Journal of Inherited Metabolic Disease

# Inhaled sargramostim induces resolution of pulmonary alveolar proteinosis in lysinuric protein intolerance

--Manuscript Draft--

| | |
|---|---|
| **Manuscript Number:** | BOLI-D-16-00215R1 |
| **Full Title:** | Inhaled sargramostim induces resolution of pulmonary alveolar proteinosis in lysinuric protein intolerance |
| **Article Type:** | Original Article |
| **Keywords:** | lysinuric protein intolerance; aminoaciduria; pulmonary alveolar proteinosis; sargramostim; granulocyte-macrophage colony-stimulating factor |
| **Manuscript Classifications:** | 90: Other amino acid disorders |
| **Corresponding Author:** | Laura M Tanner, M.D., Ph.D<br>Turku University Hospital<br>Turku, FINLAND |
| **Corresponding Author Secondary Information:** | |
| **Corresponding Author's Institution:** | Turku University Hospital |
| **Corresponding Author's Secondary Institution:** | |
| **First Author:** | Laura M Tanner, M.D., Ph.D |
| **First Author Secondary Information:** | |
| **Order of Authors:** | Laura M Tanner, M.D., Ph.D |
| | Johanna Kurko, Ph.D |
| | Maaria Tringham, Ph.D |
| | Heikki Aho, M.D, Ph.D |
| | Juha Mykkänen, Ph.D |
| | Kirsti Näntö-Salonen, M.D., Ph.D |
| | Harri Niinikoski, M.D., Ph.D |
| | Heikki Lukkarinen, M.D., Ph.D |
| **Order of Authors Secondary Information:** | |
| **Funding Information:** | Päivikki ja Sakari Sohlbergin Säätiö — Dr. Johanna Kurko |
| | Magnus Ehrnroothin Säätiö — Dr. Johanna Kurko |
| **Abstract:** | Pulmonary alveolar proteinosis (PAP) is a potentially fatal complication of lysinuric protein intolerance (LPI), an inherited disorder of cationic amino acid transport. The patients often present with mild respiratory symptoms, which may rapidly progress to acute respiratory failure responding poorly to conventional treatment with steroids and bronchoalveolar lavations (BALs). The pathogenesis of PAP in LPI is still largely unclear. In previous studies, we have shown disturbances in the function and activity of alveolar macrophages of these patients, suggesting that increasing the activity and the number of macrophages by recombinant human GM-CSF (rhuGM-CSF) might be beneficial in this patient group.<br><br>Two LPI patients with complicated PAP were treated with experimental inhaled rhuGM-CSF (sargramostim) after poor response to maximal conventional therapy. BAL fluid and cell samples from one patient were studied with light microscopy and transmission electron microscopy. |

| | |
|---|---|
| | Excellent response to therapy was observed in Patient 1 with no compliance problems or side effects. Macrophages with myelin figure -like structures were seen in her BAL sample. Slight improvement of the pulmonary function was evident also in Patient 2, but the role of sargramostim could not be properly evaluated due to the complicated clinical situation.<br><br>In conclusion, inhaled rhuGM-CSF might be of benefit in patients with LPI-associated PAP. |

Dear Chief Editor,

Attached please find our revised manuscript entitled ”Inhaled sargramostim induces resolution of alveolar proteinosis in lysinuric protein intolerance” to be considered for publication as an original article in JIMD Reports. We hope that we have been able to satisfactorily address all the issues raised by the reviewers. We appreciate your time and look forward to your response.

Best regards,

Laura Tanner

**Responses to the reviewer comments**

We thank the Reviewers for their careful reading of our manuscript and for their insightful comments. Following their suggestions, we have now added several improvements to the manuscript, which have been listed below.

**Comments by Reviewer 1.**

**Abstract: I would delete the sentence: "However further studies on this subject are warranted".**

This sentence has been deleted, as suggested.

**Introduction, line 40 replace hospitalization by management. Next page line 7 replace stimulate by promote**

The suggested changes have been made.

