The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

Utility of anti-GM-CSF antibodies in the diagnosis of pulmonary alveolar proteinosis: a case report

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ARTICLE INFO

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Key words:
pulmonary alveolar proteinosis, anti-GM-CSF, pulmonary surfactant, bronchoalveolar lavage

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ABSTRACT

Introduction

Pulmonary alveolar proteinosis (PAP) is a disease characterized by the accumulation of lipoproteinaceous material in the alveoli as a consequence of deficient processing of pulmonary surfactant. It is classified into primary, secondary, and congenital forms. Primary PAP (autoimmune origin) is characterized by the presence of antibodies against granulocyte-macrophage colony-stimulating factor (GM-CSF), while secondary PAP is due to multiple causes such as exposure to certain environmental substances. We present a case of a patient with probable mixed PAP, primary and secondary, due to exposure at the patient's workplace.

Case presentation

A 35-year-old male patient attends the outpatient clinic of pulmonology due to symptoms of exertional

dyspnea for one year. Pulmonary function tests are performed, and the chest X-ray reveals diffuse bilateral lung involvement with a ground-glass pattern. Incision and excision lung biopsy show findings compatible with predominant PAP in the left lower lobe (LLL). Additionally, a positive anti-GM-CSF antibody result is obtained. The patient is treated with bronchoalveolar lavage (BAL) and nebulized sargramostim. The patient shows satisfactory progress.

Discussion

The clinical, analytical, radiological, and histological manifestations were compatible with the diagnosis of autoimmune PAP, and there was suspicion of secondary PAP due to exposure to rock wool. The role of the laboratory, in this case, was essential for the diagnostic confirmation of PAP by performing the determination of anti-GM-CSF antibodies.

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INTRODUCTION

Pulmonary alveolar proteinosis (PAP) is a rare diffuse interstitial disease characterized by excessive accumulation of lipoproteinaceous material derived from pulmonary surfactant in the alveolar spaces and terminal bronchioles, which can range from asymptomatic to severe respiratory failure (1).

In all its forms, the physiopathological substrate lies in the accumulation of surfactant in the alveolar spaces due to deficient activity in its processing by macrophages. GM-CSF is necessary for the final differentiation and maturation of alveolar macrophages (2).

PAP is classified into three types: primary (autoimmune), secondary, and congenital. The mechanisms that lead to macrophage dysfunction differ in each clinical form.

The primary or idiopathic variant (PAPi) is the most common form, accounting for up to 90% of cases. This clinical form is characterized by the presence of neutralizing IgG antibodies against granulocyte-macrophage colony-stimulating factor (anti-GM-CSF), which blocks the bioactivity of GM-CSF in vivo (3). These antibodies against GM-CSF affect the terminal differentiation of macrophages and, therefore, prevent the growth of these cells that are responsible for eliminating surfactant in the lungs (4).

Secondary forms are associated with hematological disorders or diseases (myelodysplastic syndromes, monoclonal gammopathies, leukemias, lymphomas), non-hematological neoplastic diseases, immunodeficiencies, chronic inflammatory syndromes, infections (Mycobacterium tuberculosis, Nocardia, Pneumocystis jirovecii), or exposure to various environmental substances such as silica, aluminum, titanium, or some fertilizers (5).

In the case of congenital PAP, the problem is associated with recessive anomalies in the gene that codes for the α (CSF2RA) and β (CSF2RB) chains of the GM-CSF receptor (6). It can also be secondary to mutation of GM-CSF or mutations in genes that code for surfactant proteins B (SP-B), C (SP-C) (7) and mutation of the ATP-Binding Cassette transporter A3 (ABCA3) (8).

Next, we describe a case of mixed PAP, of both primary and possibly secondary origin, that occurred in our hospital.

CASE REPORT

A 35-year-old male with a history of smoking since he was 16 years old (8-10 cigarettes/day) and employed for about 2 years in a fireproofing company processing paint and rock wool (material with high silica content). The patient reports working with machines that grind rock wool and project dust, so exposure to this material is constant. He also reports previous episodes

of rhinorrhea with the expulsion of black soot for two days. He has been experiencing exertional dyspnea for a year and a weight loss of 6 kg during this period. Subsequently, due to exacerbation of dyspnea and respiratory difficulty, he was referred to external pulmonology consultations. He has no other relevant medical history, does not have pets, nor does he engage in related activities.

