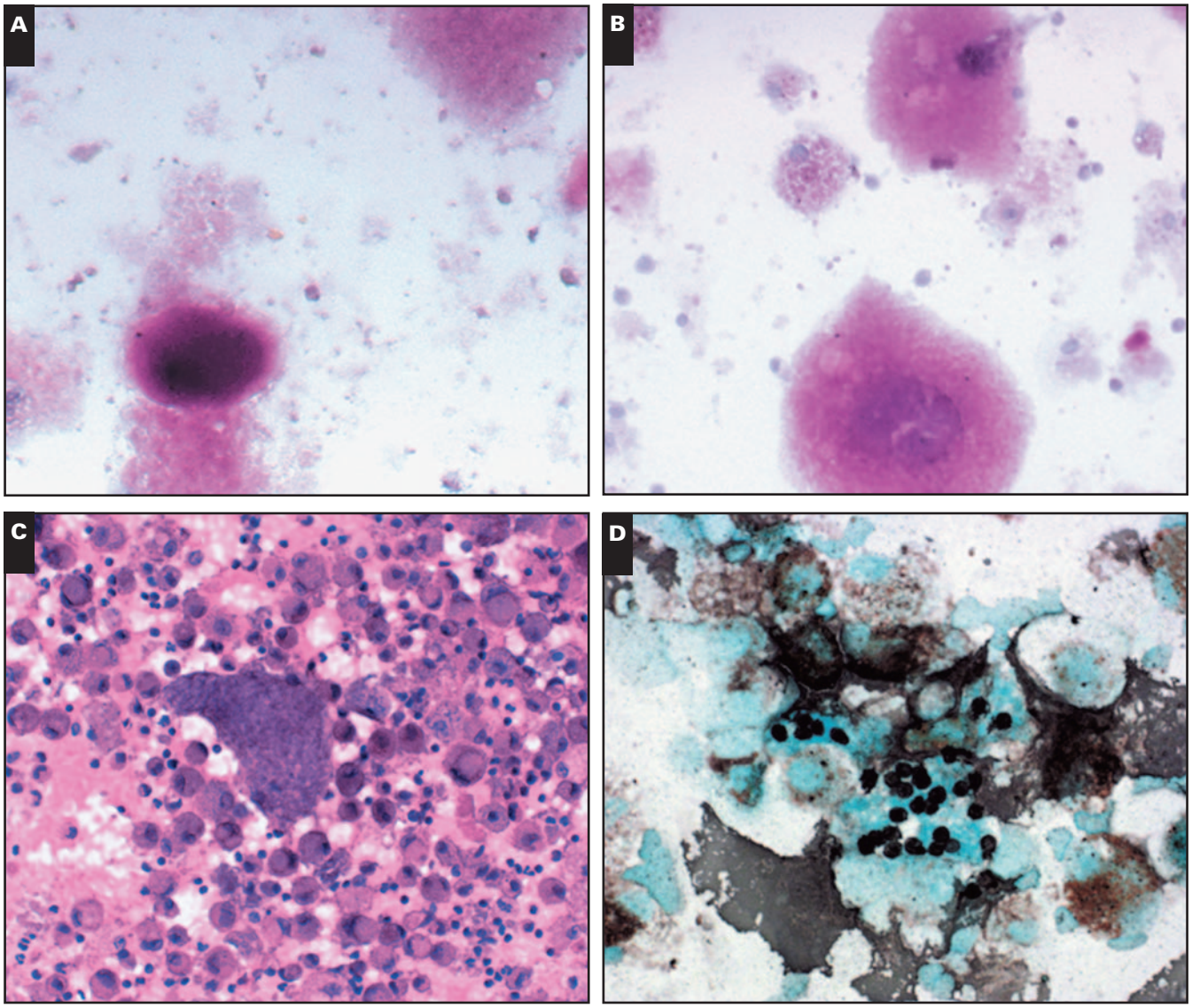

Image 2 **A**, Globular deposits of periodic acid–Schiff (PAS)+ material in a patient with alveolar proteinosis (AP) (×200). **B**, Globular deposits in AP are grossly granular and rounded but characterized by a poorly cellular, dirty background with PAS-diastase+ foamy macrophages (×200). **C**, Frothy, ill-defined material in *Pneumocystis* infection within a necrotic and inflammatory background (H&E, ×100). **D**, Silver methenamine staining highlights the presence of numerous round organisms consistent with *Pneumocystis* species (Grocott, ×100).

from the dark weakly polarized center to the periphery, also staining with PE-10.[35,36]

We first described in this study the presence of eosinophilic globular deposits of uncertain nature (possibly collagen material to be further characterized by immunocytochemical or ultrastructural analysis) in BAL fluid from a subset of patients with extensive and severe ILD, mainly related to SSc (16 of 18), but not in BAL samples from patients with other ILDs of different causes. Based on the presence of these globules, we prospectively suggested a clinically confirmed diagnosis of SSc with pulmonary ILD in 4 cases. In the SSc setting, a statistically significant correlation between the finding of globular deposits in BAL samples and radiologic identification of involvement of ILD was found. Although these preliminary observations need to be validated by further studies, pathologists should be aware that the finding of these eosinophilic globules in BAL samples may indicate an underlying severe SSc-related ILD and may be useful in suggesting this diagnostic possibility, which requires confirmation by integration with clinical and laboratory data.

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