**General: more details should be given on metabolic treatment: amount of natural protein restriction? Dosages of nitrogen scavengers? Could the authors comment on the heterogeneity of the two patients'presentation (different ages)as a potential limitation of the study?**

Dosages of nitrogen scavengers, as well as dosages of L-citrulline and L-lysine and the amount of protein restriction have now been added to the manuscript. The following sentence has been added to Discussion, Paragraph 4, Line 6:

*However, comparing the outcomes is complicated by the marked age difference between the patients.*

**Next page, line 24: anemization sounds unfrequently used.**

The sentence has been modified as follows:

*Five days after hospital admission, the patient developed severe epistaxis, which led to severe anaemia requiring red blood cell transfusions.*

**Discussion: second paragraph, line 58: NO production in LPI. Can the authors comment on the apparent contradiction between these results (low NO in LPI macrophages) and the hypothesis from the Italian groups regarding intracellular trapping and subsequent high intracellular NO?**

Since $y^+$LAT1 is the most important transporter of arginine in macrophages, capable of both influx and efflux, its defectiveness may have different impact on the macrophage NO synthesis than that of cultured fibroblasts (Mannucci et al. 2005, Ref 15877200) in which $y^+$LAT2 is known to compensate the deficient $y^+$LAT1. Therefore, since the work by

Mannucci et al. 2005 has been performed on fibroblasts, different from macrophages, we prefer to refer to the works by Sebastio et al. 2011 and Ogier de Baulny et al. 2012 introducing the prevailing hypothesis of high intracellular arginine levels in macrophages. Consequently, we modified the text in the discussion section as follows:

*We hypothesized that, in contrast to the earlier suggestions by other groups (Sebastio et al. 2011 and Ogier de Baulny et al. 2012), arginine reservoirs and subsequent NO levels may actually be diminished in LPI macrophages as a result of reduced arginine influx by the defective $y^+$LAT1 transporter, known to be the most important transporter of arginine in macrophages, including those of alveolar origin (Barilli et al. 2011, Rotoli et al. 2007). In addition, the defective CAA transport also results in impaired phagocytic activity of LPI macrophages (Barilli et al. 2012).*

**In conclusion, line 6: I would delete "small patient cohort" and replace it by "data on these 2 patients etc".**

The sentence has been modified as suggested.

**Comments by Reviewer 2:**
**Comment: Overall the case reports are well described and the conclusions are fairly supported by the clinical evidences. However, findings reported do not appear that novel, and conclusions should be modified accordingly, before the paper is suitable for publication**.

**Indeed, Barilli et al. already described in 2010 the benefits deriving from inhaled GM-CSF for an LPI patient (Orphanet J Rare Dis. 2010 Nov 26;5:32.; doi: 10.1186/1750-1172-5-32); the authors actually cite that paper, but they only refer "to the defective CAA transport by y+LAT1" observed in macrophages (line 2, page 10). The efficacy of GM-CSF, shown by those authors, needs to be clearly stated in the Introduction and Discussion.**

**Moreover, in the present ms., authors indicate that they "have recently shown disturbances in the function and activity of monocyte-derived macrophages (Kurko et al. 2015)", with reference to a dysfunction in macrophage toll-like receptor signaling. The authors forgot to cite that in another paper Barilli et al. "demonstrate that the phagocytic activity of LPI macrophages is severely impaired" (Mol Genet Metab. 2012 Apr;105(4):585-9. doi: 10.1016/j.ymgme.2012.01.008). The issue should be, hence, better discussed.**

We thank the Reviewer of these constructive comments. The following modifications have now been made to better emphasize the significance of the aforementioned reports.

*Introduction, Paragraph 3, Line 1:*

*Although the exact pathogenesis of PAP in LPI has been unclear, disturbances in the function and phagocytic activity of monocyte-derived macrophages have been demonstrated (Barilli et al. 2010, Barilli et al. 2012, Kurko et al. 2015).*

*Introduction, Paragraph 3, Line 9:*

*Barilli et al. (2010) have previously reported one Italian patient diagnosed with LPI-associated PAP at the age of 15 years, whose respiratory condition and CT showed marked improvement after rGM-CSF treatment. However, the authors were naturally unable to draw conclusions on the efficacy of rGM-CSF in LPI patients based on a single patient case.*

*Discussion, Paragraph 4, Line 1:*

*Our experiences based on two patient cases together with the case previously reported by Barilli et al. (2010) suggest that inhaled rhuGM-CSF may be useful in LPI-associated pulmonary alveolar patients.*

# Inhaled sargramostim induces resolution of pulmonary alveolar proteinosis in lysinuric protein intolerance

Laura M. Tanner[1,2], Johanna Kurko[2], Maaria Tringham[2,3], Heikki Aho[4], Juha Mykkänen[5,6], Kirsti Näntö-Salonen[6], Harri Niinikoski[6], Heikki Lukkarinen[6].

