Journal of Health Sciences

CASE REPORT Open Access

Pulmonary alveolar proteinosis with secondary *Aspergillus* infection: A case report

Fanny Fachrucha[1]*, Sita Andarini[1], Mia Elhidsi[1], Rizky Yudha Irawan[1], Romi Beginta[2], Dianiati Kusumo Sutoyo[1]

[1]Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Indonesia, Persahabatan National Respiratory Referral Hospital, Jakarta, Indonesia, [2]Department of Anatomic Pathology, Persahabatan National Respiratory Referral Hospital, Jakarta, Indonesia

ABSTRACT

Pulmonary alveolar proteinosis (PAP) is a rare disease with mostly due to autoimmune toward granulocyte-macropahge colony-stimulating factor. In some conditions, PAP followed with secondary infection. A 34-year-old woman came with progressive shortness of breath, chronic dry cough, and mild fever. The chest High-Resolution Computed Tomography showed ground-glass opacity with septal reticulation or known as the crazy-paving pattern, and a cavity on the upper lobe of the left lung. The patient underwent bronchoscopy for diagnostic and therapeutic measures and found milky appearance bronchoalveolar lavage fluid (BALF). The serum galactomannan came out positive. Fungal infection detected from the BALF culture, *Aspergillus fumigatus*, hence fulfilling the diagnosis of PAP with probable invasive pulmonary aspergillosis. The patient showed clinical improvement after undergoing whole lung lavage and given anti-fungal medications.

Keywords: Fungal infection; milky appearance bronchoalveolar lavage fluid; pulmonary alveolar proteinosis; whole lung lavage

INTRODUCTION

Pulmonary alveolar proteinosis (PAP) is a rare disease marked by deposits of surfactant components in the alveolus (1). More than 90% of PAP cases are autoimmune PAP with an increase of antibody towards granulocyte-macrophage colony-stimulating factor (GM-CSF), a cytokine affecting the final differentiation of alveolar macrophage and decreasing the function of circulating neutrophils, so besides causing surfactant deposits, it can also increase the risk of infection (1,2). Infection occurrence in PAP is 5-13% of the total number of cases and is the cause of PAP death in 18-20% of cases. One of the reported infections in PAP patients is *Aspergillus* fungal infection (1). Although we managed several cases of PAP patients in Indonesia, this is the first published report of PAP in Indonesia.

CASE REPORT

A 34-year-old woman came with history of progressive shortness of breath in the past 2 years. The symptoms were accompanied by chronic dry cough and mild fever on multiple occasions relieved by antipyretics. She did not have a history of smoking nor exposure to certain substances. She had been treated with antituberculosis agent for 6 months in 2017 and 8 months in 2018, both with acid-fast bacili negative, and inhaled bronchodilators, but her dyspnea was not improved. She had also been consuming 24 mg of methylprednisolone daily in three divided doses, for more than 12 weeks.

During the first visit to our hospital, she was dyspneic, respiratory rate 28 times/minute, oxygen saturation 88%, and inspiratory crackles during auscultation on both lungs. The Modified Medical Research Council (mMRC) score in this patient was 3. Chest X-ray showed symmetrical bilateral consolidation with peripheral, perihilar, and basal predomination (Figure 1).

The chest high-resolution computed tomography (HRCT) showed ground-glass opacity with septal reticulation or known as the crazy-paving pattern, as well as a cavity in the upper lobe of the left lung (Figure 2).

Results of rheumatoid factor, ANA, anti-dsDNA, and HIV serology examinations were negative. Lung function examination showed mild restriction (forced vital capacity [FVC] 49% predicted and severe decrease in diffusion capacity (lung diffusing capacity value for carbon monoxide [DLCO] 28% predicted). She underwent bronchoscopy for diagnostic and therapeutic measures. Transbronchial lung biopsy was not performed during the procedure due to

*Corresponding author: Fanny Fachrucha, Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Indonesia, Persahabatan National Respiratory Referral Hospital, Jakarta, Indonesia. E-mail: fanny.fachrucha@gmail.com