On physical examination, he presented with a blood pressure of 121/79 mmHg, heart rate of 84 bpm, arterial blood gas with an O2 saturation of 92%, and an inspired oxygen fraction (FiO2) of 0.21. Additionally, exertional dyspnea was identified without cough, expectoration, fever, or thermal sensation, and no orthopnea. The patient reports occasional apneas with some asphyctic awakenings. The tests to evaluate the respiratory function, including forced vital capacity (FVC), forced expiratory volume in the first second (FEV1), and FEV1/FVC ratio were normal. On laboratory analysis, increased levels of lactate dehydrogenase (LDH) of 530 mg/dL (RI: 210-425 mg/dL) and angiotensin converting enzyme (ACE) of 75 U/L (RI: 20-70 U/L) were observed. The chest X-ray and high-resolution computed tomography (HRCT) revealed diffuse bilateral lung involvement with a ground-glass pattern and smooth thickening of the interlobular septa, adopting a paving stone pattern with a tendency to consolidation in the posterior region of the middle third of the left inferior lobe Figures 1 and 2.

The rest of the blood and urine tests were normal, including complete blood count, coagulation, antinuclear antibodies (ANA), and antineutrophil cytoplasmic antibodies (ANCA), complete biochemistry with liver, renal, and bone metabolism functions. Moreover, microbiological results were negative for Gram staining, fungal cultures, and PCR for *Adenovirus (A, B, C, D, and E), Parainfluenza virus (1, 2, 3, and 4), Rhinovirus (A, B, and C), Influenza A and B viruses, Metapneumovirus, Aspergillus, Pneumocystis jirovecii, and Mycobacteria.*

With these findings, a differential diagnosis was raised between: alveolar proteinosis (primary or secondary to rock wool inhalation) as the first possibility, or less likely sarcoidosis (no lymph node or pleural involvement), lipoid pneumonia (no areas of fatty density observed, and the involvement was extensive), infectious origin (bacterial, viral, and P. jiroveci cultures were negative), or bronchioalveolar carcinoma.

After discussing the case with the immunology service, determination of anti-GM-CSF antibodies was requested due to suspicion of primary PAP, and the results were positive with a figure of 8.2 U/mL (RI: <5 U/mL). The determination of anti-GM-CSF antibodies was performed using a ClinMax™ Human GM-CSF Quantitative ELISA kit, which is a standard sandwich immunoassay designed to quantify GM-CSF present in complex biological matrices such as human serum, plasma, and buffer solution. Additionally, the patient underwent a transbronchial biopsy in the left inferior lobe, and the samples were sent to the Pathology Department. The histological studies of the lung biopsy confirmed the diagnosis of PAP.

Based on the clinical, analytical, radiological, and anatomopathological findings, the patient was diagnosed with primary pulmonary alveolar proteinosis (PAP). The secondary origin could not be demonstrated due to the absence of previous sera to determine anti-GM-CSF concentrations. Combined treatment was decided upon, consisting of bronchoalveolar lavage (BAL) techniques along with nebulized sargramostim administration.

DISCUSSION

Pulmonary alveolar proteinosis (PAP) was formerly called Rosen Castleman Liebow syndrome,

Figure 1 Chest X-ray (1.A and 1.B) showing bilateral and diffuse lung involvement