1 Department of Clinical Genetics, Turku University Hospital, Turku, Finland

2 Department of Medical Biochemistry and Genetics, University of Turku, Turku, Finland

3 Functional Foods Forum, University of Turku, Turku, Finland

4 Department of Pathology, Turku University Hospital and University of Turku, Turku, Finland

5 Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Turku, Finland

6 Department of Pediatrics, Turku University Hospital and University of Turku, Turku, Finland

Correspondence:

Laura M. Tanner, MD, PhD

Department of Clinical Genetics, Turku University Hospital

PL 52, FIN-20521 Turku, Finland

e-mail: lamaer@utu.fi

Word count: text 2510, summary 206

5 figures, 2 tables (Figure 2 is a colour picture which may be used for the front cover of the issue)

Take home message: Inhaled sargramostim induces resolution of pulmonary alveolar proteinosis associated with lysinuric protein intolerance.

Abstract

Pulmonary alveolar proteinosis (PAP) is a potentially fatal complication of lysinuric protein intolerance (LPI), an inherited disorder of cationic amino acid transport. The patients often present with mild respiratory symptoms, which may rapidly progress to acute respiratory failure responding poorly to conventional treatment with steroids and bronchoalveolar lavations (BALs). The pathogenesis of PAP in LPI is still largely unclear. In previous studies, we have shown disturbances in the function and activity of alveolar macrophages of these patients, suggesting that increasing the activity and the number of macrophages by recombinant human GM-CSF (rhuGM-CSF) might be beneficial in this patient group.

Two LPI patients with complicated PAP were treated with experimental inhaled rhuGM-CSF (sargramostim) after poor response to maximal conventional therapy. BAL fluid and cell samples from one patient were studied with light microscopy and transmission electron microscopy.

Excellent response to therapy was observed in Patient 1 with no compliance problems or side effects. Macrophages with myelin figure –like structures were seen in her BAL sample. Slight improvement of the pulmonary function was evident also in Patient 2, but the role of sargramostim could not be properly evaluated due to the complicated clinical situation.

In conclusion, inhaled rhuGM-CSF might be of benefit in patients with LPI-associated PAP.

Contributions: LT collected the clinical data. LT, JK and HA drafted the manuscript. JK and MT performed the laboratory analyses. HA performed the electron microscopy imaging and provided Figures 1 and 2. JM, KN-S and HN supervised the study. KN-S, HN and HL were responsible for the treatment and follow-up of the patients and provided clinical data. HL planned the treatment protocol. All the authors commented on and approved the manuscript.

Guarantor: Laura Tanner

Compliance with Ethics Guidelines

Conflicts of interest: Laura Tanner, Johanna Kurko, Maaria Tringham, Heikki Aho, Juha Mykkänen, Kirsti Näntö-Salonen, Harri Niinikoski and Heikki Lukkarinen declare that they have no conflict of interest.

Details of funding: Johanna Kurko has received grants from the Päivikki and Sakari Sohlberg Foundation and the Magnus Ehrnrooth Foundation. The authors confirm independence from the sponsors; the content of the article has not been influenced by the sponsors.

The Ethics Committee of the Hospital District of Southwest Finland has approved the follow-up study concerning the pathogenesis of long-term complications of LPI and informed consent has been obtained from all the patients or their parents. A written informed consent was also obtained from the patients before the initiation of the experimental rhuGM-CSF treatment. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000.

Keywords: lysinuric protein intolerance, aminoaciduria, pulmonary alveolar proteinosis, sargramostim, granulocyte-macrophage colony-stimulating factor

## Introduction

Lysinuric protein intolerance (LPI) is a rare inherited amino acid transport disorder leading to decreased plasma concentrations and increased urinary excretion of cationic amino acids (CAAs) arginine, ornithine and lysine by defective $y^+$LAT1 transporter. The patients present with a variety of symptoms including failure to thrive, postprandial hyperammonaemia and haematological and immunological abnormalities, the exact mechanism of which is still unclear. The patients may remain asymptomatic for decades with a carefully planned low-protein diet and supplementation with low-dose oral L-citrulline and ammonia-scavenging drugs. Unfortunately, the current treatment regime does not seem to protect the patients from long-term complications including renal disease and pulmonary alveolar proteinosis (PAP), a rare condition characterised by accumulation of lipoproteinaceous material in the alveoli (Parto et al. 1993, Santamaria et al. 1996). Acute episodes of PAP may occur even in those patients with excellent treatment compliance and no previous respiratory symptoms. Unlike in idiopathic PAP, granulocyte macrophage colony-stimulating factor (GM-CSF) antibodies have not been detected in the LPI patients and the pathogenesis of PAP in LPI patients has thus remained unclear.