Submitted: 09 June 2021/Accepted: 26 October 2021

DOI: https://doi.org/10.17532/jhsci.2021.1366

UNIVERSITY OF SARAJEVO
FACULTY OF HEALTH STUDIES

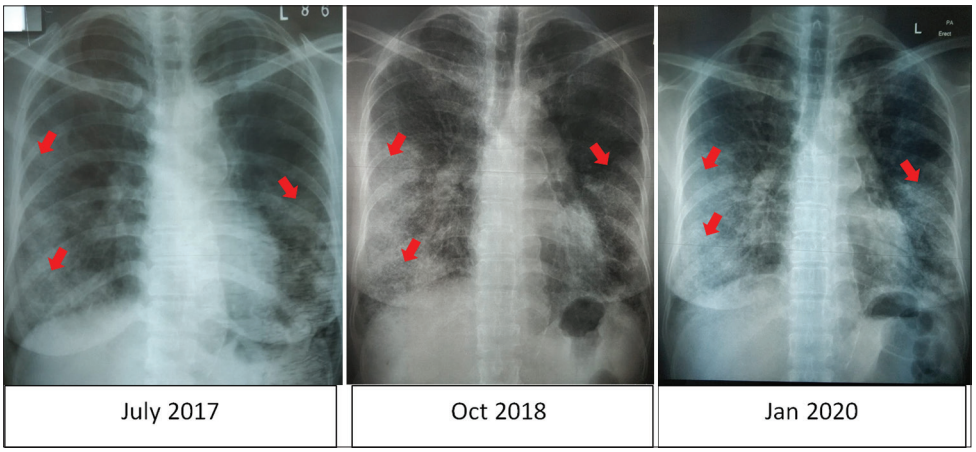

FIGURE 1. The serial chest X-ray showed bilateral symmetrical consolidation (red arrows) with predominance in the peripheral, perihilar, and basal area.

oxygen desaturation. Bronchoalveolar lavage fluid revealed milky appearance (Figures 3 and 4).

The result for BALF cellular analysis showed dirty and a paucicellular pattern (<400 cells), with neutrophilic cellular pattern. BALF sample was sent for fungal culture. Her serum galactomannan was positive. The patient was then diagnosed with PAP and underwent whole lung lavage. Confirmation of PAP ethiology could not be done because the required tests such as serum GM-CSF autoantibody, GM-CSF concentration, and other tests are not available in Indonesia. She was discharged after the procedure. Due to COVID-19 situation and our hospital designation to COVID-19 hospital, her condition was monitored using teleconsultation.

Few weeks after the procedure, the fungal culture showed *Aspergillus fumigatus* and sensitive to itraconazole. Her subjective symptoms at that time were slightly relieved. Based on the current immunocompromised status of the patient (taking high dose of corticosteroid for a long time), a cavity in the chest HRCT, positive serum galactomannan, and positive *Aspergillus* culture from BALF, she was subsequently diagnosed with PAP with probable invasive pulmonary aspergillosis and treated with anti-fungal itraconazole of 150 mg once daily, for 3 months. Her symptoms and signs showed further improvements each month. There were improvements in the mMRC score from 3 to 2 and her respiratory rate. The peripheral oxygen saturation at the resting time increased to 92-94%. However, there was no significant change in chest X-ray after 3 months of therapy. Inhaled GM-CSF was not given yet, since the hospital closed to non-COVID-19 due to COVID-19 pandemic. Limited facilities for diagnosis and management of PAP are the obstacles for this patient. The patient gave written consent for publication of this case report.

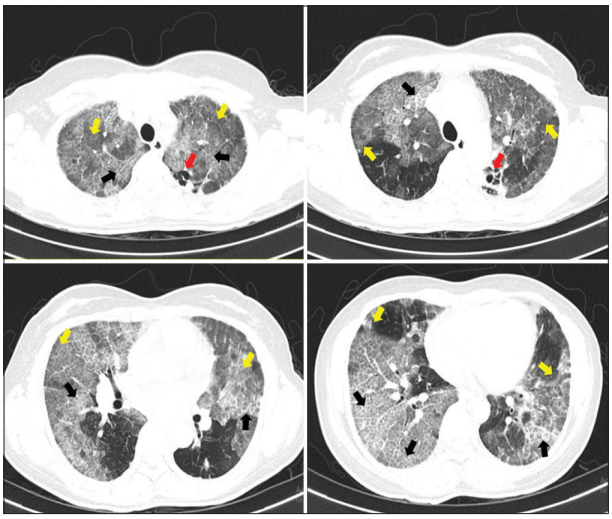

FIGURE 2. The chest high-resolution computed tomography (January 2020) showed ground-glass opacity (yellow arrows) with reticulation changes (black arrows), a combination known as crazy paving pattern, and cavities on the upper lobe of the left lung (red arrow).

