Medizinische Fakultät

der

Universität Duisburg-Essen

Aus der Abteilung Pneumologie/Allergologie

der Ruhrlandklinik Essen-Heidhausen

WASH OUT KINETICS AND EFFICACY OF A MODIFIED LAVAGE TECHNIQUE FOR PULMONARY ALVEOLAR PROTEINOSIS

Inaugural-Dissertation

zur

Erlangung des Doktorgrades der Medizin

durch die Medizinische Fakultät

der Universität Duisburg-Essen

Vorgelegt von

Francesco Tommaso Bonella

aus Trento, Italien

2014

Dekan:	Herr Univ.-Prof. Dr. med. J. Buer
1.Gutachter:	Herr Prof. Dr. med. U. Costabel
2.Gutachter:	Herr Univ.-Prof. Dr. med. K.W. Schmid

Tag der mündlichen Prüfung: 10. Dezember 2014

Publications related to the thesis

Original research articles

1. Bonella, F., Bauer, P.C., Griese, M., Ohshimo, S., Guzman, J., Costabel, U. (2011): Pulmonary alveolar proteinosis: new insights from a single-center cohort of 70 patients. Respir Med. 105,1908-1916.
2. Bonella, F., Bauer, P.C., Griese, M., Wessendorf, T.E., Guzman, J., Costabel U. (2012): Wash-out kinetics and efficacy of a modified lavage technique for alveolar proteinosis. Eur Respir J. 40,1468-1474.

Chapter in a textbook

1. Bonella, F., Theegarten, D., Guzman, J., Costabel, U. (2011): Alveolar Lipoproteinosis Syndromes. In Cordier, J.F. (Ed): Orphan Lung Diseases. Ed. European Respiratory Society Monograph. Vol. 54: S.171-186.

Abstracts

1. Bonella, F., Ohshimo, S., Bauer, P.C., Griese, M., Guzman, J., Costabel, U. (2008): Alveolarproteinose – Update in Germany. Pneumologie 62, 58s.
2. Bauer, P.C., Bonella, F., Bonzel, P., Costabel, U. (2008) Eine modifizierte Technik der therapeutischen Lavage bei Alveolarproteinose. (2008): Pneumologie 62, 58s.
3. Bonella, F., Ohshimo, S., Bauer, P.C., Griese, M., Guzman, J., Costabel, U. (2008): Pulmonary alveolar proteinosis: single centre experience with 55 patients. Am. J. Respir. Crit. Care Med. 177, A879.

4. Bauer, P., Bonella, F., Bonzel, P., Costabel, U. (2008): Whole-lung lavage by a modified technique in pulmonary alveolar proteinosis. Am J Respir Crit Care Med 177, A879.
5. Bonella, F., Cai, M., Ohshimo, S., Bauer, P., Guzman, J., Costabel, U. (2009): Determinants of protein concentration in whole lung lavage of patients with pulmonary alveolar proteinosis. Eur Respir J 34 Suppl. 53, 827s.
6. Bonella, F., Bauer, P.C., Costabel, U. (2010): Die therapeutische Lavage bei Alveolarproteinose: Einfluss der Technik auf die Proteinenkonzentration der Spülflüssigkeit. Pneumologie 64, 172s.

CONTENTS:

1. INTRODUCTION

1.1. Definition of Pulmonary alveolar proteinosis

Pulmonary alveolar proteinosis (PAP), first described in 1958 by Rosen and Castelman (Rosen et al., 1958), is a rare diffuse parenchymal lung disease characterised by abundant accumulation of surfactant-derived phospholipids and protein components within the alveoli and the distal airways. This leads to a progressive impairment of gas exchange and respiratory insufficiency. PAP has been reported in the medical literature under various terms: alveolar proteinosis, alveolar lipoproteinosis, alveolar phospholipidosis, pulmonary alveolar lipoproteinosis, and pulmonary alveolar phospholipoproteinosis. PAP is best viewed as a syndrome composed of a heterogeneous group of disorders (Huizar and Kavuru, 2009).

1.2. Epidemiology

The true prevalence of PAP is unknown, with current understanding based on about 900 reported cases (Inoue et al., 2008; Seymour and Presneill, 2002; Xu et al., 2009). Through a national registry in Japan the prevalence of human alveolar proteinosis has been estimated to be 6.2 per 1.000.000. The median age at onset is 51 years in the Japanese cohort (Inoue et al., 2008), 10 years older than previously reported (Seymour and Presneill, 2002; Xu et al., 2009). A few cases have been reported in infants and children. The reported male to female

ratio varies from 2:1 (Inoue et al., 2008; Xu et al., 2009) to 3:1 (Seymour and Presneill, 2002), and smokers are predominantly affected (reported rate: 56-80%) (Inoue et al., 2008; Seymour and Presneill, 2002). Secondary PAP is much rarer than the primary PAP, which comprises 90 % of all reported cases (Carey and Trapnell, 2010; Huizar and Kavuru, 2009; Ishii et al., 2011).

1.3. Pathology

PAP is a prototypical example of an alveolar filling process. Macroscopically the lung shows yellow-tan color and filled alveolar spaces (Travis et al., 2002.). On histopathology, the alveolar spaces are filled with a characteristic eosinophilic acellular, finely granular material that stains with periodic acid-Schiff (PAS) stain and is diastase-negative (Costabel et al., 2007; Travis et al., 2002.). Usually the lung is diffusely affected, but in some cases a patchy involvement is found. Typically there is little inflammation or interstitial fibrosis. Hyperplastic or detached Type II pneumocytes, foamy macrophages, cholesterol clefts and ghost cells can be found (Rosen et al., 1958; Travis et al., 2002.). The alveolar filling material stains with antibodies to surfactant apoprotein.

On electron microscopy, the abnormal material consists predominantly of unusual tubular, myelin-like, multilamellated structures, which are similar to the tubular myelin found in normal lungs but without the intersecting membranes of normal tubular myelin. Structures that relate to cell debris are also present. Lamellar bodies of normal lungs are only minor components (Carey and Trapnell,

2010; Costabel and Guzman, 2005; Seymour and Presneill, 2002). Biochemical analysis of the material, mainly obtained from bronchoalveolar lavage (BAL) fluid, demonstrated that total phospholipids are increased, with a relative decrease in phosphatidylcholine and phosphatidylglycerol, and a relative increase in sphingomyelin and phosphatidylinositol. The proteins are mainly of molecular weights between 30,000 and 62,000, typical of glycoproteins and Immunoglobulins (IgA and IgG) (Onodera et al., 1983). Surfactant proteins (SP) A, B and D are increased. The relative abundance of SP- A isoforms varies markedly from patient to patient and is different from normals (Carey and Trapnell, 2010; Honda et al., 1995; Seymour and Presneill, 2002).

1.4 Classification and pathogenesis

Recently a new PAP classification has emerged based on the important progress in our understanding of the pathogenesis (**table 1**).

Primary PAP disorders are caused by impaired Granulocyte macrophage colony-stimulating factor (GM-CSF) signaling. GM-CSF plays a critical role in the regulation of surfactant homeostasis, alveolar macrophage maturation and function, lung host defense, and innate immunity (Carey and Trapnell, 2010).

Autoimmune PAP is characterized by the loss of GM-CSF signaling due to the presence of neutralizing anti GM-CSF antibodies (Carey and Trapnell, 2010; Costabel and Guzman, 2005; Kitamura et al., 1999). GM-CSF is essential for normal surfactant turnover by activating the alveolar macrophages and increasing

Table 1 PAP classification according to the pathogenesis

Clinical type	Pathogenesis
Primary PAP	**Impaired GM-CSF signaling**:
Autoimmune	GM-CSF autoantibody
Hereditary	GM-CSF receptor α/β chain mutations
Secondary PAP	**Reduction in number and function of alveolar macrophages**
Inhalation exposure	Inorganic dust: Aluminum, Cement, Silica, Titanium, Indium, Tin
	Organic dust: Sawdust, Fertilizer/agricultural dust, Bakery flour
	Fumes: Synthetic plastic, Gasoline
	Others: Varnish, Chlorine, Petroleum, Cleaning products
Infections	Cytomegalovirus *Mycobacterium tuberculosis* Nocardiosis *Pneumocystis jiroveci* HIV
Hematologic disorders	Myelodysplastic syndrome Acute lymphatic leukemia Acute myeloid leukemia Chronic myeloid leukemia Hairy cell leukemia Hodgkin's disease Non-Hodgkin's lymphoma Multiple myeloma Essential thrombocythemia Polycythemia vera Amyloidosis Fanconi's anemia
Other malignancies	Adenocarcinoma Glioblastoma Melanoma
Immunologic diseases	Monoclonal gammopathy Selective IgA deficiency Severe combined immunodeficiency
Miscellaneous	Membranous nephrophaty Dermatomyositis Lung transplantation Lysinuric protein intolerance
PAP-like diseases	**Impaired surfactant production**
SP-B and SP-C mutations	SP-B and SP-C deficiency
ABCA3 mutations	Abnormal surfactant

the alveolar macrophages and increasing their rate of surfactant clearance (Trapnell and Whitsett, 2002). In-vivo and in-vitro data showed that GM-CSF binding to specific receptors on alveolar macrophages stimulates the terminal differentiation of the macrophages through the nuclear transcription factor PU.1 (Bonfield et al., 2003). This GM-CSF signaling is the critical process for the catabolism of surfactant by alveolar macrophages. It is likely that the anti-GM-CSF antibody is pathogenic in the development of the disease through its ability to inhibit the activity of endogenous GM-CSF, leading to a state of functional GM-CSF deficiency (Trapnell and Whitsett, 2002). The summary of current evidence suggests that adult idiopathic PAP is an autoimmune disease caused by decreased availability of functional GM-CSF due to CM-CSF blocking activity of a neutralizing autoantibody (Carey and Trapnell, 2010; Costabel and Guzman, 2005; Kitamura et al., 1999).

Hereditary PAP occurs in children and is caused by mutations in genes encoding for the GM-CSF receptor (Carey and Trapnell, 2010; Suzuki et al., 2010). The GM-CSF receptor β chain plays a critical role in surfactant homeostasis in humans (Carey and Trapnell, 2010). Hereditary PAP associated with absence of GM-CSF receptor β chains on blood leukocytes was reported in infants presenting with respiratory failure (Dirksen et al., 1997). A point mutation within CSF2RB encoding the GM-CSF receptor β chain has been sporadically detected in children with PAP (Suzuki et al., 2011). Hereditary PAP caused by

abnormalities or absence of the GMCSF receptor α chain has also been reported (Suzuki et al., 2008; Suzuki et al., 2010).

Secondary PAP may develop in association with inhalation of dusts and fumes, with infections such as nocardiosis, histoplasmosis, mycobacteriosis and pneumocystosis, with malignancies, particularly lymphoma and leukemia, and finally in association with immunodeficiency (table 1).

The pathogenesis of secondary PAP is poorly understood. The associated diseases presumably cause the syndrome by reducing either the number or certain functions of alveolar macrophages, thereby impairing alveolar-macrophage mediated surfactant clearance. Another pathogenetic hypothesis is based on an acquired loss of GM-CSF signaling. In children with acute myeloid leukemia and PAP, the loss of GM-CSF stimulation of alveolar macrophage-mediated surfactant clearance was due to defective expression of the GM-CSF receptor (Dirksen et al., 1998).

Hematological disorders constitute 90% of all secondary PAP causes (Ishii et al., 2011). Among these, myelodysplastic syndrome (MDS) is the most frequent accounting for 65% of secondary PAP (Ishii et al., 2011). In one patient with acute lymphoid leukemia, PAP occurred after marked decrease of myeloid cell numbers during the neutropenic stage of consolidation chemotherapy (Pamuk et al., 2003). This suggests that the reduction of the number of alveolar macrophages affects the capacity of removing surfactant from the lungs. In support of this, a recent

study reported that depletion of alveolar macrophage numbers increased pulmonary surfactant levels in rats (Forbes et al., 2007). PAP also develops in mice with severe combined immunodeficiency (Jennings et al., 1995).

With respect to dust and fume exposure, PAP developed in rats exposed to inhaled silica although the mechanism was not determined (Heppleston et al., 1970). Patients with secondary PAP have been considered autoantibody-negative, primarily based on studies of the large cohort of Japanese patients (Inoue et al., 2008). However, the secondary cases in the Japanese cohort which were all tested negative for the autoantibody have been limited to those with hematologic or autoimmune comorbidity. A recent report by Cummings et al (Cummings et al., 2010) about the occurrence of autoimmune alveolar proteinosis in indium workers supports the hypothesis that an inhaled agent may be the trigger for the development of autoimmune PAP. The mechanism by which dust exposure may induce GM-CSF antibody formation needs further investigation. Therefore it is essential to obtain a detailed occupational and environmental history in every patient newly diagnosed with PAP.