The LPI PAP patients often present initially with mild respiratory symptoms, which may progress rapidly and lead to pulmonary insufficiency and multi-organ failure. To date, the only treatment options, i.e. repeated bronchoalveolar lavations (BALs) and systemic corticosteroids, have shown limited effectiveness (Santamaria et al. 2004, Ceruti et al. 2004). During the last ten years, four Finnish pediatric patients from our cohort of over 40 Finnish LPI patients have been diagnosed with acute PAP. Three of them died despite immediate management in an intensive care unit (Table 1). The incidence of PAP has been even larger in other LPI cohorts. Valimahamed-Mitha and colleagues have reported a series of 14 pediatric patients with LPI, out of which ten fulfilled the diagnostic criteria of PAP and six died of pulmonary failure (Valimahamed-Mitha et al. 2015). In a group of nine Italian LPI patients reported by Santamaria and colleagues, one patient died of respiratory insufficiency and five other patients had signs of lung involvement when studied with high-resolution computed tomography (HRCT) imaging despite being asymptomatic at the time of the study (Santamaria et al. 1996).

Although the exact pathogenesis of PAP in LPI has been unclear, disturbances in the function and phagocytic activity of monocyte-derived macrophages have been demonstrated (Barilli et al. 2010, Barilli et al. 2012, Kurko et al. 2015). Thus, accumulation of proteinous material into the lungs may

be caused by insufficient clearance of proteins by poorly functioning alveolar macrophages. Inhaled granulocyte macrophage colony-stimulating factor (GM-CSF) is used off-label in patients with idiopathic PAP with anti-GM-CSF antibodies to activate and attract monocyte-derived macrophages into the lungs. We hypothesized that increasing the activity and the number of alveolar macrophages in the alveolar fluid by recombinant human GM-CSF (rhuGM-CSF) inhalation could promote the resolution of PAP also in LPI. Barilli et al. (2010) have previously reported one Italian patient diagnosed with LPI-associated PAP at the age of 15 years, whose respiratory condition and CT showed marked improvement after rGM-CSF treatment. However, the authors were naturally unable to draw conclusions on the efficacy of rGM-CSF in LPI patients based on a single patient case.

Here, we describe two Finnish LPI patients, one child and one adult, with complicated PAP treated with experimental inhaled rhuGM-CSF (Sargramostim, Leukine®, Genzyme) after already receiving maximal conventional therapy.

## Patients and methods

### Patients

The research was conducted according to the principles of the Declaration of Helsinki. A written informed consent was obtained from the patients before the initiation of the experimental rhuGM-CSF treatment.

### Methods

#### BAL fluid and cell sample collections

BAL fluid was collected routinely from Patient 1. After Cyto-Tek and Cytospin cytocentrifugations of the BAL sample, routine Papanicolaou, May-Grünwald-Giemsa, Prussian blue and periodic acid -Schiff (PAS)-stained slides were prepared and studied under a light microscope. In addition, a part of the BAL fluid was filtered through a sterile gauze. The filtered cells were centrifuged 250 g for ten minutes, washed with ice cold HBSS and suspended in the RPMI-1640 medium with a GlutaMAX supplement (Invitrogen, Life Technologies, Carlsbad, CA, USA) and 10% FBS before the following experiments.

#### Transmission electron microscopy

The centrifuged cell pellet was fixed with 5% glutaraldehyde overnight and osmium tetroxide was added to fix the sample for 2 h. The sample was then dehydrated with ethanol and embedded with propylenoxid in epoxy resin. Ultrathin sections contrasted with uranyl acetate and lead citrate were studied under the Jeol JEM-1400Plus transmission electron microscope (Jeol, Tokyo, Japan).

Histology

The cells for histopathological examination were fixed in 10% buffered formalin, centrifuged and pre-embedded in agar. Then, the agar blocks were further embedded in paraffin and routine 4 μm thick histological sections were cut on slides. The sections were stained with hematoxylin and eosin, and PAS, and studied under a light microscope.