Figure 1.A

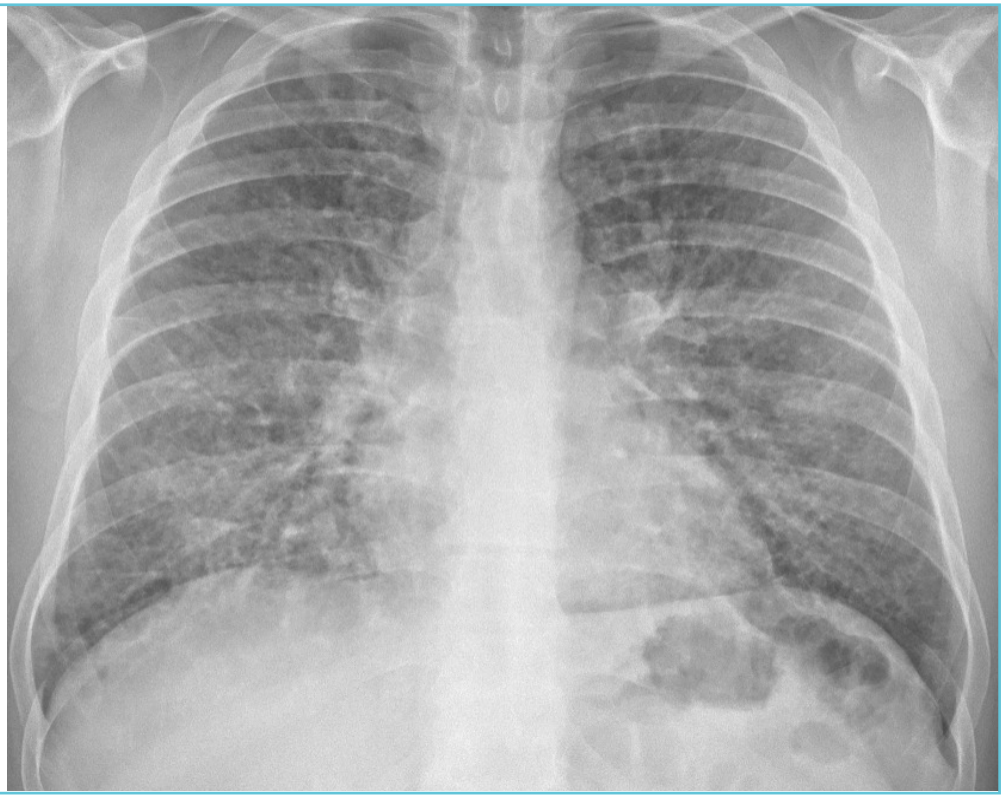


Figure 1.B

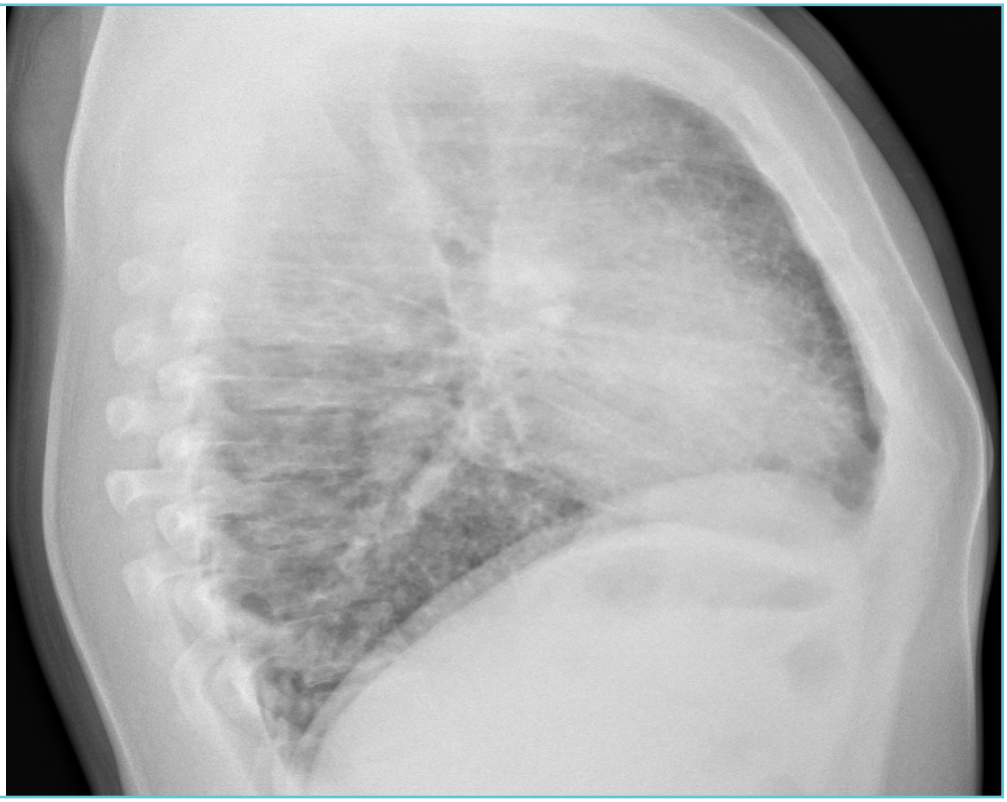


Figure 2 **High-resolution computed tomography (HRCT) of the chest (2.A and 2.B) showing bilateral and diffuse lung involvement with ground-glass opacities of a patchy appearance, with smooth thickening of the interlobular septa, adopting a reticular pattern known as "crazy paving"**