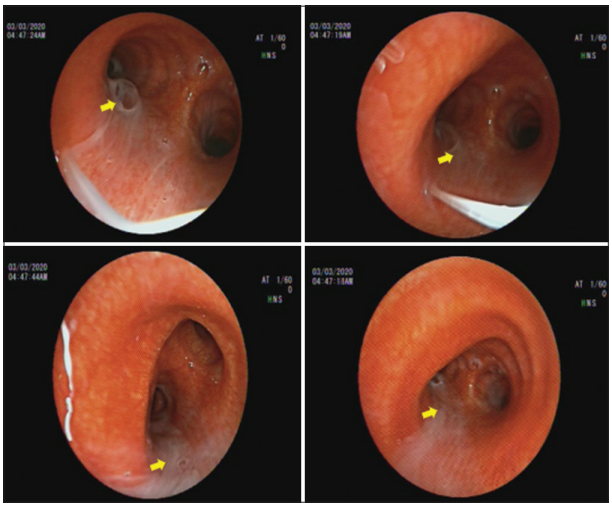

FIGURE 3. Bronchus branches and bronchoalveolar lavage fluid with milky appearance (yellow arrows).

DISCUSSION

PAP is a rare disease. The prevalence ranged from 4 to 40 cases in every 1 million population with an incidence of 0.2-0.42 cases for every 1 million population every year (1,2). In general, PAP is a condition with deposition of surfactant, a lipoprotein, in the alveolar space and can be accompanied by alveolar macrophage dysfunction (1-3). In patients with PAP, the gas exchange process is disrupted and causes various respiratory issues with the increased risk of secondary infection (1,2).

PAP can be classified into three types based on its pathophysiology as primary, secondary, and congenital. In primary PAP, there is a disturbance of GM-CSF signaling, causing a reduction in surfactant clearance. GM-CSF is a

cytokine with an important role in surfactant homeostasis and is secreted by many cells, including the type II alveolar epithelial, and can be classified further as autoimmune (increase in anti-GM-CSF antibody) or hereditary (CSF2RA or CSF2RB gene mutation) (1,2). More than 90% of PAP cases are autoimmune (1). In secondary PAP, there is a decrease in the number and/or function of the alveolar macrophages due to certain health conditions or substance exposures, such as myelodysplastic syndrome, myeloid leukemia (acute and chronic), cigarette smoke exposure, and inhalation of silica or other mineral and metal particles (1,2,4). PAP can also be caused by the abnormality of surfactant production as in congenital PAP (4). In our patient, it is hard to find the etiology of PAP due to limitations in supporting examinations needed such as anti-GM-CSF antibody, GM-CSF level, or genetic testing.

FIGURE 4. Left: Normal saline, right: Bronchoalveolar lavage fluid with opaque and milky appearance.

Prognosis of PAP varied as the disease progression is unpredictable. PAP could progress into one of the following patterns: Progressive deterioration, stable condition, or spontaneous resolution (in 5-25% cases) (1,2). Prognosis in autoimmune and hereditary PAP (both belong to primary PAP, caused by GM-CSF signaling deficiency) tends to be better compared to secondary and congenital PAP (2). The poor prognosis of patients with secondary PAP is probably related to the underlying disease (2,5). In cases reported by Zhang *et al*., patients with PAP secondary to tuberculosis (TB) had better prognosis than those with PAP secondary to myelodysplastic syndrome. Patients with TB had improvements after antituberculosis treatments (5). Based on a large comprehensive meta-analysis in all types of PAP patients, the 2-year survival rate is 78%, the 5-year survival rate is 75%, and the 10-year survival rate is 68% (2). However, since the implementation of whole lung lavage therapy, the 5-year survival rate in autoimmune PAP increased to almost 95% (4). The main cause of death (72%) is from respiratory failure in disease deterioration and 18% from uncontrollable infection (1,2).

Diagnosis of PAP syndrome could be confirmed by performing bronchoscopy and obtaining BALF on patients with suspected clinical history and high-resolution CT findings. Opaque and milky gross appearance of BALF is characteristic of PAP and could confirm the diagnosis when anti-GM-CSF autoantibody test is not readily available, mainly in resource-limited setting (6,7) Microscopic and

TABLE 1. Diagnostic criteria for pulmonary alveolar proteinosis (1,2,4,6,8).