With regard to other forms of secondary PAP, lysinuric protein intolerance is a very rare disease caused by mutations in the SLC7A7 gene, mainly occurring in Finnish children (Torrents et al., 1999). PAP and interstitial lung disease represent the major cause of an unfavorable clinical course and fatal outcome (Ceruti et al., 2007).

The prognosis of secondary PAP is worse than that of autoimmune PAP (Huizar and Kavuru, 2009; Ishii et al., 2011). Ishii et al observed a median survival time of only 20 months (Ishii et al., 2011). In our cohort of 70 patients we registered a death rate of 50 % in patients with secondary PAP most of them secondary to hematological disorders (Bonella et al., 2011).

PAP-like conditions are due to impaired surfactant production. Such disorders include recessive mutations in the genes encoding for SP-B (Griese et al., 2005; Nogee et al., 1994; Tredano et al., 1999), SP-C (Griese et al., 2005; Stevens et al., 2005; Tredano et al., 2004) or ABCA3 (Saugstad et al., 2007; Weichert et al., 2011).

SP-B deficiency caused by SP-B mutations presents as unexplained acute respiratory failure in full-term neonates. Since SP-B is required for processing of pro-SP-C, mature SP-C is also reduced, thus impairing alveolar surface tension (Carey and Trapnell, 2010). This condition is incompatible with life.

Spontaneous and hereditary mutations in the gene encoding for SP-C result in a poorly defined interstitial lung disease in children and adults that can cause respiratory failure and death (Brasch et al., 2004; Griese et al., 2005; Stevens et al., 2005; Tredano et al., 2004). This disease results in gross distortion of lung structure due to widening of alveolar walls and extensive fibrosis (Carey and Trapnell, 2010).

ABCA3 is an integral membrane lipid transporter located on the limiting membrane of lamellar vesicles in alveolar type 2 cells. Alterations in the gene encoding ABCA3 result in various clinical presentations ranging from respiratory failure and death in neonates to interstitial lung disease in adolescents (Carey and Trapnell, 2010; Saugstad et al., 2007; Weichert et al., 2011).

1.5. Clinical presentation

The majority of patients (70-90%) suffers from slowly increasing dyspnea on exertion and cough (Inoue et al., 2008; Prakash UB, 1987; Shah et al., 2000). Less frequently (30-50%) fever, weight loss, fatigue and chest pain are seen.

The physical examination is typically unremarkable but may reveal inspiratory crackles and clubbing (15-20%). Cyanosis or evidence of cor pulmonale is rare (<5%) (Hazouard E, 2000; Inoue et al., 2008; Prakash UB, 1987; Seymour and Presneill, 2002; Shah et al., 2000).

Recently a Disease Severity Score, based on the presence of symptoms and degree of reduction in PaO2, has been proposed to stratify the patients, from least severe (DSS-1) to most severe (DSS-5) (Inoue et al., 2006; Inoue et al., 2008). Its utility needs to be further investigated.

1.6. Diagnosis and differential diagnosis

The diagnosis can usually be established by BAL (Costabel et al., 2007; Inoue et al., 2008; Seymour and Presneill, 2002). In our centre which has a large

experience with BAL, the diagnosis was made by BAL in 74 % of our cohort of 70 patients with acquired PAP (Bonella et al., 2011). On gross examination, the BAL fluid has a characteristic milky appearance. On light microscopy, the striking features are: acellular globules (basophilic on May-Grünwald-Giemsa and positive with PAS staining), few and foamy macrophages, and large amounts of cell debris showing weak PAS staining.

Electron microscopy is not usually required to establish the diagnosis but if performed the BAL sediment shows characteristic myelin-like multilamellated structures, debris and foamy macrophages.

Whit regards to the radiology, chest radiograph is not pathognomonic. Typical are diffuse bilateral symmetrical alveolar infiltrates with air bronchograms. The shadowing may be cloudy and butterfly -or batwing- like, as a result of the more prominent involvement of the perihilar regions. Less commonly, unilateral infiltrates or a reticulonodular pattern may be seen. Lymphadenopathy and pleural lesions are rare (Goldstein et al., 1998; Lee et al., 1997).

The HRCT shows airspace filling in variable and patchy distribution. The distinctive features are ground-glass opacities (GGO) sharply demarcated from normal lung, creating a 'geographical' pattern, GGO with intralobular lines and interlobular septal thickening, often in polygonal shapes, called 'crazy paving', and areas of consolidation with air bronchograms, surrounded by GGO. Ishii et al recently compared HRCT scan findings between autoimmune PAP and secondary PAP (Ishii et al., 2009). Although the major HRCT scan finding was GGO in both

in patients with autoimmune and secondary PAP, the appearance of the GGO was distinctive: a patchy geographic pattern of crazy paving with lower lung field predominance was typical for autoimmune PAP (71%), whereas a diffuse pattern with even distribution was more common in secondary PAP (62%). Some cases showed overlapping features (Ishii et al., 2009).

With regards to the laboratory tests, although GM-CSF autoantibodies can be detected in healthy individuals, they appear at very low levels (<3 mcg/ml) (Uchida et al., 2009). Serological diagnosis of primary PAP by demonstration of autoantibodies against GM-CSF has an excellent sensitivity and specificity for the autoimmune variant of primary PAP. Interestingly, some studies have identified GM-CSF neutralizing antibodies in patients with malignancies (Sergeeva et al., 2008), inflammatory conditions (Han et al., 2009), or secondary alveolar proteinosis due to dust exposure (Cummings et al., 2010). The prognostic value of GM-CSF antibodies needs to be further investigated.

Serum lactate dehydrogenase (LDH) is increased in 82 % of patients (Seymour and Presneill, 2002), but is nonspecific for PAP. LDH has been found to reflect the dynamic changes in disease severity during treatment after therapeutic lavage or spontaneous resolution (Seymour et al., 2003; Seymour and Presneill, 2002).

Elevation of serum and BAL tumor biomarkers such as carcinoembryonic antigen (CEA) may also reflect the severity of disease (Hirakata et al., 1995). Serum levels of SP-A and SP-D can be increased but this is also not specific for

the disease, since high levels have also been reported in patients with idiopathic pulmonary fibrosis (Honda et al., 1995). SP-A has been found to correlate with the severity of disease (Seymour et al., 2003).

At present, the most promising diagnostic and prognostic biomarker for PAP is KL-6, a mucin-like glycoprotein. Serum and BAL levels are extremely high in PAP, higher than in patients with other interstitial lung disease (Nakajima et al., 1998), independently from the nature of PAP. Recently, Inoue et al (Inoue et al., 2008) reported a good correlation of KL-6 with the disease severity score in 284 patients with autoimmune PAP (Inoue et al., 2008). Serum levels of KL-6, SP-D, SP-A, and CEA are elevated to a similar degree in autoimmune and secondary PAP (Inoue et al., 2008; Ishii et al., 2011).

Taken together, the diagnosis of PAP should be suspected in a patient with slowly developing dyspnea, a 'butterfly' pattern of acinar shadowing on the chest radiograph and characteristic findings on HRCT (crazy paving) pattern. The introduction of a simple blood test for measuring GM-CSF autoantibody levels facilitates the diagnosis of autoimmune PAP (Bonfield, Russell, et al., 2002; Kitamura et al., 1999; Kitamura et al., 2000; Uchida et al., 2009; Uchida et al., 2004). The test has a reported sensitivity and specificity for autoimmune PAP close to 100% (Presneill et al., 2004). Other blood tests like LDH, CEA, SP-A, SP-D or KL-6 are not yet validated for diagnostic purposes. Bronchoscopy with BAL, cytological analysis and transbronchial biopsy should be performed early in most patients, and special stains and cultures should be performed to rule out

infection by common and opportunistic microbial pathogens (Costabel et al., 2007). The diagnosis is usually confirmed by the characteristic BAL findings.

1.7. Clinical course and treatment

Spontaneous remission is rare in PAP, occurring in only 5-10 % of patients (Bonella et al., 2011; Inoue et al., 2008; Seymour and Presneill, 2002). Treatment is indicated when respiratory symptoms impair the quality of life or when lung function deteriorates, but established criteria do not exist.

The treatment of choice is whole lung lavage (WLL), which is almost always effective (Huizar and Kavuru, 2009; Luisetti et al., 2010) but is adopted as an institutional procedure in only a limited number of specialized clinical centers. WLL is not standardized and no prospective clinical trials have been performed. Since its introduction in the 1960s by Ramirez et al (Ramirez et al., 1963), the technique has been improved through the application of manual or mechanical chest percussion (Hammon et al., 1993), also in combination with postural changes (Perez and Rogers, 2004). Clinically significant improvement in radiologic appearance, PaO_2, lung volumes and DLCO is seen in 84 % of patients following the first therapeutic lavage (Seymour and Presneill, 2002). In secondary PAP, WLL, although feasible, usually provides only transient benefit (Luisetti et al., 2010). Recently WLL has been reported as ineffective in a patient exposed to indium being GM-CSF antibody negative (Cummings et al., 2010). However, at

least one case of long-lasting remission following WLL has been described in PAP associated with lysinuric protein intolerance (Ceruti et al., 2007).

The treatment with exogenous GM-CSF has still to be considered experimental. Two prospective, open-label, uncontrolled trials (Seymour et al., 2001; Venkateshiah et al., 2006) and several anecdotal reports have shown that daily subcutaneous administration of recombinant human GM-CSF is effective in about 50 % of patients with autoimmune PAP. The administration of aerosolized GM-CSF seems to be more effective, as shown in a retrospective case series (Wylam et al., 2006), and recently in a controlled prospective trial of 50 patients with a response rate of 62% (Tazawa et al., 2010). It is unclear, whether the pre-treatment blood levels of GM-CSF antibodies are able to predict a response to such treatment since two groups reported conflicting data (Bonfield, Kavuru, et al., 2002; Seymour et al., 2003; Venkateshiah et al., 2006).

A combined therapy with WLL and plasmapheresis is able to reduce the titer of GM-CSF antibodies (Kavuru et al., 2003; Luisetti et al., 2009), but the data on clinical efficacy are controversial (Kavuru et al., 2003; Luisetti et al., 2009).

B-lymphocyte depletion is an interesting option for autoimmune PAP. Rituximab is a humanized monoclonal antibody that by binding CD20 selectively decreases the B-cell pool. Rituximab has been administered in one PAP patient at the dose of 1 g i.v. on days 1 and 15 (Borie et al., 2009). A long-lasting depletion of B Lymphocytes was achieved; titer and activity of neutralizing GM-SCF antibodies were markedly decreased. The clinical picture and gas exchange

parameters were also improved. A prospective, nonrandomized, open-label trial of rituximab in 10 patients with primary PAP showed that this drug was well-tolerated and effectively ameliorated lung disease; a reduction in anti-GM-CSF IgG levels in the lung correlated with disease changes, suggesting that disease pathogenesis is related to autoantibody levels in the target organ (Kavuru et al., 2011; Malur et al., 2012).

1.8. Outcome and prognosis

Prognosis of PAP has improved considerably with introduction of whole lung lavage. Seymur and Presneill (Seymour and Presneill, 2002) reported a significantly ($p<0.04$) greater survival rate in 146 of 231 PAP patients who underwent WLL than in the 85 who did not (94±2% versus 85±5%, respectively). The median number of lavages was two, with a median interval of 15 months between the two procedures, and disease recurrence was observed in 80% of PAP patients within 3 years of the procedure. In our series 52% of patients achieved remission after 1 WLL (Bonella et al., 2011), similarly to the 55% reported by Luisetti et al (Luisetti et al., 2010).

Comparing the demographic and disease-related features of patients who did or did not respond to therapeutic lavage, there are no differences in gender, region of origin, duration of symptoms, smoking status, and time from diagnosis to lavage (Seymour and Presneill, 2002). In our series of 70 patients, nonsmokers needed an average number of 2.4 lavages per patient, exsmokers an average

number of 3.8, and smokers required 5 lavages per patient to achieve long lasting remission (Bonella et al., 2011).

PAP may be complicated by infections such as nocardiosis, cryptococcosis, mucormycosis and others. In the era of therapeutic lavage these complications are rare. There have been single reports of progressive interstitial pulmonary fibrosis developing in patients previously affected by alveolar proteinosis. Lung transplantation may be an option for these patients, although recurrence of disease has been reported in one patient 3 years after double-lung transplantation (Parker and Novotny, 1997).

The data about the clinical course of secondary PAP are poor. In our centre we noted a marked difference in the incidence of PAP related death between primary PAP and secondary PAP (8 % vs 50 %) (Bonella et al., 2011). For secondary PAP associated with hematologic malignancy, the prognosis is linked to the underlying disease and is generally worse than in autoimmune PAP (Huizar and Kavuru, 2009; Ishii et al., 2011). For PAP associated with exposure, data on prognosis and treatment are scarce.