Figure 2.A

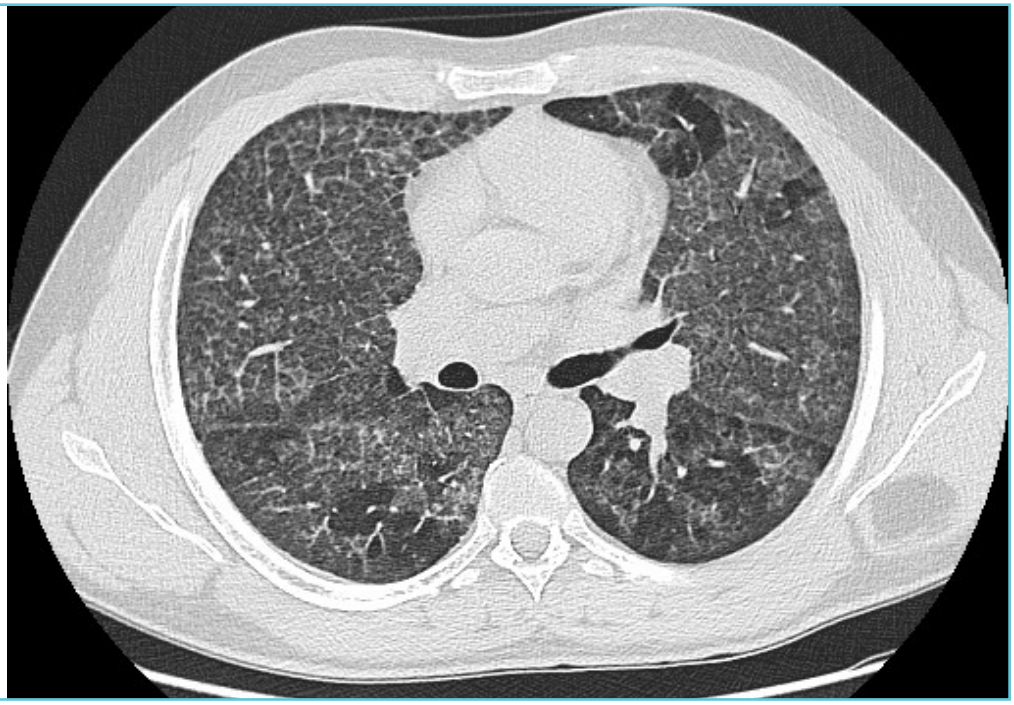
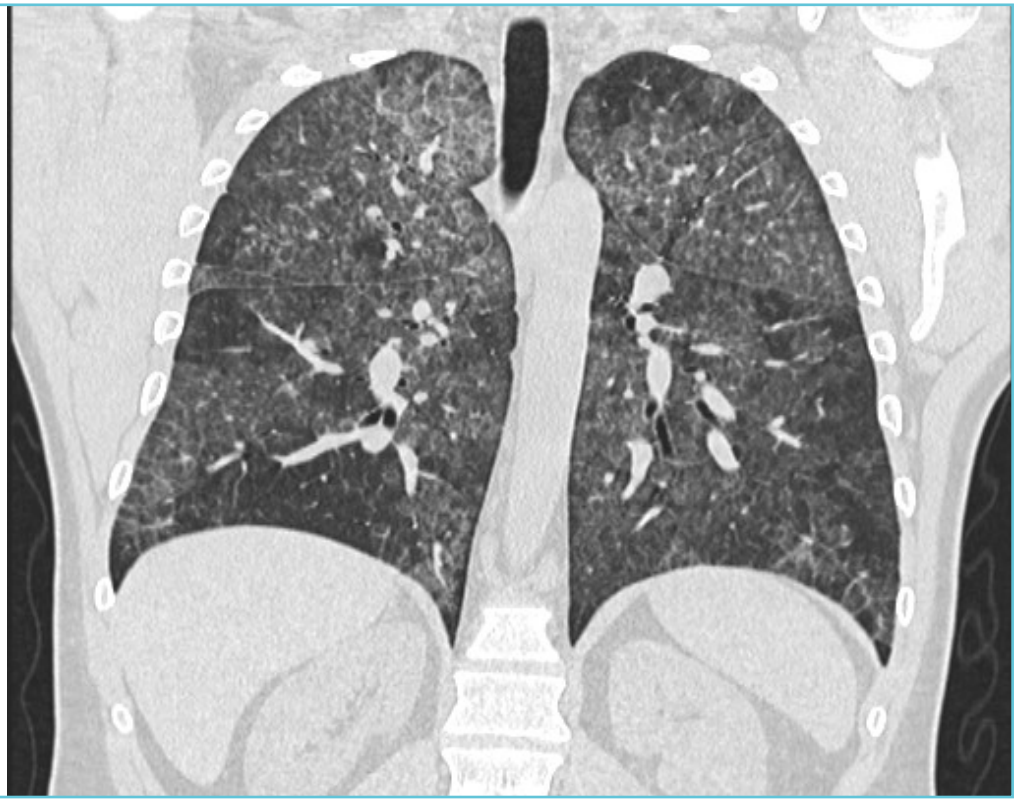