Main diagnostic criteria	
Clinical history	Slowly progressive dyspnea with or without cough and fatigue
Chest high-resolution CT	Ground-glass opacities with intralobular lines and interlobular septal thickening (crazy paving pattern)
BAL fluid (gross appearance)	Opaque and milky gross appearance, usually containing large amounts of sediment
BAL fluid (microscopic)	Granular, acellular, lipoproteinaceous globules after May-Grunwald-Giemsa and PAS staining, and foamy alveolar macrophages and red after oil-red-O staining. Lamellar bodies on electron microscopy
Transbronchial biopsy (no longer necessary, can be considered if the BALF is not characteristic)	Macroscopically: geographic pattern of 2–3-cm grayish-yellow regions of firm consolidation with fatty material exudates Microscopically: Alveoli and terminal airways filled with a fine eosinophilic material that stains for surfactant proteins, with occasional lymphocyte infiltration and fibrosis
Etiological confirmation criteria	
Serum GM-CSF autoantibody (autoimmune PAP)	≥5 μg/mL: Autoimmune PAP<5 μg/mL: Other etiologies
Serum GM-CSF concentration and/or GM-CSF signaling (hereditary PAP)	>10 pg/mL: Hereditary PAP<7 pg/mL: Other etiologies
Genetic testing (congenital PAP)	Mutations in genes required for surfactant production (SFTPB, SFTPC, ABCA3, or NKX2-1)
Medical history and genetic testing (secondary PAP)	Disease or condition known to cause secondary PAP (chronic myeloid leukemia, myelodysplastic syndromes, and other malignant and non-malignant hematological diseases, non-hematological malignancies, immune deficiency syndromes, chronic inflammatory syndromes, and chronic infections) Mutations in genes associated with the development of secondary PAP (SLLC7A7 and MARS) might be found
Other nonspecific characteristics	
Chest X-ray	Diffuse bilateral symmetrical infiltrates in a perihilar and basal distribution, without signs of heart failure
Pulmonary function tests	Decreased forced vital capacity, reduction of DLCO

CT: Computed tomography, BAL: Bronchoalveolar lavage, PAS: Periodic acid-Schiff, GM-CSF: Granulocyte-macrophage colony-stimulating factor, PAP: Pulmonary alveolar proteinosis, DLCO: Diffusion capacity of the lung for carbon monoxide

histopathological examination of BALF and transbronchial biopsy could be considered when gross appearance of BAL is not characteristic (6). The microscopic BALF examination will reveal Periodic acid-Schiff (PAS)-positive granular, acellular, lipoprotein materials, while the biopsy will show small airways filled with eosinophilic material microscopically (8). However, etiological confirmation of PAP can only be done with specific tests such as anti-GM-CSF autoantibody, serum GM-CSF, genetic testing, and others (6). Summary of the diagnosis criteria is shown in Table 1.

Secondary infection is the most common complication occurring in autoimmune PAP and is life-threatening (2). Around 5-13% of PAP patients have a secondary infection. Infections can be caused by general pathogens (*Streptococcus*, *Haemophilus*, and *Enterobacter*) and opportunistic pathogens (*Mycobacterium*, *Nocardia*, *Actinomyces*, *Aspergillus*, and *Cryptococcus*) (1). From the many pathogens, *Aspergillus*, *Mycobacterium*, and *Nocardia* are the most common infection source in autoimmune PAP (9). From Japanese national PAP registry, most of the infections in autoimmune PAP are caused by *Aspergillus* spp. (2). While the immunodeficiency from autoimmune PAP can cause *Aspergillus* infection, aspergillosis could potentially become a risk factor for autoimmune PAP (9).

Invasive pulmonary aspergillosis, a type of pulmonary *Aspergillus* infection, can be classified into "proven," "probable," or "possible" based on some criteria adapted from the revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) (10,11). (Table 2)

In our case, the corticosteroid consumed (24 mg of methylprednisolone daily) is equivalent to 30 mg of prednisone daily (equals to 0.67 mg/kgBW/day with body weight of 45 kg) for more than 12 weeks (12). Combined with the presence of cavity in chest HRCT and positive culture and serum galactomannan, this patient meets the criteria for probable invasive pulmonary aspergillosis, and treated with oral itraconazole 150 mg once daily, for 3 months.