1.9. Aim of the study

This study aimed to provide data about kinetics of the protein wash out during WLL, to identify factors influencing the protein concentration in the recovered fluid, and to assess the efficacy of a modified lavage technique (MLT) in comparison to the classical lavage technique (CLT)

2. METHODS

2.1. Study population

This study was conducted at the Ruhrlandklinik, a referral centre for the diagnosis and therapy of PAP in Germany. The characteristics of the 42 PAP patients (14 male, 28 female) are summarized in the table 2. The study was approved by the Ethic Committee of the University of Duisburg-Essen (approval number 06-3170 and 10-4397 for the subjects included in the project EuPAPNet). Informed consent was obtained from the patients.

2.2. Whole lung lavage techniques

110 WLLs were performed in 33 patients according to the classical technique described by Ramirez et al (Ramirez, 1966; Ramirez et al., 1963). 70 WLLs in 9 patients were performed with a modification reported by Bingisser et al (Bingisser et al., 1998) (**table 3**). Most of the patients received consecutive WLLs during the course of their disease. WLL with both techniques were performed using the same materials (tube, instilled solution) and following the same anesthesiological protocol (drugs and monitoring procedures). For one complete WLL procedure, both lungs were lavaged separately on two different days. The mean interval was 10±3 days.

Table 2 Demographics and features of the cohort.

Characteristics	N=42
Smoking habits at first lavage	
-never	5
-ex	20
-current	17
Previous dust/fume exposure*	23
Pulmonary function at diagnosis	
FEV1, %pred. (n=42)	73 ± 15**
FVC, % pred. (n=42)	75 ± 15**
TLC, % pred. (n=38)	77 ± 16**
DLCO, % pred. (n=38)	45 ± 17**
Blood gas analysis at diagnosis	
PaO2, mmHg (n=39)	66 ± 15**
PaCO2, mmHg (n=39)	35 ± 4**
(A-a)DO2, mmHg (n=39)	36 ± 15**
DSS grade at first lavage	
DSS 1 (no symptoms and PaO2 ≥ 70 mmHg)	0
DSS 2 (symptomatic and PaO2≥70 mmHg)	14
DSS 3 (60mmHg ≤ PaO2 < 70 mmHg)	10
DSS 4 (50mmHg ≤ PaO2 < 60 mmHg)	9
DSS 5 (PaO2 < 50 mmHg)	6
Serum biomarkers†	
GM-CSF Ab, mcg/mL (n=18)	52 ± 16 (28-86)***
LDH, U/L (n=40)	360 ± 171 (126-894)***
CEA, ng/mL (n=32)	14 ± 10 (2-27)***
KL-6, U/mL (n=22)	2978 ± 2488 (830-6950)***

N= number of patients.

DSS= disease severity score (Inoue et al., 2008).

*Aluminum dust, bakery flour dust, cement dust, cleaning products, gasoline fumes, paint, petroleum, saw dust, silica (glass grinding), synthetic plastic fumes, varnish.

** Data are mean ± SD

***Data are mean ± SD (range)

† Reference values for serum biomarkers are indicated in the methods.

Table 3 Allocation of the patients and patients´ features according to WLL technique.

	CLT	MLT	p
Patients (n)	33	9	
Gender (M/F)	23/10	5/4	ns
Age, years (mean ± SD)	44 ± 11	43 ± 9	ns
BMI, kg/m^2 (mean ± SD)	25 ± 4	25 ± 5	ns
Current smokers (n)	14	3	ns
Previous dust/fumes exposure (n)	20	3	ns
TLC, % pred (mean ± SD)	77± 10	77± 27	ns
DLCO, % pred (mean ± SD)	48 ± 20	41 ± 10	ns
PaO2, mmHg (mean ± SD)	65 ± 15	69 ± 18	ns
Time from diagnosis to first WLL, days (mean) (range)	530 (5-3691)	261 (5-876)	ns
Lavaged lung (right/left)	55/55	33/37	

CLT=classical lavage technique; MLT=modified lavage technique; ns=not significant

2.2.1 Classical whole lung lavage technique

The procedure is illustrated in the **figure 1**. The patient underwent double-lumen intubation. Ventilation with 100% oxygen using a volume-controlled ventilator (Servo; Siemens; Danvers, Mass) was started. An indwelling arterial catheter was placed. The tube was tested for leaks by single-lung ventilation. A flexible fiberoptic bronchoscope was used to ascertain proper tube position initially and during the procedure. The lung to be washed was clamped for 5 min to allow oxygen absorption. Saline solution at body temperature was instilled into the non-ventilated lung with a tidal washing volume of 1000 ± 200 mL during each cycle. After recovering the opaque fluid over a closed silicone tube system, the next washing cycle was begun. The optical density (OD) was measured in each

recovered tidal volume to monitor the progress of the lavage procedure (Paschen et al., 2005). The recovery rate of each cycle was accurately documented. The lavage cycles were continued until the optical density reached the target value of < 0.4 OD, or until a plateau was reached. In general, 30 to 60 L were needed to achieve this.

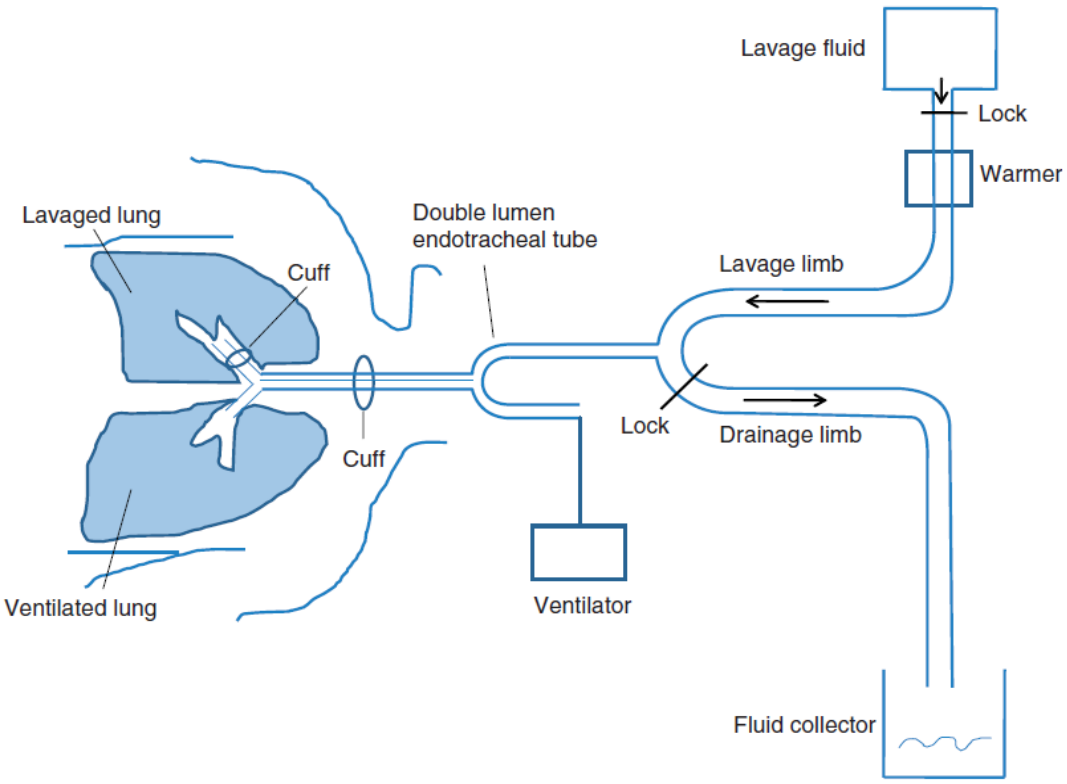

Figure 1. Classical whole lung lavage technique. For the description see the text. Modified from Luisetti et al, 2010 (Luisetti et al., 2010).

2.2.2 Modified whole lung lavage technique

The intubation procedure and the infusion-recovery cycle at the beginning of the lavage procedure were the same as for the CLT. When the target value of < 0.4 OD was reached with the classical procedure, controlled manual ventilation was applied during one infusion-recovery cycle as follows (see **figure 2**): at first 500 ml of saline solution were instilled, and then the ventilation was started. A tidal volume of 300 ml of room air was delivered by the bag 5 times consecutively, without fluctuations. After having recovered the first 500 ml of instilled saline solution, the rest of the fluid (500 ml) of this cycle was instilled to remove the foam in the lavaged lung and airways and recovered, and the next cycle was started. Subsequently, the lavage was continued until the target value 0.4 OD was reached for the second time.

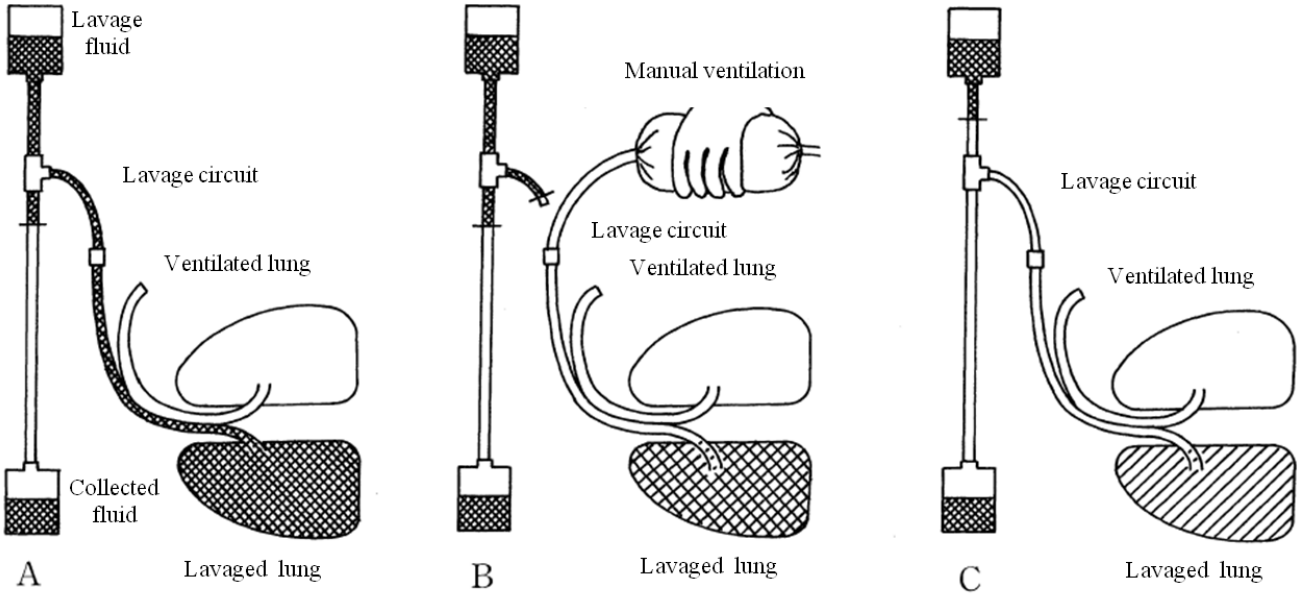

Figure 2 Modified whole lung lavage technique. A) The procedure begins according to the classical technique. B) When the target value of turbidity (< 0.4 OD) has been reached, 500 ml saline are instilled, the manual ventilation will be applied 5 times, and the fluid will be recovered. C) After instillation of further 500 ml of saline to remove the foam in the lavaged lung and airways, the fluid is recovered and the lavage continued according to the classical technique. Modified from Ishikawa et al 2002 (Ishikawa et al., 2002).

2.3. Laboratory measurements

2.3.1 GM-CSF autoantibody in serum

GM-CSF autoantibody concentration was measured by enzyme linked immunosorbent assay (ELISA) as previously reported (Latzin et al., 2005; Uchida et al., 2009). The detection limit of our assay is 0.2 mcg/mL. Values <10 mcg/mL are considered normal.

2.3.2. Biomarkers in serum and BAL

KL-6 was measured by ELISA (Eisai Co. Ltd., Tokyo, Japan) as described previously (Takahashi et al., 1998) in serum and BAL. LDH and CEA were measured in serum only. Normal serum ranges in our laboratory are: < 620 U/ml for KL-6, < 200 IU/l for LDH and < 2.5 ng/mL for CEA .

2.3.3. Rapid turbidity assessment

The optical density of the recovered fluid was measured at a wave length of 405 nm (EPAC 6140, Eppendorf, Germany).

2.3.4. Protein concentration

The recovered fluid was centrifugated at 1720 g for 10 minutes, in order to separate water insoluble particulate materials including cells and debris, as described before (Onodera et al., 1983). In the supernatant, the total protein concentration was measured with a spectrophotometer in portions standardized in amount (10 ml) and in duplicate (Konelab T series for U/CSF protein, Thermo Fisher Scientific, Finland).