**Results**

In the BAL sample of Patient 1, a total of 520 million cells per litre were detected. Of these, 55% were macrophages, 42% lymphocytes and the remaining 3% neutrophils. Cytological bronchoalveolar preparations showed macrophages which contained PAS-positive granules. Similar granules were also seen around the cells. In addition, the histological sections contained macrophages with PAS-positive granules. (Figure 1) In a sample studied with electron microscopy, several macrophages containing lysosomes as well as myelin figure-like structures were observed (Figure 2).

Patient 1.

The patient is a girl who was diagnosed with LPI at the age of 1 year after her older sister had been diagnosed with LPI. At that time, she had no clinical findings except for the characteristic urinary amino acid profile and elevated serum ferritin. Her treatment regime consisted of oral L-citrulline (100-200 mg/kg/day), sodium benzoate (125-250 mg/kg/day) and protein-restricted diet (1-1.2 g/g/day) supplemented with calcium carbonate, vitamins, L-lysine hydrochloride (13-17 mg/kg/day) and commercial carbohydrate energy supplements. She had mild growth retardation, mild proteinuria and recurrent urinary tract infections but was previously otherwise quite healthy and regularly participated in sports activities.

At the age of 12 years, she had an acute episode of dyspnea and cough without symptoms of a respiratory infection. HRCT imaging suggested acute PAP. She was treated with repeated BALs and high-dose corticosteroids with a partial response but she remained dependent on supplemental oxygen. Hypertonic saline and salbutamol inhalations were not of significant benefit. After several large-volume BALs, her respiratory function gradually improved and supplemental oxygen was discontinued three months after the initial episode. However, only a couple of months later her pulmonary function started to worsen again and she developed dyspnea and cough. A new BAL was performed. Three weeks later, the patient developed symptoms of pneumonia, and a PCR test performed on the lavation fluid was shown to be positive for human herpesvirus 6 (HHV-6) and *Mycoplasma pneumoniae*. She was treated in an intensive care unit with intravenous antibiotics and BALs which were of benefit, and her condition improved. However, regular BALs were continued as a prophylactic treatment.

During the following years, her condition remained relatively stable; she was able to participate in sports activities to some extent and the glucocorticoid treatment was gradually weaned off. At the age of 16, her pulmonary function again decreased rapidly and she developed progressive dyspnea and poor exercise tolerance. The response to high-dose corticosteroids was poor. HRCT revealed an acute episode of PAP. BAL was performed several times and surfactant was given to prevent atelectasis after the procedure. Chest physiotherapy was also initiated to improve lung function. After six months, her condition had not improved and she remained dependent on supplemental oxygen. Due to a poor overall prognosis, an experimental treatment with inhaled rhuGM-CSF (sargramostim) was offered.

The treatment started with 125 μg of rhuGM-CSF in 2 ml of saline given twice a day with PARI BOY nebuliser every other week for 12 weeks. Inhalation was well tolerated. Blood leukocyte counts and liver enzymes were followed up weekly, but no adverse effects were observed during the treatment. After no more than two weeks on rhuGM-CSF, her lung function had significantly improved and the radiographic findings had ameliorated (Figure 3 and Figure 4). After three courses (one week each) with rhuGM-CSF, supplemental oxygen was discontinued and tapering off corticosteroids was initiated. Two years after the treatment the pulmonary function has remained normal and no relapses have occurred. The patient is currently able to participate normally in sports activities and inhaled asthma medications have been discontinued. The respiratory function before and after the rhuGM-CSF treatment are summarized in Table 2.

Patient 2.