Choice of therapy for PAP depended on its etiology and severity of symptoms. For primary and secondary PAP patients (not including congenital PAP) with severe clinical manifestations, one of the primary therapies of choice is whole lung lavage (2). Whole lung lavage aims to remove the accumulation of surfactant components and has become the gold standard for autoimmune PAP therapy (1,2). There is no consensus or guideline correlated with whole lung lavage indications and procedures (1). Whole lung lavage can be considered in patients with deterioration of symptoms, desaturation, or reduced DLCO, aiming to improve oxygenation, relieve symptoms, and improve quality of life (2). Whole lung lavage is a safe procedure with minimal complications if performed by a trained operator, and with proper facilities (1). According to reports, whole lung lavage improves symptoms, increases activity tolerance, increases PaO2 and A-aDO2, improves lung physiological parameters, and radiological findings, although not very effective for rarer type of PAP (1,2). Usually, we only need one to two procedures (4). Moreover, patients with PAP and aspergillosis should

TABLE 2. Diagnostic criteria for invasive pulmonary aspergillosis (10,11).

Diagnosis	Criteria
Proven	Meet one of the three criteria below: Microscopic analysis of sterile material: histopathologic, cytopathologic, or direct microscopic examination of a lung specimen obtained by needle aspiration or biopsy showing hyphae with associated tissue damage Culture of sterile material: Positive *Aspergillus* culture from specimen obtained by a sterile procedure from a normally sterile and clinically or radiologically abnormal site consistent with an infectious disease (excluding BAL fluid, cranial sinus cavity specimen, and urine) Tissue nucleic acid diagnosis: amplification of *Aspergillus* DNA by PCR combined with DNA sequencing
Probable	Meet at least one host factor, one clinical feature, and one mycological evidence criteria: Host factors: 1. Recent history of neutropenia (<0.5 × 10^9 cells/L or < 500 cells/mm3 for > 10 days) temporally related to the onset of invasive fungal disease 2. Active or recent hematologic malignancy, excluding aplastic anemia 3. Receipt of an allogeneic stem cell transplant 4. Receipt of a solid organ transplant 5. Prolonged use of corticosteroids at a dose of ≥ 0.3 mg/kg of prednisone equivalent for ≥ 3 weeks in the past 60 days (excluding patients with allergic bronchopulmonary aspergillosis) 6. Treatment with recognized T-cell immunosuppressants (such as calcineurin inhibitors, tumor necrosis factor-a blockers, lymphocyte-specific monoclonal antibodies, immunosuppressive nucleoside analogues) or B-cell immunosuppressants (such as Bruton's tyrosine kinase inhibitors) in the past 90 days 7. Inherited severe immunodeficiency (such as chronic granulomatous disease, STAT 3 deficiency, or severe combined immunodeficiency) 8. Acute graft-versus-host disease Grade III or IV involving the gut, lungs, or liver that is refractory to first-line treatment with steroids 9. Chemotherapy 10. Advanced AIDS Clinical features: The presence of one of the four following patterns on CT: 1. Dense, well-circumscribed lesion (s) with or without a halo sign 2. Air crescent sign 3. Cavity 4. Wedge-shaped and segmental or lobar consolidation Mycological evidence: 1. *Aspergillus* species recovered by culture from sputum, BAL, bronchial brush, or aspirate 2. Microscopical detection of fungal elements in sputum, BAL, bronchial brush, or aspirate indicating a mold 3. Galactomannan antigen detected in plasma, serum, BAL, or CSF 4. Two positive *Aspergillus* PCR tests on plasma, serum, whole blood, or BAL fluid
Possible	Meet at least one host factor and one clinical feature, without any mycological evidence criteria, as in probable

BAL: Bronchoalveolar lavage, PCR: Polymerase chain reaction, AIDS: Acquired immunodeficiency syndrome, CT: Computed tomography, CSF: Cerebrospinal fluid

be treated with antifungal therapy. According to some reports, PAP patients experience spontaneous resolution after aspergillosis treatment (9,13).

Aside from whole lung lavage, other promising autoimmune PAP therapies are inhaled GM-CSF supplementation, plasmapheresis (to eliminate antibody), and rituximab (anti-cell B monoclonal antibody to reduce antibody secretion). Further studies about these are still needed (2).