2.3.5. Statistical analysis

All variables were evaluated for a normal distribution using the Kolmogorov-Smirnov test and for equal variance using the Levene median test. The area under the curve was calculated with trapezoidal method and verified with integration of regression equation (IRE). The following variables had no normal distribution: Disease severity score (DSS), volume of instilled fluid, protein concentration and amount of removed proteins (AUC). Therefore these variables are expressed as median (50^{th} percentile) and interquartile range (IQR) (25^{th}-75^{th} percentile). Categorical data are presented as either a percentage of the total or numerically, as appropriate. Statistical comparisons of parametric data were made with Student's t-test for two group comparisons. Nonparametric data were compared with the Wilcoxon test. Comparisons of categorical data were made with Chi-squared or Fischer's exact test. Longitudinal data of parametric data (biomarkers and lung function tests) were compared with the paired T-test. The comparison of the means in the same subjects at different times was performed with one way repeated measures ANOVA and the comparison of repeated measures between the techniques (MLT vs CLT) using general linear model (GLM) for repeated measures. Spearman's or Pearson´s coefficient was obtained for all correlations. Partial correlation analysis (with covariates) and regression analysis were used to confirm the correlations. All tests were two sided and p values of less than 0.05 were considered to indicate statistical significance.

3. RESULTS

There was a linear correlation between the protein concentration determined with the quantitative method (Konelab) and the optical density (**figure 3**).

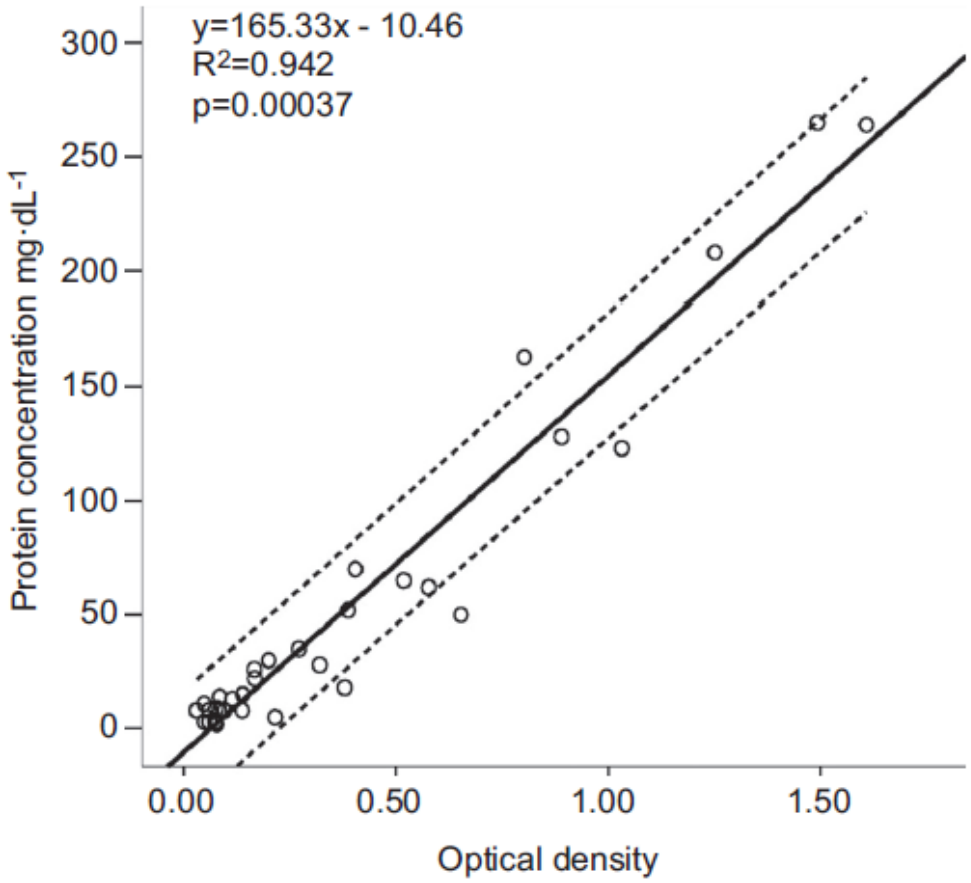

Figure 3. Regression curve fit showing the linear correlation (bold line) between the protein concentration (assessed by Konelab quantitative analysis) and the optical density of the spectrophotometric absorption in the first recovered portion of 6 consecutive WLL. Linear equation with 95% confidence interval (CI) (dashed lines), correlation coefficient and significance are also shown.

3.1. Kinetics of the wash out process

3.1.1. Protein concentration

The median protein concentration in the first portion of the recovered fluid of 180 WLL was 460 (15-3907) mg/dL. There was no correlation of this initial protein concentration with age, BMI, smoking habits. An inverse correlation was seen with TLC (n=126, r=-0.222, p=0.012) and PaO2 (n=142, r=-0.214, p=0.01). The initial protein concentration correlated with the DSS (n=142, r=0.3, p=0.002), and inversely with the duration of disease, defined as interval between the diagnosis and the first treatment with WLL (n=42, r=-0.4, p=0.012). There was also a correlation with serum LDH (n=149, r=0.5, p=0.0001) and BALF KL-6 concentration (n=26, r=0.44, p=0.02). No correlations were found with serum CEA or GM-CSF autoantibody levels.

The median protein concentration in the final portion of the recovered fluid was 26 (4-71) mg/dL.

3.1.2. Protein amount

The median amount of proteins removed from the lung by one WLL was 17.5 (7.2-41) g. This was not affected by gender, age, BMI, the WLL being performed in the left or right lung, smoking habits, or a history of dust exposure (data not shown).

The removed protein amount correlated inversely with DLCO (n=54, r=-0.44, p=0.001) and PaO2 (n=142, r=-0.243, p=0.004) and directly with the DSS

(n=142, r=0.3, p=0.0001), serum LDH (n=149, r=0.53, p=0.0001), and BAL KL-6 levels (n=26, r=0.533, p=0.005). No correlations were found with serum KL-6, CEA and GM-CSF antibodies.

3.1.3. Other results

The protein concentration in the last recovered portion was higher in patients exposed to dust/fume than in those not exposed, with a median value of 24 (9-57) mg/dL vs 9 (3-21) mg/dL (p=0.00013); the instilled volume per WLL between the two groups did not differ (25 (16-32) L vs 27 (20-4) L; p=0.08) and was not considered as covariate.

9 patients underwent 6 consecutive WLLs. 6 patients received CLT and 3 patients MLT. The mean interval between multiple WLLs in this subgroup of 9 pts that were lavaged 6 times was 138±105 days. The initial protein concentration in the recovered fluid did not change significantly from the first to the last WLL (**figure 4a**), but the final protein concentration significantly decreased with the third WLL (**figure 4b**). The amount of removed protein declined with consecutive WLLs (**figure 4c**) but the instilled volume did not change (p=0.2) (**figure 4d**).

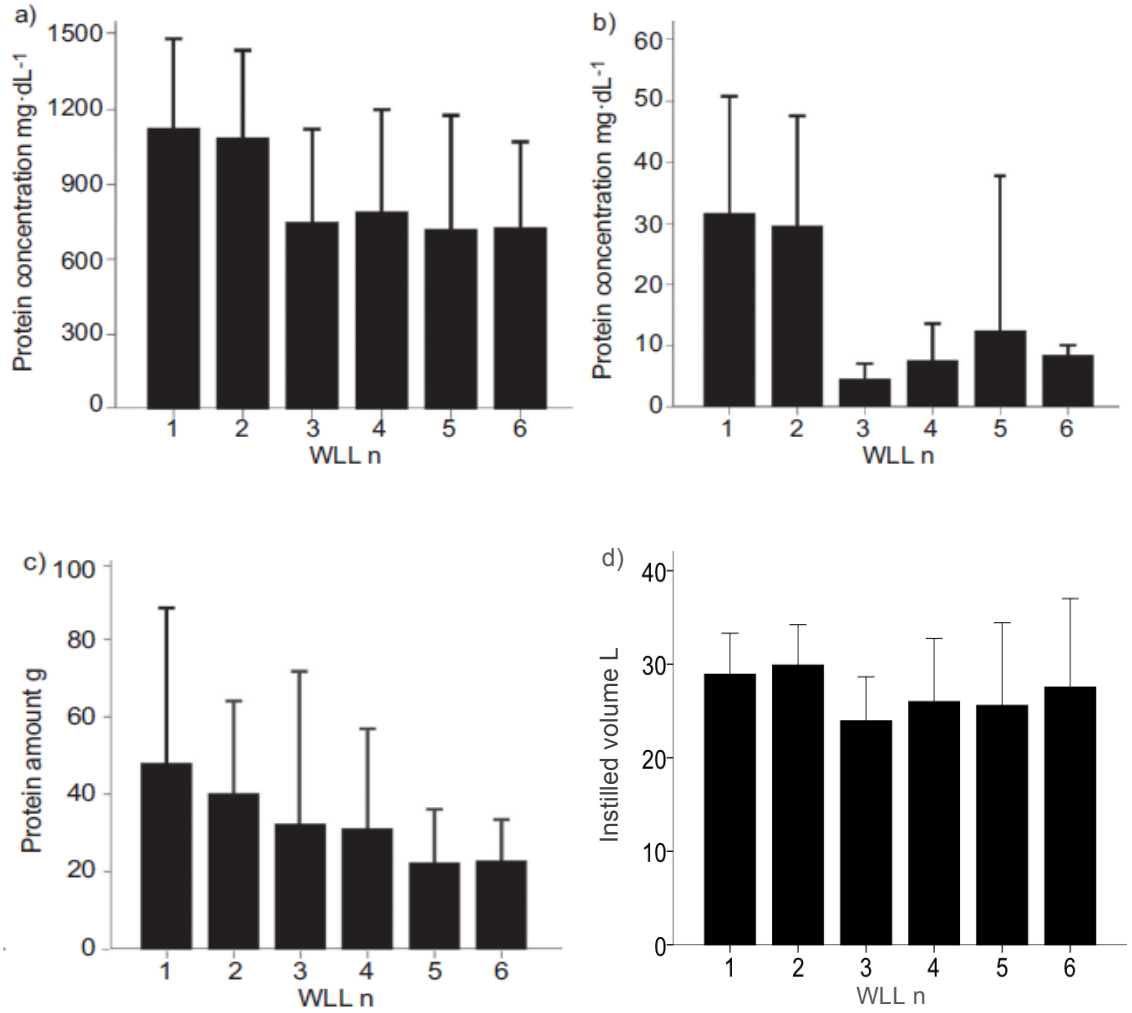

Figure 4. Change of protein wash out kinetics in 9 patients who underwent 6 consecutive WLLs, independently from the technique applied: (a) initial protein concentration, (b) final protein concentration, (c) protein amount and (d) instilled volume.

3.2. Comparison of the whole lung lavage techniques.

The results from the comparison of the WLL techniques are summarized in **table 4.** The patients who received MLT had a significantly lower final protein

concentration (median 9 (3-20) mg/dL) than those receiving CLT (median 22 (5-58) mg/dL; p=0.0002). The amount of proteins removed with WLL was significantly greater in patients who received MLT than in those receiving CLT (median 22.6 vs 13.7 g, respectively, p=0.0001).

The time range between the first and second WLL was significantly prolonged in patients that underwent MTL in comparison to those receiving CLT (225 vs 84 days, p=0.011) (Table 4).

In patients undergoing up to 6 consecutive WLLs, the amount of instilled volume necessary to reach the target final protein concentration remained higher for MLT than CLT. Only a tendency to decline was shown with the application of repeated modified WLLs (**figure 5**).

After the second WLL, only a tendency was seen for a negative correlation between initial protein concentration and the time to next WLL in the group receiving MLT, but not in those receiving CLT (first to second WLL: r=-0.5, p=0.067 for MLT vs r=-0.226, p=0.36 for CTL; second to third WLL: r=-0.5, p=0.067 for MLT vs r=0.007, p=0.978 for CLT).

Table 4. Results from the comparison of WLL techniques.

	CLT		MLT		
	number of WLL	**Mean* ± SD (range)**	**number of WLL**	**Mean* ± SD (range)**	**p**
Instilled Volume, liter	110	15 (4-40)	70	40 (21-71)	0.0003†
Initial protein concentration, mg/dL	110	460 (15-3906)	70	458 (41-3810)	0.77†
Final protein concentration, mg/dL	110	21 (1-593)	70	9 (1-39)	0.0002†
Amount of removed protein, mg	110	13780 (350-32015)	70	22580 (2920-26860)	0.0001†
Time range 1st - 2nd WLL, days	29	84 ± 168	9	225 ± 151	0.011§
Time range 2nd - 3rd WLL, days	18	270 ± 276	3	260 ± 298	ns§
Time range 3rd - 4th WLL, days	13	236 ± 286	3	211 ± 120	ns§
Number of WLL per patient, n		3.2 ± 2		4.2 ± 5	ns§

CLT=classical lavage technique; MLT=modified lavage technique; ns=not significant

* or 50th percentile for non normal distributed variables

† Mann-Witney non parametric test

§ Student t-test

There was an inverse correlation between the final protein concentration and the total volume instilled (r=-0.257, p=0.00049). When corrected for volume, the difference in the final protein concentration between the two techniques still remained significant (p=0.001).