The patient was a 58-year-old woman who was diagnosed with LPI in her early childhood. Her treatment regime consisted of a protein-restricted diet (about 1 g/kg/day) together with oral L-citrulline (about 2500 mg/day), L-lysine (750 mg/day), sodium benzoate (2000 mg/day) and calcium and vitamin supplements. At the age of 49 years, a statin therapy was initiated for combined hyperlipidemia and mild renal insufficiency was diagnosed at the age of 50 years. She also had a tendency to bruising and nosebleeds. At the age of 57 years, she complained of prolonged cough and dyspnea. A BAL was performed twice but the symptoms persisted and the patient also had an episode of pneumonia. Radiological findings were consistent with PAP. The patient was given oral and inhaled steroids without any effect. The patient became dependent on supplementary oxygen and was subsequently hospitalized. HRCT and pulmonary function tests could not be performed due to her poor general condition. Inhaled rhuGM-CSF treatment (125 μg) was initiated with the protocol described above, along with oral prednisolone (40 mg x 1), furosemide (40 mg x1) and antibiotics (oral doximycin and intravenous moxifloxacin). The clinical condition of the patient improved enough for her to be released from hospital although still dependent on supplementary oxygen, and significant radiographic improvement was also observed (Figure 5). Two weeks later, she was readmitted because of severe dyspnea and elevated CRP (90 mg/L). A treatment with intravenous cefuroxime was initiated and the prednisolone dose was doubled to 40 mg x 2. A chest radiograph showed mildly increased alveolar opacity of the left lung. Five days after hospital admission, the patient developed severe epistaxis, which led to severe anaemia requiring red blood cell transfusions. After two weeks, the patient was again released from hospital as her CRP had normalized. The rhuGM-CSF dose was increased to 250 μg x 1 and inhaled corticosteroid was also initiated. With this regime, her condition seemed to remain stable. However, two months later she was again admitted to hospital due to severe epistaxis, and posterior tamponade was performed. Shortly afterwards, she was diagnosed with fulminant staphylococcal septicaemia, of which she subsequently died.

## Discussion

Pulmonary alveolar proteinosis is a relatively poorly known clinical entity, which can develop with a variety of mechanisms including genetic defects of the GM-CSF protein or its receptor, neutralizing anti-GM-CSF antibodies, infections and malignancies (Campo et al. 2012). Patients with LPI are predisposed to secondary PAP, the prognosis of which seems to be especially poor in

paediatric patients (Parto et al. 1993, Parto et al. 1994). The initial symptoms of PAP may be very subtle, making the diagnosis challenging. However, the disease can rapidly progress to a life-threatening multi-organ failure and a careful monitoring of any LPI patient with dyspnea, unexplained fatigue or fever in absence of other signs of an infection is therefore warranted.

Toll-like receptor signalling, especially a response to viral DNA, has been shown to be impaired and nitric oxide (NO) production decreased in LPI monocyte-derived macrophages reinforcing the role of macrophages in the development of secondary complications of LPI (Kurko et al. 2015). We hypothesized that, in contrast to the earlier suggestions by other groups (Sebastio et al. 2011 and Ogier de Baulny et al. 2012), arginine reservoirs and the subsequent NO levels may actually be diminished in LPI macrophages as a result of reduced arginine influx by the defective $y^+$LAT1 transporter, known to be the most important transporter of arginine in macrophages, including those of alveolar origin (Barilli et al. 2011, Rotoli et al. 2007). In addition, the defective CAA transport also results in impaired phagocytic activity of LPI macrophages (Barilli et al. 2012). This may, in turn, lead to an impaired clearance of phospholipoproteinaceous material from the alveoli. It is interesting that iNOS synthesizing NO from arginine is expressed in the airway epithelial cells where the gene encoding $y^+$LAT1 is also expressed (Thomassen and Kavuru 2001, Rotoli et al. 2005). Therefore, NO synthesis in the LPI airway cells could be distorted affecting for example the production of cytokines in alveolar macrophages. Since NO is known to be involved in several inflammatory lung diseases (Thomassen and Kavuru 2001), it would be reasonable to measure NO levels in the airway aspirate or bronchoalveolar lavage fluid of the LPI patients with PAP.

In the study by Douda and colleagues, a supplementation with surfactant protein D (SP-D) and GM-CSF increased the uptake of protein and dying cells *ex vivo* but GM-CSF increased the number of spontaneously generated granulomas (Douda et al. 2009). The authors therefore suggested that GM-CSF might not be a suitable mode of treatment for patients with LPI-associated PAP. The 2-year-old LPI patient in their report had large amounts of cholesterol, cholesterol crystals and lipid-laden macrophages in the airways and the authors suggested that therapies aiming to decrease the amount of cholesterol in the airways might be beneficial instead. On the other hand, in the study by Ohashi and colleagues, the GM-CSF inhalation therapy improved lung clearance and decreased the amount of protein in BAL fluid in a patient with autoimmune alveolar proteinosis (Ohashi et al. 2012). Furthermore, in 2014, Yu and colleagues reported a patient with autoimmune PAP treated successfully with a combination of whole lung lavation and inhaled GM-CSF (Yu et al. 2014). It is therefore logical to assume that rhuGM-CSF might also be useful in patients with LPI-associated

PAP. New, non-invasive therapeutic options would be warmly welcomed as the effectiveness of corticosteroids and whole lung lavation has proven to be inadequate in this patient group. Although repeated whole lung lavage has been shown to alleviate symptoms and reduce hypoxia in PAP, it does not actually stop the progression of the underlying disease (Gao et al. 2014). Also, general anaesthesia is required to perform the whole lung lavage, which may present a challenge due to the pre-existing respiratory failure.