Figure 2.B



in honor of the authors of a publication of a series of 27 cases in 1958 that described pulmonary infiltrates of proteinaceous material and PAS-positive respiratory specimens (9).

This disease has an estimated incidence of 0.2-0.4 cases per million people per year and a worldwide prevalence of nearly seven cases per million people (10). It usually presents between the third and sixth decades of life.

It is a heterogeneous entity, characterized by productive or dry cough, dyspnea, fever, fatigue, and chest pain. Additionally, it can be associated with polycythemia, hypergammaglobulinemia, elevated LDH, elevated tumor markers (CEA, CA 19.9), as well as the presence of serum antibodies (anti-GM-CSF), both in serum and in BAL in the case of primary PAP (1). Its clinical course is variable and ranges from spontaneous resolution to death from infections or progressive respiratory failure (11).

The diagnosis includes clinical evaluation, respiratory function tests, HRCT, determination of anti-GM-CSF autoantibody levels, and genetic testing.

Regarding treatment, BAL is currently the gold standard, although the therapeutic approach will depend on the diagnosis and severity of the disease (12).

The detection of high levels of type IgG anti-GM-CSF antibodies by latex agglutination or ELISA techniques in peripheral blood and BAL (13) is currently accepted as a useful tool in the diagnosis of PAPi, with a sensitivity of 100% and a specificity of 98%. Levels above 5 U/mL are consistent with the diagnosis, even in asymptomatic phases. Its knowledge has also guided the development of new treatment strategies for PAPi, such as the administration of this cytokine exogenously, as an alternative or complementary therapy to BAL.

The applicability of anti-GM-CSF in monitoring and as a marker of treatment response is still under discussion. A possible correlation between anti-GM-CSF antibody titers and the extent of the disease has been sought, but the studies carried out have yielded contradictory results (14).

On the other hand, it has been seen that the inhalation of industrial dust, especially silica, titanium, and aluminum, induces the appearance of autoantibodies associated with rheumatoid arthritis, systemic lupus erythematosus, scleroderma, and glomerulonephritis. In the case of PAP, it could be considered that occupational inhalation of dust induced the appearance of anti-GM-CSF antibodies, but such an association is not clear, as there are Japanese studies of patients exposed to industrial dust who develop PAP but without the presence of anti-GM-CSF antibodies (15).

In conclusion, PAP is a rare disease that often poses diagnostic difficulties and, in many cases, requires confirmation through lung biopsy to obtain a definitive diagnosis. With the available scientific evidence, the determination of anti-GM-CSF antibodies has proven to be a useful tool in the diagnosis of PAPi, and its involvement in the long-term monitoring of anti-GM-CSF serum levels could clarify its usefulness as an early detector of recurrences and would allow for individualized values and cutoff points preceding the establishment of clinical symptoms, although studies need to be continued to corroborate this.