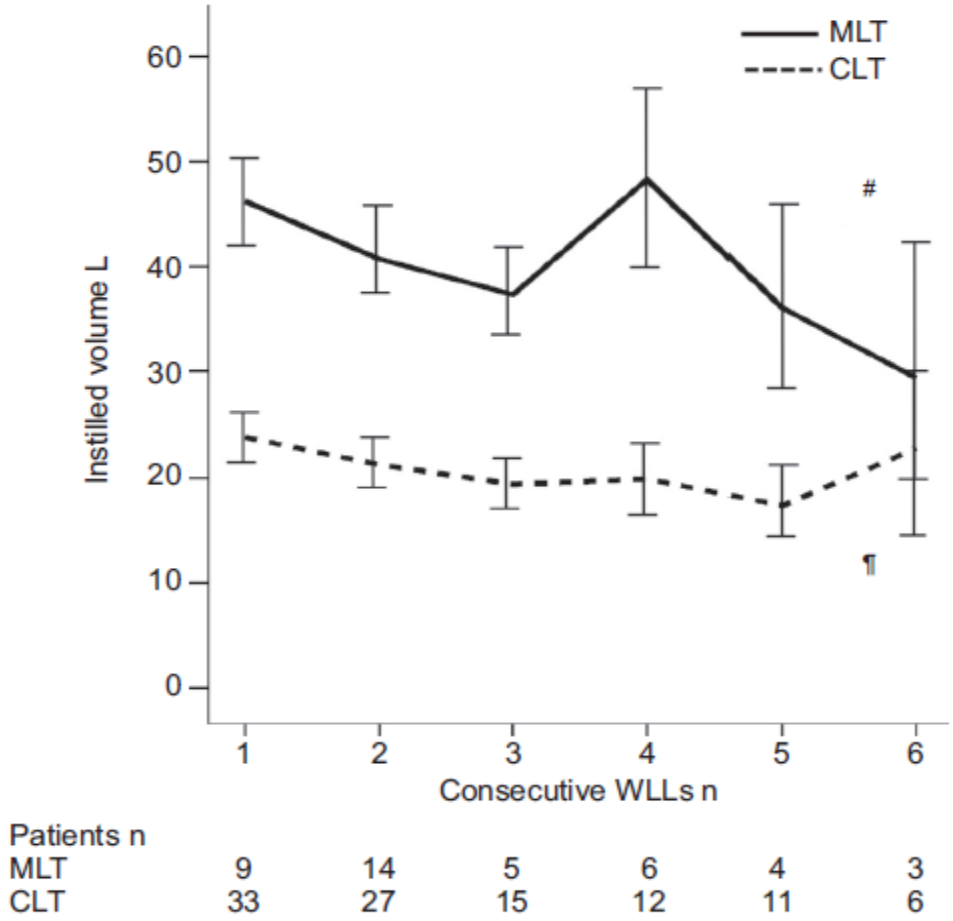

Figure 5. Mean instilled volume in up to 6 consecutive WLLs, according to the applied technique. The error bars indicate the 95% CI of the mean. The p values in the graphic refer to the significance for the comparison in each group (ANOVA). The difference between groups remained significant in each measure (overall p=0.044) (general linear model test between subjects). CLT=classical lavage technique; MLT=modified lavage technique.

The **figure 6** shows the effect of the manual ventilation on the protein wash out. The amount of removed proteins, represented by the area under the curve

(AUC) in figure 6, was significantly greater with MLT than with the CLT (see table 3). The volume instilled and recovered through CLT did not exceed 40 L, while the volume reached up to 84 L with MLT in one session, which is the reason why more material was removed with this technique. When corrected for volume, the difference in the amount of removed proteins between CLT and MLT was no longer significant (p=0.121).

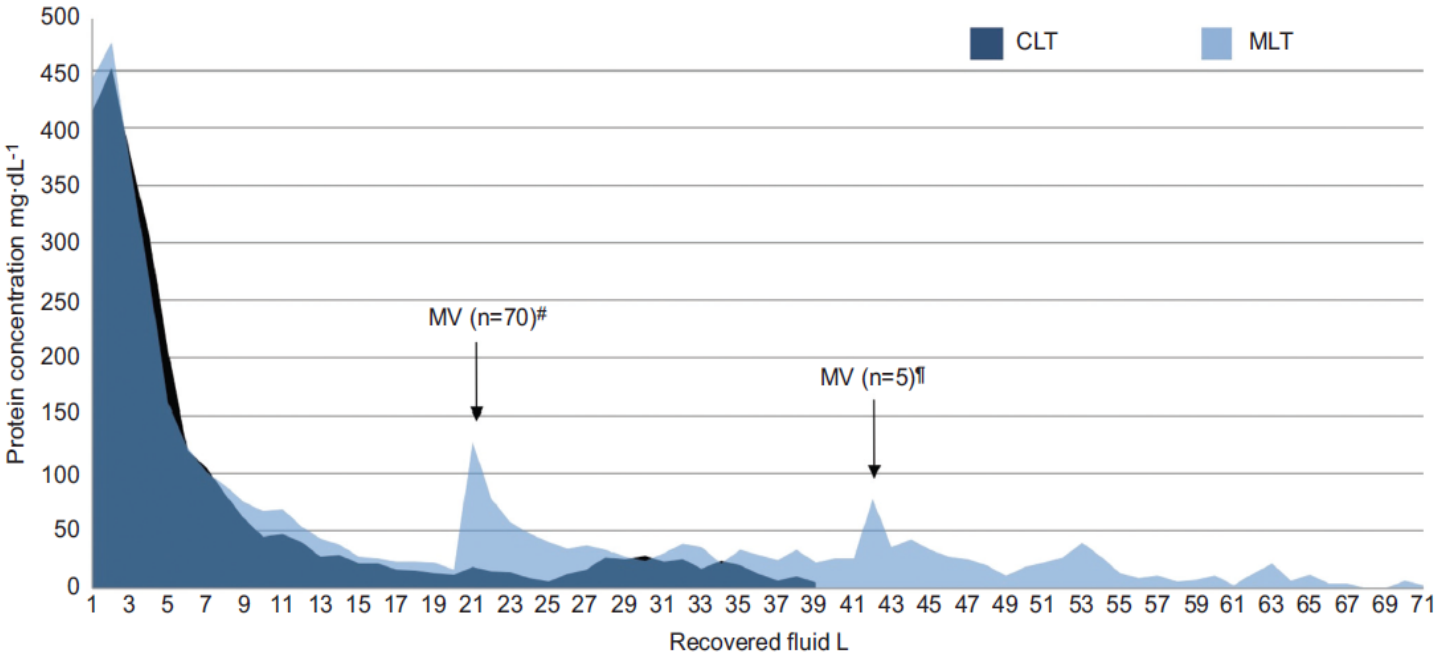

Figure 6. Comparison between the classical (CLT) (n=110 procedures) and the modified (MLT) WLL technique (n=70 procedures) in removing proteins from the lung. The amount of removed proteins is represented by the area under the curve (AUC). The arrows indicate when the manual ventilation (MV) was applied during the procedure, mostly after the 21st and the 41st liter. Statistics are described in the text. # In 70 WLL procedures the manual ventilation was applied only once

during the lavage.¶ In 5 WLL procedures the manual ventilation was applied twice during the lavage.

4. DISCUSSION

Since its first application in the 1960´s (Ramirez et al., 1963; Seard et al., 1970; Wasserman et al., 1968), WLL is still the treatment of choice in patients with PAP (Cheng et al., 2002; Ioachimescu and Kavuru, 2006; Kavuru and Popovich, 2002; Morgan, 2004; Paschen et al., 2005; Selecky et al., 1977; Seymour and Presneill, 2002; Shah et al., 2000; Trapnell et al., 2003). In this study, we provide detailed data on kinetics of protein wash out in the recovered fluid (initial and final protein concentration, amount of removed proteins) of 180 WLLs in a cohort of 42 adult PAP patients. We also compared the wash out efficacy of two different lavage techniques in terms of quantity of removed proteins from the lung and time interval from first to second WLL. To the best of our knowledge, this is the largest single centre study regarding WLL reported worldwide.

In our PAP cohort, we observed an exponential decay of the protein concentration during the lavage, similarly to data published before (Beccaria et al., 2004; Onodera et al., 1983; Perez and Rogers, 2004; Rodi et al., 1995): the median initial protein concentration was at least 20 fold higher than the final concentration. We did not find a correlation with age, BMI or lavaged side. The initial value of the protein concentration showed an inverse correlation with TLC

(p=0.012), PO2 (p=0.01) and a direct correlation with the disease severity score (DSS) (p=0.002). This may be explained by the degree of filling of the alveolar space. The inverse correlation between the protein concentration in the initial recovered fluid and the duration of disease (p=0.012), measured as time from diagnosis to first treatment with WLL, seems to indicate that patients with a more severe alveolar impairment, or higher protein accumulation speed, tend to undergo WLL earlier. Due to the low number of patients who received subsequent WLLs (21 patients received 2 WLL and 16 patient 3 WLL), only a tendency was seen for a negative correlation between initial protein concentration and time to next WLL in the group receiving MTL, but not in those receiving CLT. Therefore, it can only be speculated that a deeper removal of proteins during the first WLL through a more effective technique than CTL may result in a delayed accumulation of proteins in the alveoli over time.

Furthermore, the observed correlation between the initial protein concentration, duration of disease and well established biomarkers for PAP (serum LDH and BAL KL6 levels) (Lin et al., 2008; Seymour et al., 2003) suggests that also the initial protein concentration could have a role as biomarker.

The median protein concentration of 26 mg/dl at the end of the lavage is consistent with that reported by Paschen (10 mg/dL) (Paschen et al., 2005) and by Alberti (20-80 mg/dL) (Alberti et al., 1996). The amount of protein removed from the lungs (17.5 g) was compatible with the range reported by Paschen (2-33

g) (Paschen et al., 2005) and by Ceruti (6.5-8.5 g) (Ceruti et al., 2007), and was not affected by gender, age, BMI, dust exposure or smoking habits.

Moreover, we found that a history of dust exposure, but not smoking, was associated with a higher residual protein concentration in the recovered fluid. This needs further investigation because the protein concentration in the first recovered portion shows only a tendency to be higher in patients exposed to dusts and fumes.

The effect of consecutive WLL on the protein kinetics has not been investigated before. We found a progressive decline in the amount of removed protein and a better clearance with consecutive procedures.

Finally, we showed that a modified WLL technique with manual ventilation can remove a larger amount of protein and reduce the residual protein concentration in the fluid more than the classical technique. The amount of proteins removable from the lungs depends on the instilled volume. The manual ventilation in the middle of the procedure seems to mobilize additional proteins from the alveoli. The magnitude of the second protein concentration peak (after manual ventilation) was about one third of the initial (**figure 6**); then the wash out curve declined as usual. An ideal technique of WLL should remove the largest amount of protein with the lowest instilled volume, in order to reduce the duration of anesthesia and the risk of complications, like overspill of lavage fluid into the ventilated lung, barotrauma, hydropneumothorax and severe acidosis (Shah et al.,

2000). Even if MLT is not the ideal lavage technique, it seems to be more effective in prolonging the time to the second WLL compared to the classical technique

There are several limitations of this study. First, there is an imbalance in the number of patients that was assigned to the different technique. Second, we did not perform systematically gel electrophoresis/Western blot analysis to separate the proteins; therefore we cannot exclude an influence of aberrant lipoproteins on the kinetics of the procedure.

In summary, this study supports the concept that the kinetics of protein removal from the lungs can be easily estimated by spectrophotometry of the effluent and can provide biochemical variables of clinical interest for the outcome. The clearance of the fluid through WLL appears be affected by a history of dust exposure, but not by smoking. Applying manual ventilation during the procedure can enhance the efficacy of WLL, even if it does not reduce the amount of volume to be instilled.

5. SUMMARY

Whole lung lavage (WLL) is the standard treatment for pulmonary alveolar proteinosis (PAP). This study aimed to provide data about kinetics of the protein wash out, to identify factors influencing the protein concentration in the recovered fluid, and to assess the efficacy of a modified lavage technique.

Samples from 180 WLLs of 42 adult PAP patients were collected. 110 WLL were performed according to the classical technique. In 70 WLL repeated manual ventilation was applied during the procedure. Spectrophotometry was used to measure the protein concentration in the recovered fluid.