Our experiences based on two patient cases together with the case previously reported by Barilli et al. (2010) suggest that inhaled rhuGM-CSF may be useful in LPI-associated pulmonary alveolar patients. In Patient 1, an excellent response to therapy was observed with no compliance problems or side effects. In Patient 2, the role of rhuGM-CSF could not be properly evaluated due to the complicated clinical situation, but slight improvement in the pulmonary function was observed also in this patient. However, comparing the outcomes is complicated by the marked age difference between the two patients.

In conclusion, the data on these two patients suggest that inhaled rhuGM-CSF might be of benefit in patients with LPI-associated PAP responding inadequately to high-dose corticosteroids and lung lavations. Such PAP often carries a dismal prognosis, especially in paediatric patients. However, further studies on this subject are warranted.

References

Barilli A, Rotoli BM, Visigalli R et al. (2010) In Lysinuric Protein Intolerance system y+L activity is defective in monocytes and in GM-CSF -differentiated macrophages. *Orphanet J Rare Dis* 5:32.

Barilli A, Rotoli BM, Visigalli R, Bussolati O, Gazzola GC, Dall´Asta V. (2011) Arginine transport in human monocytic leukemia THP-1 cells during macrophage differentiation. *J Leukoc Biol* 90:293-303.

Barilli A, Rotoli BM, Visigalli R et al. (2012) Impaired phagocytosis in macrophages from patients affected by lysinuric protein intolerance. *Mol Genet Metab* 105:585-589.

Campo I, Kadija Z, Mariani F et al. (2012) Pulmonary alveolar proteinosis: diagnostic and therapeutic challenges. *Multidiscip Respir Med* 7:4.

Ceruti M, Rodi G, Stella GM et al. (2007) Successful whole lung lavage in pulmonary alveolar proteinosis secondary to lysinuric protein intolerance: a case report. *Orphanet J Rare Di*s 2:14.

Douda DN, Farmakovski N, Dell S, Grasemann H, Palaniyar N. (2009) SP-D counteracts GM-CSF -mediated increase of granuloma formation by alveolar macrophages in lysinuric protein intolerance. *Orphanet J Rare Dis* 4:29

Gao F, Lu GC, Zhou XY, Yu Z, Wang HM, Bian T. (2014) Repeated whole-lung lavage for unremitting pulmonary alveolar proteinosis: an eight-year follow-up of a case. *Genet Mol Res* 13:6135-6141.

Kurko J, Vähä-Mäkilä M, Tringham M et al. (2015) Dysfunction in macrophage toll-like receptor signalling caused by an inborn error of cationic amino acid transport. *Mol Immunol* 67;416-425.

Ogier de Baulny H, Schiff M, Dionisi-Vici C. (2012) Lysinuric protein intolerance (LPI): a multi organ disease by far more complex than a classic urea cycle disorder. *Mol Genet Metab* 106:12-17.

Ohashi K, Sato A, Takada T et al. (2012) Direct evidence that GM-CSF inhalation improves lung clearance in pulmonary alveolar proteinosis. *Respir Med* 106:284-293.

Parto K, Kallajoki M, Aho H, Simell O. (1994) Pulmonary alveolar proteinosis and glomerulonephritis in lysinuric protein intolerance: case reports and autopsy findings of four pediatric patients. *Hum Pathol* 25:400-407.

Parto K, Svedström E, Majurin ML, Härkönen R, Simell O. (1993) Pulmonary manifestations in lysinuric protein intolerance. *Chest* 104:1176-1182.

Rotoli BM, Bussolati O, Sala R, Gazzola GC, Dall´asta V. (2005) The transport of cationic amino acids in human airway cells: expression of system y+L activity and transepithelial delivery of NOS inhibitors. *FASEB J* 19:810-812.

Rotoli BM, Dall´Asta V, Barilli A et al. (2007) Alveolar macrophages from normal subjects lack the NOS-related system y+ for arginine transport. *Am J Respir Cell Mol Biol* 37:105-112.