TAKE-HOME MESSAGES

- Importance of clinical laboratory in determining anti-GM-CSF as a confirmatory method for primary PAP.
- Different types of PAP lead to the accumulation of pulmonary surfactant and dysfunction of macrophages.

- PAP is a rare disease that can present with an asymptomatic clinical picture or progress to respiratory failure.
- Importance of using BAL as the treatment of choice in patients with PAP.

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Compliance with ethical standards

Conflict of interest: The authors have declared that no conflict of interest exists.

Author contributions: All authors were involved in the diagnosis and management of this case.

Funding: The authors have not declared a specific grant for this research from any funding agency or source in the public, commercial or not-for-profit sectors.

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REFERENCES

1. Trapnell BC, Whitsett JA, Nakata K. Pulmonary alveolar proteinosis. N Engl J Med. 2003;349(26):2527-2539. doi:10.1056/NEJMra023226

2. Carey B, Trapnell BC. The molecular basis of pulmonary alveolar proteinosis. Clin Immunol. 2010;135(2):223-235. doi:10.1016/j.clim.2010.02.017

3. Jouneau S, Kerjouan M, Briens E, et al. Pulmonary alveolar proteinosis. Rev Mal Respir. 2014;31(10):975-991. doi:10.1016/j.rmr.2014.08.009

4. McCarthy C, Avetisyan R, Carey BC, Chalk C, Trapnell BC. Prevalence and healthcare burden of pulmonary alveolar proteinosis. Orphanet J Rare Dis. 2018;13(1):129. doi:10.1186/s13023-018-0846-y

5. Ben-Dov I, Segel MJ. Autoimmune pulmonary alveolar proteinosis: clinical course and diagnostic criteria. Autoimmun Rev. 2014;13(4-5):513-517. doi:10.1016/j.autrev.2014.01.046

6. Suzuki T, Maranda B, Sakagami T, et al. Hereditary pulmonary alveolar proteinosis caused by recessive CSF2RB mutations. Eur Respir J. 2011;37(1):201-204. doi:10.1183/09031936.00090610

7. Nogee LM, Dunbar AE 3rd, Wert SE, Askin F, Hamvas A, Whitsett JA. A mutation in the surfactant protein C gene is associated with familial interstitial lung disease. N Engl J Med. 2001;344(8):573-579. doi:10.1056/NEJM200102223440805

8. Deutsch GH, Young LR, Deterding RR, et al. Diffuse lung disease in young children: application of a novel classification scheme. Am J Respir Crit Care Med. 2007;176(11):1120-1128. doi:10.1164/rccm.200703-393OC

9. Rosen SH, Castleman B, Liebow AA. Pulmonary alveolar proteinosis. N Engl J Med. 1958 Jun 5;258(23):1123-42. doi: 10.1056/NEJM195806052582301.

10. Salvaterra E, Campo I. Pulmonary alveolar proteinosis: from classification to therapy. Breathe (Sheff). 2020;16(2):200018. doi:10.1183/20734735.0018-2020

11. Riley L, Wang T, Ataya A. Pulmonary Alveolar Proteinosis (PAP). Am J Respir Crit Care Med. 2019;200(8):P16-P17. doi:10.1164/rccm.2008P16

12. Talamoni H, Pisapia N, Cortes L, Arellano M, Wainstein E, Svetliza G, et al. Therapeutic bilateral lung lavage in an adolescent with pulmonary alveolar proteinosis. Case report. Arch Argent Pediatr. 2020;118(3):e324-28. doi: 10.5546/aap.2020.e324.

13. Uchida K, Nakata K, Carey B, et al. Standardized serum GM-CSF autoantibody testing for the routine clinical diagnosis of autoimmune pulmonary alveolar proteinosis. J Immunol Methods. 2014;402(1-2):57-70. doi 10.1016/j.jim.2013.11.011

14. Nei T, Urano S, Itoh Y, et al. Light chain (κ/λ) ratio of GM-CSF autoantibodies is associated with disease severity in autoimmune pulmonary alveolar proteinosis. Clin Immunol. 2013;149(3):357-364. doi: 10.1016/j.clim.2013.10.002

15. Borie R, Danel C, Debray MP, et al. Pulmonary alveolar proteinosis. Eur Respir Rev. 2011;20(120):98-107. doi: 10.1183/09059180.00001311