The initial protein concentration in the recovered fluid was 460 mg/dL, the final concentration was 26 mg/dL, and the total amount of removed proteins during a lavage was 17.5 g. A history of dust exposure was associated with a higher residual protein concentration in the recovered fluid ($p=0.00013$). The amount of removed proteins correlated inversely with the diffusing capacity of the lung for carbon monoxide (DLCO) ($p=0.001$) and the partial pressure of oxygen in the blood (PaO_2) ($p=0.004$). The modified technique removed a greater amount of proteins than the classical technique and prolonged the time to relapse ($p=0.011$).

The exposure to dust seems to influence the kinetics of the protein wash out. Applying manual ventilation during the procedure can enhance the efficacy of WLL.

6. REFERENCES

1. Alberti, A., Luisetti, M., Braschi, A., Rodi, G., Iotti, G., Sella, D., Poletti, V., Benori, V., and Baritussio, A. (1996): Bronchoalveolar lavage fluid composition in alveolar proteinosis. Early changes after therapeutic lavage. Am J Respir Crit Care Med 154, 817-820.
2. Beccaria, M., Luisetti, M., Rodi, G., Corsico, A., Zoia, M. C., Colato, S., Pochetti, P., Braschi, A., Pozzi, E., and Cerveri, I. (2004): Long-term durable benefit after whole lung lavage in pulmonary alveolar proteinosis. Eur Respir J 23, 526-531.
3. Bingisser, R., Kaplan, V., Zollinger, A., and Russi, E. W. (1998): Whole-lung lavage in alveolar proteinosis by a modified lavage technique. Chest 113, 1718-1719.
4. Bonella, F., Bauer, P. C., Griese, M., Ohshimo, S., Guzman, J., and Costabel, U. (2011): Pulmonary alveolar proteinosis: New insights from a single-center cohort of 70 patients. Respir Med 105, 1908-1916.
5. Bonfield, T. L., Kavuru, M. S., and Thomassen, M. J. (2002): Anti-GM-CSF titer predicts response to GM-CSF therapy in pulmonary alveolar proteinosis. Clin Immunol 105, 342-350.

6. Bonfield, T. L., Raychaudhuri, B., Malur, A., Abraham, S., Trapnell, B. C., Kavuru, M. S., and Thomassen, M. J. (2003): PU.1 regulation of human alveolar macrophage differentiation requires granulocyte-macrophage colony-stimulating factor. Am J Physiol Lung Cell Mol Physiol 285, 1132-1136.
7. Bonfield, T. L., Russell, D., Burgess, S., Malur, A., Kavuru, M. S., and Thomassen, M. J. (2002): Autoantibodies against granulocyte macrophage colony-stimulating factor are diagnostic for pulmonary alveolar proteinosis. Am J Respir Cell Mol Biol 27, 481-486.
8. Borie, R., Debray, M. P., Laine, C., Aubier, M., and Crestani, B. (2009): Rituximab therapy in autoimmune pulmonary alveolar proteinosis. Eur Respir J 33, 1503-1506.
9. Brasch, F., Griese, M., Tredano, M., Johnen, G., Ochs, M., Rieger, C., Mulugeta, S., Muller, K. M., Bahuau, M., and Beers, M. F. (2004): Interstitial lung disease in a baby with a de novo mutation in the SFTPC gene. Eur Respir J 24, 30-39.
10. Carey, B., and Trapnell, B. C. (2010): The molecular basis of pulmonary alveolar proteinosis. Clin Immunol 135, 223-235.
11. Ceruti, M., Rodi, G., Stella, G. M., Adami, A., Bolongaro, A., Baritussio, A., Pozzi, E., and Luisetti, M. (2007): Successful whole lung lavage in pulmonary alveolar proteinosis secondary to lysinuric protein intolerance: a case report. Orphanet J Rare Dis 2, 14-20.

12. Cheng, S. L., Chang, H. T., Lau, H. P., Lee, L. N., and Yang, P. C. (2002): Pulmonary alveolar proteinosis: treatment by bronchofiberscopic lobar lavage. Chest 122, 1480-1485.
13. Costabel, U., and Guzman, J. (2005): Pulmonary alveolar proteinosis: a new autoimmune disease. Sarcoidosis Vasc Diffuse Lung Dis 22 Suppl 1, 67S-73S.
14. Costabel, U., Guzman, J., Bonella, F., and Oshimo, S. (2007): Bronchoalveolar lavage in other interstitial lung diseases. Semin Respir Crit Care Med 28, 514-524.
15. Cummings, K. J., Donat, W. E., Ettensohn, D. B., Roggli, V. L., Ingram, P., and Kreiss, K. (2010): Pulmonary alveolar proteinosis in workers at an indium processing facility. Am J Respir Crit Care Med 181, 458-464.
16. Dirksen, U., Hattenhorst, U., Schneider, P., Schroten, H., Gobel, U., Bocking, A., Muller, K. M., Murray, R., and Burdach, S. (1998): Defective expression of granulocyte-macrophage colony-stimulating factor/interleukin-3/interleukin-5 receptor common beta chain in children with acute myeloid leukemia associated with respiratory failure. Blood 92, 1097-1103.
17. Dirksen, U., Nishinakamura, R., Groneck, P., Hattenhorst, U., Nogee, L., Murray, R., and Burdach, S. (1997): Human pulmonary alveolar proteinosis associated with a defect in GM-CSF/IL-3/IL-5 receptor common beta chain expression. J Clin Invest 100, 2211-2217.

18. Forbes, A., Pickell, M., Foroughian, M., Yao, L. J., Lewis, J., and Veldhuizen, R. (2007): Alveolar macrophage depletion is associated with increased surfactant pool sizes in adult rats. J Appl Physiol 103, 637-645.
19. Goldstein, L. S., Kavuru, M. S., Curtis-McCarthy, P., Christie, H. A., Farver, C., and Stoller, J. K. (1998): Pulmonary alveolar proteinosis: clinical features and outcomes. Chest 114, 1357-1362.
20. Griese, M., Schumacher, S., Tredano, M., Steinecker, M., Braun, A., Guttentag, S., Beers, M. F., and Bahuau, M. (2005): Expression profiles of hydrophobic surfactant proteins in children with diffuse chronic lung disease. Respir Res 6, 80-90.
21. Hammon, W. E., McCaffree, D. R., and Cucchiara, A. J. (1993): A comparison of manual to mechanical chest percussion for clearance of alveolar material in patients with pulmonary alveolar proteinosis (phospholipidosis). Chest 103, 1409-1412.
22. Han, X., Uchida, K., Jurickova, I., Koch, D., Willson, T., Samson, C., Bonkowski, E., Trauernicht, A., Kim, M. O., Tomer, G., Dubinsky, M., Plevy, S., Kugathsan, S., Trapnell, B. C., and Denson, L. A. (2009): Granulocyte-macrophage colony-stimulating factor autoantibodies in murine ileitis and progressive ileal Crohn's disease. Gastroenterology 136, 1261-1271.

23. Hazouard E, Jacquemain C, Rivoire B, Besnier JM, de Muret A, Diot P. (2000): Digital clubbing associated with primary alveolar proteinosis: possible implication of growth factors. Presse Med 29, 999.

24. Heppleston, A. G., Wright, N. A., and Stewart, J. A. (1970): Experimental alveolar lipo-proteinosis following the inhalation of silica. J Pathol 101, 293-307.

25. Hirakata, Y., Kobayashi, J., Sugama, Y., and Kitamura, S. (1995): Elevation of tumour markers in serum and bronchoalveolar lavage fluid in pulmonary alveolar proteinosis. Eur Respir J 8, 689-696.

26. Honda, Y., Kuroki, Y., Matsuura, E., Nagae, H., Takahashi, H., Akino, T., and Abe, S. (1995): Pulmonary surfactant protein D in sera and bronchoalveolar lavage fluids. Am J Respir Crit Care Med 152, 1860-1866.

27. Huizar, I., and Kavuru, M. S. (2009): Alveolar proteinosis syndrome: pathogenesis, diagnosis, and management. Curr Opin Pulm Med 15, 491-498.

28. Inoue, Y., Nakata, K., Arai, T., Tazawa, R., Hamano, E., Nukiwa, T., Kudo, K., Keicho, N., Hizawa, N., Yamaguchi, E., Eda, R., Oishi, K., Maeda, Y., Koreeda, Y., Kodo, N., and Sakatani, M. (2006): Epidemiological and clinical features of idiopathic pulmonary alveolar proteinosis in Japan. Respirology 11 Suppl, 55S-60S.

29. Inoue, Y., Trapnell, B. C., Tazawa, R., Arai, T., Takada, T., Hizawa, N., Kasahara, Y., Tatsumi, K., Hojo, M., Ichiwata, T., Tanaka, N., Yamaguchi, E., Eda, R., Oishi, K., Tsuchihashi, Y., Kaneko, C., Nukiwa, T., Sakatani, M., Krischer, J. P., and Nakata, K. (2008): Characteristics of a large cohort of patients with autoimmune pulmonary alveolar proteinosis in Japan. Am J Respir Crit Care Med 177, 752-762.

30. Ioachimescu, O. C., and Kavuru, M. S. (2006): Pulmonary alveolar proteinosis. Chron Respir Dis 3, 149-159.

31. Ishii, H., Tazawa, R., Kaneko, C., Saraya, T., Inoue, Y., Hamano, E., Kogure, Y., Tomii, K., Terada, M., Takada, T., Hojo, M., Nishida, A., Ichiwata, T., Trapnell, B. C., Goto, H., and Nakata, K. (2011): Clinical features of secondary pulmonary alveolar proteinosis: pre-mortem cases in Japan. Eur Respir J 37, 465-468.

32. Ishii, H., Trapnell, B. C., Tazawa, R., Inoue, Y., Akira, M., Kogure, Y., Tomii, K., Takada, T., Hojo, M., Ichiwata, T., Goto, H., and Nakata, K. (2009): Comparative study of high-resolution CT findings between autoimmune and secondary pulmonary alveolar proteinosis. Chest 136, 1348-1355.

33. Ishikawa, N., Kondo, K., Oguri, T., Kamitsuna, M., Sakurai, J., Fujitaka, K., Yamasaki, M., Maeda, H., Isobe, T., and Kohno, N. (2002): Usefulness of the modified lavage technique of Bingisser and KL-6 monitoring in a patient with pulmonary alveolar proteinosis. Intern Med 41, 381-385.

34. Jennings, V. M., Dillehay, D. L., Webb, S. K., and Brown, L. A. (1995): Pulmonary alveolar proteinosis in SCID mice. Am J Respir Cell Mol Biol 13, 297-306.

35. Kavuru, M. S., Bonfield, T. L., and Thomassen, M. J. (2003): Plasmapheresis, GM-CSF, and alveolar proteinosis. Am J Respir Crit Care Med 167, 1036-1037.

36. Kavuru, M. S., Malur, A., Marshall, I., Barna, B. P., Meziane, M., Huizar, I., Dalrymple, H., Karnekar, R., and Thomassen, M. J. (2011): An open-label trial of rituximab therapy in pulmonary alveolar proteinosis. Eur Respir J 38, 1361-1367.

37. Kavuru, M. S., and Popovich, M. (2002): Therapeutic whole lung lavage: a stop-gap therapy for alveolar proteinosis. Chest 122, 1123-1124.

38. Kitamura, T., Tanaka, N., Watanabe, J., Uchida, Kanegasaki, S., Yamada, Y., and Nakata, K. (1999): Idiopathic pulmonary alveolar proteinosis as an autoimmune disease with neutralizing antibody against granulocyte/macrophage colony-stimulating factor. J Exp Med 190, 875-880.

39. Kitamura, T., Uchida, K., Tanaka, N., Tsuchiya, T., Watanabe, J., Yamada, Y., Hanaoka, K., Seymour, J. F., Schoch, O. D., Doyle, I., Inoue, Y., Sakatani, M., Kudoh, S., Azuma, A., Nukiwa, T., Tomita, T., Katagiri, M., Fujita, A., Kurashima, A., Kanegasaki, S., and Nakata, K. (2000): Serological diagnosis of idiopathic pulmonary alveolar proteinosis. Am J Respir Crit Care Med 162, 658-662.

40. Latzin, P., Tredano, M., Wust, Y., de Blic, J., Nicolai, T., Bewig, B., Stanzel, F., Kohler, D., Bahuau, M., and Griese, M. (2005): Anti-GM-CSF antibodies in paediatric pulmonary alveolar proteinosis. Thorax 60, 39-44.

41. Lee, K. N., Levin, D. L., Webb, W. R., Chen, D., Storto, M. L., and Golden, J. A. (1997): Pulmonary alveolar proteinosis: high-resolution CT, chest radiographic, and functional correlations. Chest 111, 989-995.