CONCLUSIONS

Here, we reported a first case of PAP with probable pulmonary aspergillosis in Indonesia. Delay in diagnosis and therapy of interstitial lung diseases, especially PAP, is common in Indonesia. In this case, she was misdiagnosed as lung tuberculosis and obstructive lung diseases. Limited diagnostic modalities such as HRCT examination, serum anti-GM-CSF antibody level, and serum GM-CSF in Indonesia also hampered the determination of PAP etiology and proper management. In our case, PAP was diagnosed from characteristic gross appearance of BALF with suspected clinical presentation and HRCT. Whole lung lavage and itraconazole could effectively reduce shortness of breath, improve oxygenation, and improve radiological findings in PAP patients with secondary infection. A clinical guideline standard for PAP and whole lung lavage technique is also needed to maximize success.

DECLARATION OF INTERESTS

Nothing to declare.

REFERENCES

1. Jouneau S, Menard C, Lederlin M. Pulmonary alveolar proteinosis. Respirology 2020;25(8):816-26.
http://doi.org/10.1111/resp.13831
2. Trapnell BC, Nakata K, Bonella F, Campo I, Griese M, Hamilton J, et al. Pulmonary alveolar proteinosis. Nat Rev 2019;5(1):16.
http://doi.org/10.1038/s41572-019-0066-3
3. Trapnell BC, Whitsett JA, Nakata K. Pulmonary alveolar proteinosis. N Engl J Med 2003;349(26):2527-39.
http://doi.org/10.1056/NEJMra023226
4. Borie R, Danel C, Debray MP, Taille C, Dombret MC, Aubier M, et al. Pulmonary alveolar proteinosis. Eur Respir Rev 2011;20(120):98-107.
http://doi.org/10.1183/09059180.00001311
5. Zhang D, Tian X, Feng R, Guo X, Wang P, Situ Y, et al. Secondary pulmonary alveolar proteinosis: A single-center retrospective study (a case series and literature review). BMC Pulm Med 2018;18(1):15.
http://doi.org/10.1186/s12890-018-0590-z
6. Kumar A, Abdelmalak B, Inoue Y, Culver DA. Pulmonary alveolar proteinosis in adults: Pathophysiology and clinical approach. Lancet Respir Med 2018;6(7):554-65.
http://doi.org/10.1016/S2213-2600(18)30043-2
7. Kumar A, Abdelmalak B, Inoue Y, Culver D. Blood testing in the diagnosis of pulmonary alveolar proteinosis authors' reply. Lancet Respir Med 2018;6(11):e55.
http://doi.org/10.1016/S2213-2600(18)30373-4
8. Trapnell BC, Luisetti M. Pulmonary alveolar proteinosis syndrome. In: Murray J, Nadel J, Broaddus V, Mason R, Ernst J, King T, editors. Murray and Nadel's Textbook of Respiratory Medicine. Philadelphia, PA: Elsevier Saunders; 2016.
9. Arai T, Inoue Y, Akira M, Nakata K, Kitaichi M. Autoimmune pulmonary alveolar proteinosis following pulmonary aspergillosis. Intern Med 2015;54(24):3177-80.
http://doi.org/10.2169/internalmedicine.54.5034
10. Donnelly J, Chen S, Kauffman C, Steinbach W, Baddley J, Verweij P, et al. Revision and update of the consensus definitions of invasive fungal disease from the European organization for research and treatment of cancer and the mycoses study group education and research consortium. Clin Infect Dis 2019;71(6):1367-76.
http://doi.org/10.1093/cid/ciz1008
11. Kousha M, Tadi R, Soubani A. Pulmonary aspergillosis: A clinical review. Eur Respir Rev 2011;20(121):156-74.
http://doi.org/10.1183/09059180.00001011
12. Liu D, Ahmet A, Ward L, Krishnamoorthy P, Mandelcorn E, Leigh R, et al. A practical guide to the monitoring and management of the complications of systemic corticosteroid therapy. Allergy Asthma Clin Immunol 2013;9(1):30.
http://doi.org/10.1186/1710-1492-9-30
13. Lin KP, Sheng WH, Wang CP, Chang WL, Chang SC. Resolution of secondary pulmonary alveolar proteinosis following treatment of rhinocerebral aspergillosis. Int J Infect Dis 2010;14(Suppl 3):e246-9.
http://doi.org/10.1016/j.ijid.2009.10.004