42. Lin, F. C., Chen, Y. C., and Chang, S. C. (2008): Clinical importance of bronchoalveolar lavage fluid and blood cytokines, surfactant protein D, and Kerbs von Lungren 6 antigen in idiopathic pulmonary alveolar proteinosis. Mayo Clin Proc 83, 1344-1349.

43. Luisetti, M., Kadija, Z., Mariani, F., Rodi, G., Campo, I., and Trapnell, B. C. (2010): Therapy options in pulmonary alveolar proteinosis. Ther Adv Respir Dis 4, 239-248.

44. Luisetti, M., Rodi, G., Perotti, C., Campo, I., Mariani, F., Pozzi, E., and Trapnell, B. C. (2009): Plasmapheresis for treatment of pulmonary alveolar proteinosis. Eur Respir J 33, 1220-1222.

45. Malur, A., Kavuru, M. S., Marshall, I., Barna, B. P., Huizar, I., Karnekar, R., and Thomassen, M. J. (2012): Rituximab therapy in pulmonary alveolar proteinosis improves alveolar macrophage lipid homeostasis. Respir Res 13, 46-52.

46. Morgan, C. (2004): The benefits of whole lung lavage in pulmonary alveolar proteinosis. Eur Respir J 23, 503-505.

47. Nakajima, M., Manabe, T., Niki, Y., and Matsushima, T. (1998): Serum KL-6 level as a monitoring marker in a patient with pulmonary alveolar proteinosis. Thorax 53, 809-811.

48. Nogee, L. M., Garnier, G., Dietz, H. C., Singer, L., Murphy, A. M., deMello, D. E., and Colten, H. R. (1994): A mutation in the surfactant protein B gene responsible for fatal neonatal respiratory disease in multiple kindreds. J Clin Invest 93, 1860-1863.

49. Onodera, T., Nakamura, M., Sato, T., and Akino, T. (1983): Biochemical characterization of pulmonary washings of patients with alveolar proteinosis, interstitial pneumonitis and alveolar cell carcinoma. Tohoku J Exp Med 139, 245-263.

50. Pamuk, G. E., Turgut, B., Vural, O., Demir, M., Hatipoglu, O., Unlu, E., Altaner, S., Gerenli, M., and Cakir, B. (2003): Pulmonary alveolar proteinosis in a patient with acute lymphoid leukemia regression after G-CSF therapy. Leuk Lymphoma 44, 871-874.

51. Parker, L. A., and Novotny, D. B. (1997): Recurrent alveolar proteinosis following double lung transplantation. Chest 111, 1457-1458.

52. Paschen, C., Reiter, K., Stanzel, F., Teschler, H., and Griese, M. (2005): Therapeutic lung lavages in children and adults. Respir Res 6, 138-147.

53. Perez, A. th, and Rogers, R. M. (2004): Enhanced alveolar clearance with chest percussion therapy and positional changes during whole-lung lavage for alveolar proteinosis. Chest 125, 2351-2356.

54. Prakash UB, Barham SS, Carpenter HA, Dines DE, Marsh HM. (1987): Pulmonary alveolar phospholipoproteinosis: experience with 34 cases and a review. . Mayo Clin Proc 62, 499-518.

55. Presneill, J. J., Nakata, K., Inoue, Y., and Seymour, J. F. (2004): Pulmonary alveolar proteinosis. Clin Chest Med 25, 593-613.

56. Ramirez, J. (1966): Bronchopulmonary lavage. New techniques and observations. Dis Chest 50, 581-588.

57. Ramirez, J., Schultz, R. B., and Dutton, R. E. (1963): Pulmonary Alveolar Proteinosis: a new technique and rationale for treatment. Arch Intern Med 112, 419-431.

58. Rodi, G., Iotti, G., Galbusera, C., Mencherini, S., Raimondi, F., and Braschi, A. (1995): Whole lung lavage. Monaldi Arch Chest Dis 50, 64-66.

59. Rosen, S. H., Castleman, B., and Liebow, A. A. (1958): Pulmonary alveolar proteinosis. N Engl J Med 258, 1123-1142.

60. Saugstad, O. D., Hansen, T. W., Ronnestad, A., Nakstad, B., Tollofsrud, P. A., Reinholt, F., Hamvas, A., Coles, F. S., Dean, M., Wert, S. E., Whitsett, J. A., and Nogee, L. M. (2007): Novel mutations in the gene encoding ATP binding cassette protein member A3 (ABCA3) resulting in fatal neonatal lung disease. Acta Paediatr 96, 185-190.

61. Seard, C., Wasserman, K., Benfield, J. R., Cleveland, R. J., Costley, D. O., and Heimlich, E. M. (1970): Simultaneous bilateral lung lavage (alveolar washing) using partial cardiopulmonary bypass. Am Rev Respir Dis 101, 877-884.

62. Selecky, P. A., Wasserman, K., Benfield, J. R., and Lippmann, M. (1977): The clinical and physiological effect of whole-lung lavage in pulmonary alveolar proteinosis: a ten-year experience. Ann Thorac Surg 24, 451-461.

63. Sergeeva, A., Ono, Y., Rios, R., and Molldrem, J. J. (2008): High titer autoantibodies to GM-CSF in patients with AML, CML and MDS are associated with active disease. Leukemia 22, 783-790.

64. Seymour, J. F., Doyle, I. R., Nakata, K., Presneill, J. J., Schoch, O. D., Hamano, E., Uchida, K., Fisher, R., and Dunn, A. R. (2003): Relationship of anti-GM-CSF antibody concentration, surfactant protein A and B levels, and serum LDH to pulmonary parameters and response to GM-CSF therapy in patients with idiopathic alveolar proteinosis. Thorax 58, 252-257.

65. Seymour, J. F., and Presneill, J. J. (2002): Pulmonary alveolar proteinosis: progress in the first 44 years. Am J Respir Crit Care Med 166, 215-235.

66. Seymour, J. F., Presneill, J. J., Schoch, O. D., Downie, G. H., Moore, P. E., Doyle, I. R., Vincent, J. M., Nakata, K., Kitamura, T., Langton, D., Pain, M. C., and Dunn, A. R. (2001): Therapeutic efficacy of granulocyte-macrophage colony-stimulating factor in patients with idiopathic acquired alveolar proteinosis. Am J Respir Crit Care Med 163, 524-531.

67. Shah, P. L., Hansell, D., Lawson, P. R., Reid, K. B., and Morgan, C. (2000): Pulmonary alveolar proteinosis: clinical aspects and current concepts on pathogenesis. Thorax 55, 67-77.

68. Stevens, P. A., Pettenazzo, A., Brasch, F., Mulugeta, S., Baritussio, A., Ochs, M., Morrison, L., Russo, S. J., and Beers, M. F. (2005): Nonspecific interstitial pneumonia, alveolar proteinosis, and abnormal proprotein trafficking resulting from a spontaneous mutation in the surfactant protein C gene. Pediatr Res 57, 89-98.

69. Suzuki, T., Maranda, B., Sakagami, T., Catellier, P., Couture, C. Y., Carey, B. C., Chalk, C., and Trapnell, B. C. (2011): Hereditary pulmonary alveolar proteinosis caused by recessive CSF2RB mutations. Eur Respir J 37, 201-204.

70. Suzuki, T., Sakagami, T., Rubin, B. K., Nogee, L. M., Wood, R. E., Zimmerman, S. L., Smolarek, T., Dishop, M. K., Wert, S. E., Whitsett, J. A., Grabowski, G., Carey, B. C., Stevens, C., van der Loo, J. C., and Trapnell, B. C. (2008): Familial pulmonary alveolar proteinosis caused by mutations in CSF2RA. J Exp Med 205, 2703-2710.

71. Suzuki, T., Sakagami, T., Young, L. R., Carey, B. C., Wood, R. E., Luisetti, M., Wert, S. E., Rubin, B. K., Kevill, K., Chalk, C., Whitsett, J. A., Stevens, C., Nogee, L. M., Campo, I., and Trapnell, B. C. (2010): Hereditary pulmonary alveolar proteinosis: pathogenesis, presentation, diagnosis, and therapy. Am J Respir Crit Care Med 182, 1292-1304.

72. Takahashi, T., Munakata, M., Suzuki, I., and Kawakami, Y. (1998): Serum and bronchoalveolar fluid KL-6 levels in patients with pulmonary alveolar proteinosis. Am J Respir Crit Care Med 158, 1294-1298.

73. Tazawa, R., Trapnell, B. C., Inoue, Y., Arai, T., Takada, T., Nasuhara, Y., Hizawa, N., Kasahara, Y., Tatsumi, K., Hojo, M., Ishii, H., Yokoba, M., Tanaka, N., Yamaguchi, E., Eda, R., Tsuchihashi, Y., Morimoto, K., Akira, M., Terada, M., Otsuka, J., Ebina, M., Kaneko, C., Nukiwa, T., Krischer, J. P., Akazawa, K., and Nakata, K. (2010): Inhaled granulocyte/macrophage-colony stimulating factor as therapy for pulmonary alveolar proteinosis. Am J Respir Crit Care Med 181, 1345-1354.

74. Torrents, D., Mykkanen, J., Pineda, M., Feliubadalo, L., Estevez, R., de Cid, R., Sanjurjo, P., Zorzano, A., Nunes, V., Huoponen, K., Reinikainen, A., Simell, O., Savontaus, M. L., Aula, P., and Palacin, M. (1999): Identification of SLC7A7, encoding y+LAT-1, as the lysinuric protein intolerance gene. Nat Genet 21, 293-296.

75. Trapnell, B. C., and Whitsett, J. A. (2002): Gm-CSF regulates pulmonary surfactant homeostasis and alveolar macrophage-mediated innate host defense. Annu Rev Physiol 64, 775-802.

76. Trapnell, B. C., Whitsett, J. A., and Nakata, K. (2003): Pulmonary alveolar proteinosis. N Engl J Med 349, 2527-2539.

77. Travis, W.D., Colby, T.V., Koss, M.N., Rosado-de-Christenson, M.L., and Müller, N.L. (2002.). Pulmonary alveolar proteinosis. In T.E. King (Ed.), Non-neoplastic disorders of the lower respiratory tract. Atlas of Non-tumor Pathology (pp. 169-176). Washington, DC: American Registry of Pathology.

78. Tredano, M., Griese, M., Brasch, F., Schumacher, S., de Blic, J., Marque, S., Houdayer, C., Elion, J., Couderc, R., and Bahuau, M. (2004): Mutation of SFTPC in infantile pulmonary alveolar proteinosis with or without fibrosing lung disease. Am J Med Genet A 126, 18-26.

79. Tredano, M., van Elburg, R. M., Kaspers, A. G., Zimmermann, L. J., Houdayer, C., Aymard, P., Hull, W. M., Whitsett, J. A., Elion, J., Griese, M., and Bahuau, M. (1999): Compound SFTPB 1549C-->GAA (121ins2) and 457delC heterozygosity in severe congenital lung disease and surfactant protein B (SP-B) deficiency. Hum Mutat 14, 502-509.

80. Uchida, K., Nakata, K., Suzuki, T., Luisetti, M., Watanabe, M., Koch, D. E., Stevens, C. A., Beck, D. C., Denson, L. A., Carey, B. C., Keicho, N., Krischer, J. P., Yamada, Y., and Trapnell, B. C. (2009): Granulocyte/macrophage-colony-stimulating factor autoantibodies and myeloid cell immune functions in healthy subjects. Blood 113, 2547-2556.

81. Uchida, K., Nakata, K., Trapnell, B. C., Terakawa, T., Hamano, E., Mikami, A., Matsushita, I., Seymour, J. F., Oh-Eda, M., Ishige, I., Eishi, Y., Kitamura, T., Yamada, Y., Hanaoka, K., and Keicho, N. (2004): High-affinity autoantibodies specifically eliminate granulocyte-macrophage colony-stimulating factor activity in the lungs of patients with idiopathic pulmonary alveolar proteinosis. Blood 103, 1089-1098.

82. Venkateshiah, S. B., Yan, T. D., Bonfield, T. L., Thomassen, M. J., Meziane, M., Czich, C., and Kavuru, M. S. (2006): An open-label trial of granulocyte macrophage colony stimulating factor therapy for moderate symptomatic pulmonary alveolar proteinosis. Chest 130, 227-237.

83. Wasserman, K., Blank, N., and Fletcher, G. (1968): Lung lavage (alveolar washing) in alveolar proteinosis. Am J Med 44, 611-617.

84. Weichert, N., Kaltenborn, E., Hector, A., Woischnik, M., Schams, A., Holzinger, A., Kern, S., and Griese, M. (2011): Some ABCA3 mutations elevate ER stress and initiate apoptosis of lung epithelial cells. Respir Res 12, 4-15.

85. Wylam, M. E., Ten, R., Prakash, U. B., Nadrous, H. F., Clawson, M. L., and Anderson, P. M. (2006): Aerosol granulocyte-macrophage colony-stimulating factor for pulmonary alveolar proteinosis. Eur Respir J 27, 585-593.

86. Xu, Z., Jing, J., Wang, H., Xu, F., and Wang, J. (2009): Pulmonary alveolar proteinosis in China: a systematic review of 241 cases. Respirology 14, 761-766.

7. APPENDIX

7.1. Tables

7.2. Figures

8. ABBREVIATIONS

BALF	Bronchoalveolar lavage fluid
CLT	Classical lavage technique
DLCO	Diffusing capacity of the lung for carbon monoxide
DSS	Disease severity score
ELISA	Enzyme-linked immunosorbent assay
FEV1	Forced expiratory volume in one second
GM-CSF	Granulocyte macrophage colony stimulating factor
HRCT	High resolution computed tomography
KL-6	Krebs von den Lungen-6
MLT	Modified lavage technique
OD	Optical density
PAP	Pulmonary alveolar proteinosis
ROC	Receiver operating characteristic
SP	Surfactant protein
TBB	Transbronchial biopsy
TLC	Total lung capacity
VC	Vital capacity
WLL	Whole lung lavage

9. ACKNOWLEDGEMENTS

I arrived in Germany in 2007 from Italy and I was uncertain about my future. In the period of working and living here, I gradually understood the role I could play in the work of scientific research and the responsibility that this entails. I must express my hearty appreciation to my tutor Prof. Dr. U. Costabel for the kind and continuous guidance on my research work, for the financial support of my projects and the possibility to present the results around the world.

I would also like to thank Dr. P.C. Bauer for his kind assistances and teaching whole lung lavage.

I have also to thank the BAL laboratory team, especially Mrs. Tran Ngoc Lan, Mrs. B. Tamoschus, Mrs Vavrilova and Mrs. A. Wiener for the technical support and cooperation.

10. CURRICULUM VITAE

Der Lebenslauf ist in der Online-Version aus Gründen des Datenschutzes nicht enthalten.

11. PUBLICATIONS

Original Articles (first author)

1. Bonella, F., Volpe, A., Caramaschi, P., Nava, C., Ferrari, P., Schenk, K., Ohshimo, S., Costabel, U., Ferrari, M. (2011): Surfactant protein D and KL-6 serum levels in systemic sclerosis: correlation with lung and systemic involvement. Sarcoidosis Vasc Diffuse Lung Dis 28:27-33.
2. Bonella, F., Bauer, P.C., Griese, M., Ohshimo, S., Guzman, J., Costabel, U. (2011): Pulmonary alveolar proteinosis: New insights from a single-center cohort of 70 patients. Respir Med 105:1908-1916.
3. Bonella, F., Bauer, P.C., Griese, M., Wessendorf, T.E., Guzman, J., Costabel, U. (2012): Wash-out kinetics and efficacy of a modified lavage technique for alveolar proteinosis. Eur Respir J 40:1468-1474.
4. Bonella, F., Ohshimo, S., Cai, M., Griese, M., Guzman, J., Costabel, U. (2013): Serum KL-6 is a predictor of outcome in pulmonary alveolar proteinosis. Orphanet J Rare Dis 8:53-63.
5. Bonella, F., Wessendorf, T.E., Costabel, U. (2013): Clinical experience with pirfenidone for the treatment of idiopathic pulmonary fibrosis. Dtsch Med Wochenschr 138:518-523.

Original Articles (coauthor)

1. Ferrari, M., Benini, L., Brotto, E., Locatelli, F., De Iorio, F., Bonella, F., Tacchella, N., Corradini, G., Lo Cascio, V., Vantini, I. (2007): Omeprazole reduces the response to capsaicin but not to methacholine in asthmatic patients with proximal reflux. Scand J Gastroenterol 42:299-307.
2. Miori, R., Paolazzi, G., Albertazzi, R., Manica, P., Inchiostro, S., Bonella, F., Bortolotti, R., Mariani, A.M., Bortoli P. (2008): Phytothermotherapy with fermenting alpine grass in knee osteoarthritis: mid-long term results. Reumatismo 60:282-289.

3. Ferrari, M., Bonella, F., Benini, L., Ferrari, P., De Iorio, F., Testi, R., Lo Cascio, V. (2008): Acid reflux into the esophagus does not influence exercise-induced airway narrowing in bronchial asthma. Br J Sports Med 42:545-549.
4. Ohshimo, S., Bonella, F., Cui, A., Costabel, U., Guzman, J. (2009): Significance of bronchoalveolar lavage for the diagnosis of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 179:1043–1047.
5. Ohshimo,S., Grammann, N., Starke, K., Bonella, F., Cui, A., Teschler, H., Kohno, N., Guzman, J., Costabel, U. (2009): Serum KL-6 as a novel disease marker in cystic fibrosis. Sarcoidosis Vasc Diffuse Lung Dis 26:47-53.
6. Cui, A., Anhenn, O., Theegarten, D., Ohshimo, S., Bonella, F., Sixt, S.U., Peters, J., Sarria, R., Guzman, J., Costabel, U. (2010): Angiogenic and angiostatic chemokines in idiopathic pulmonary fibrosis and granulomatous lung disease. Respiration 80:372-378.
7. Ohshimo, S., Bonella, F., Sommerwerck, U., Teschler, H., Kamler, M., Jakob, H.G., Kohno, N., Guzman, J., Costabel, U. (2011) Comparison of serum KL-6 versus bronchoalveolar lavage neutrophilia for the diagnosis of bronchiolitis obliterans in lung transplantation. J Heart Lung Transplant 30:1374-1380.
8. Tardivo, S., Zerman, T., Frizzera, S., Locatelli, F., Ferrari, P., Schenk, K., Bonella, F., Tomaello, L., Posenato, C., Meneghello, L., Ferrari, M. (2012): Self-reported asthma and respiratory symptoms among Italian amateur athletes. Eur J Sport Sci 12:96-102.
9. Kida, Y., Ohshimo, S., Ota, K., Tamura, T., Otani, T., Une, K., Sadamori, T., Iwasaki, Y., Bonella, F., Hattori, N., Hirohashi, N., Guzman, J., Costabel, U., Kohno, N., Tanigawa, K. (2012): KL-6, a human MUC1 mucin, as a prognostic marker for diffuse alveolar hemorrhage syndrome. Orphanet J Rare Dis 7:99-105.

10. Horimasu, Y., Hattori, N., Ishikawa, N., Kawase, S., Tanaka, S., Yoshioka, K., Yokoyama, A., Kohno, N., Bonella, F., Guzman, J., Ohshimo, S., Costabel, U. (2012) Different MUC1 gene polymorphisms in German and Japanese ethnicities affect serum KL-6 levels. Respir Med 106:1756-1764.
11. Theegarten, D., Müller, H.M., Bonella, F., Wohlschlaeger, J., Costabel, U. (2012): Diagnostic approach to interstitial pneumonias in a single centre: report on 88 cases. Diagn Pathol 7:160-168.
12. Cai, M., Bonella, F., Dai, H., Sarria, R., Guzman, J., Costabel, U. (2013): Immunobiology. Macrolides inhibit cytokine production by alveolar macrophages in bronchiolitis obliterans organizing pneumonia. Immunobiology. Immunobiology 218:930-937.
13. Cai, M. Bonella, F., He, X., Sixt, S.U., Sarria, R., Guzman, J., Costabel, U. (2013): CCL18 in serum, BAL fluid and alveolar macrophage culture supernatant in interstitial lung diseases. Respir Med 107:1444-52.
14. Ohshimo, S., Ishikawa, N., Horimasu, Y., Hattori, N., Hirohashi, N., Tanigawa, K., Kohno, N., Bonella, F., Guzman, J., Costabel, U. (2014): Baseline KL-6 predicts increased risk for acute exacerbation of idiopathic pulmonary fibrosis. Respir Med doi: 10.1016/j.rmed. 2014.04.009.
15. Horimasu, Y., Hattori, N., Ishikawa, N., Tanaka, S., Bonella, F., Ohshimo, S., Guzman, J., Costabel, U., Kohno, N.(2014): Differences in serum SP-D levels between German and Japanese subjects are associated with SFTPD gene polymorphisms. BMC Med Genet 15:4. doi: 10.1186/1471-2350-15-4.

Reviews and Editorials

1. Biasi, D., Carletto, A., Caramaschi, P., Bonella, F., Barbara, V., Pacor, M.L., Barbara, L.M. (2003): Neutrophils in rheumatoid inflammation. Recenti Prog Med 94:25-30.
2. Caramaschi, P., Biasi, D., Lestani, M., Carletto, A., Bonella, F., Bambara, L.M. (2004): Erdheim-Chester disease. Recenti Prog Med 95:104-107.
3. Costabel, U., Guzman, J., Bonella, F., Ohshimo, S. (2007): BAL in other granulomatous diseases. Semin Respir Crit Care Med 28:514-524.
4. Bauer, P., Bonella, F., Guzman, J., Costabel, U. (2009): Die Bronchoalveoläre Lavage aus klinischer Sicht. Der Pneumologe 6:233-242.
5. Freytag, T., Bonella, F., Costabel, U. (2010): Lungensarkoidose. Diagnostik und Therapie. Internistische Praxis 4:725-734.
6. Costabel, U., Bonella, F., Ohshimo, S., Guzman, J. (2010): Diagnostic modalities in sarcoidosis: BAL, EBUS, and PET. Semin Respir Crit Care Med 31:404-408.
7. Costabel, U., Bonella, F. (2011): Treatment of pulmonary fibrosis. New substances and new interventions. Internist (Berl.) 52:1422-1428.
8. Costabel, U., Bonella, F., Guzman, J. (2012): Chronic hypersensitivity pneumonitis. Clin Chest Med 33: 151-163.
9. Ohshimo, S., Bonella, F., Guzman, J., Costabel, U. (2012): Hypersensitivity pneumonitis. Immunol Allergy Clin North Am 32:537-556.
10. Bonella, F., Caramaschi, P. (2013): The ambitious goal of validating prognostic biomarkers for systemic sclerosis-related interstitial lung disease. J Rheumatol 40:1034-1036.
11. Antoniou, K.M., Margaritopoulos, G.A., Tomassetti, S., Bonella, F., Costabel, U., Poletti,V. (2014): Interstitial lung disease. Eur Respir Rev Mar 23:40-54.
12. Bonella, F., Costabel, U. (2014) Biomarkers in connective tissue disease-associated interstitial lung disease. Semin Respir Crit Care Med 35:181-200.

13. Wuyts, W.A., Cavazza, A., Rossi, G., Bonella, F., Sverzellati, N., Spagnolo, P. (2014) Differential diagnosis of usual interstitial pneumonia - when is it truly idiopathic? Eur Res Rev in press.

Chapters in Textbooks and Monographs:

1. Bonella, F., Ohshimo, S., Bauer, P., Guzman, J., Costabel, U. (2010): Bronchoalveolar lavage. In: Strausz, J., Bolliger, C.T. (Eds): Interventional Pneumology. Ed. European Respiratory Society Monograph. Vol.48; S. 59-72.
2. Bonella, F., Uzaslan, E., Guzman, J., Costabel, U. (2010): Bronchoalveolar lavage in drug induced lung disease: an update. In: Camus, P., Rosenow III, E.C. (Eds): Drug Induced and Iatrogenic Lung Disease. Ed. Hodder Arnold. S. 32-42. London NW1 3BH, England.
3. Bonella, F., Theegarten, D., Guzman, J., Costabel, U. (2011): Alveolar lipoproteinosis syndromes. In Cordier, J.F. (Ed): Orphan Lung Diseases. Ed. European Respiratory Society Monograph. Vol.54: S.171-186.

Case Reports:

1. Bonella, F. (2005): Casi pro diagnosi. Fibrosi polmonare idiopatica. Case report n° 1. Momenti di Medicina e Chirurgia 8: S126.
2. Schroers, R., Bonella, F., Tötsch, M., Costabel, U. (2010): A female soccer player with recurrent haemoptysis and iron deficiency anaemia: idiopathic pulmonary haemosiderosis (IPH)-case report and literature review. BMJ Case Reports doi:10.1136/bcr.06.2009.1969.
3. Vanderhelst, E., Bonella, F., Wissing, K., Verfaillie, G., Geers, C., Vincken, W.(2012): An incident case of primary acquired pulmonary alveolar proteinosis. Acta Clin Belg 67:135-136.
4. Vanderhelst, E., Hanon, S., Verbanck, S., Schuermans, D., Wissing, K., Bonella, F., Vincken, W. (2012): Whole-lung lavage: a successful treatment for restoring acinar ventilation distribution in primary acquired pulmonary alveolar proteinosis. Respiration 84:70-74.