Santamaria F, Brancaccio G, Parenti G et al. (2004) Recurrent fatal pulmonary alveolar proteinosis after heart-lung transplantation in a child with lysinuric protein intolerance. *J Pediatr* 145:268-272.

Santamaria F, Parenti G, Guidi G et al. (1996) Early detection of lung involvement in lysinuric protein intolerance: role of high-resolution computed tomography and radioisotopic methods. *Am J Respir Crit Care Med* 153:731-735.

Sebastio G, Sperandeo MP, G. Andria G. (2011) Lysinuric protein intolerance: reviewing concepts on a multisystem disease. *Am J Med Genet C Semin Med Genet* 157:54-62.

Thomassen MJ, Kavuru MS. (2001) Human alveolar macrophages and monocytes as a source and target for nitric oxide. *Int Immunopharmacol* 1:1479-1490.

Valimahamed-Mitha S, Berteloot L, Ducoin H, Ottolenghi C, de Lonlay P, de Blic J. (2015) Lung involvement in children with lysinuric protein intolerance. *J Inherit Metab Dis* 38:257-263.

Yu Hy, Sun Xf, Wang Yx, Xu Zj, Huang H. (2014) Whole lung lavage combined with granulocyte-macrophage colony stimulating factor inhalation for an adult case of refractory alveolar proteinosis. *BMC Pulm Med* 14:87.

| | Age at diagnosis | Age at PAP | Presenting signs of PAP | Treatment for PAP | Result |
|---|---|---|---|---|---|
| 1 | 1 y | 1. 12 y<br>2. 16 y | 1. Dyspnea, dry cough<br>2. Poor exercise tolerance, progressive hypoxia | 1. Large-dose corticosteroids, BAL, supplementary oxygen, saline inhalations, salbutamol<br>2. BAL, peroral steroids and antibiotics, saline inhalations, sargramostim | 1. partial recovery (decreased exercise tolerance, need for regular lung lavations)<br>2. normal pulmonary function, able to participate to sports activities normally 1.5 years after the last episode of PAP |
| 2 | 1 y | 16 y | Fever, cough, epistaxis, hematemesis | Intravenous antibiotics, large-dose corticosteroids, ventilatory support | Multi-organ failure and death two days after admission to hospital |
| 3 | 3 mo | 3 mo | Dry cough, poor eating | Supplemental oxygen, corticosteroids, BAL | Died at the age of 12 months |
| 4 | 8 mo | 4y 8mo | Fever, fatigue, poor appetite, enlarged liver and spleen, enlarged lymph nodes | Intravenous antibiotics, large-dose corticosteroids, BAL | Died ten days after admission to hospital |

Table 1.
Summary of the four Finnish pediatric patients with LPI-related PAP followed up at Turku University Hospital between years 1997 and 2014. Patient n:o 1 is Patient 1 described in the Results section. BAL, bronchoalveolar lavation.

| Date | VC | VC%Ref | FVC(L) | FVC%Ref | FEV1(L) | FEV1%Ref | FEV1/FVC | FEV1/FVC%Ref | PEF(L/min) | PEF%Ref |
|---|---|---|---|---|---|---|---|---|---|---|
| DAY-82 | 1,69 | 55 | 1,68 | 53 | 1,59 | 56 | 94 | 105 | 7,5 | 133 |
| DAY-70 | 1,75 | 56 | 1,65 | 51 | 1,53 | 52 | 92,61 | 101 | 5,56 | 97 |
| **DAY-69 BAL** | | | | | | | | | | |
| DAY-34 | 1,56 | 51 | 1,52 | 48 | 1,39 | 49 | 92 | 102 | 6,06 | 107 |
| DAY-28 | 1,64 | 53 | 1,62 | 51 | 1,5 | 52 | 92 | 103 | 5,78 | 102 |
| **DAY-27 BAL** | | | | | | | | | | |
| DAY-7 | 1,7 | 55 | 1,59 | 50 | 1,5 | 52 | 94 | 105 | 5,89 | 104 |
| **DAY0** | **1,66** | **53** | **1,59** | **49** | **1,49** | **51** | **93,24** | **104** | **6,15** | **107** |
| DAY+14 | 1,62 | 52 | 1,62 | 51 | 1,5 | 53 | 93 | 104 | 6,52 | 116 |
| DAY+28 | 1,58 | 51 | 1,56 | 49 | 1,43 | 50 | 91 | 102 | 6,38 | 113 |
| DAY+42 | 1,71 | 55 | 1,68 | 53 | 1,55 | 54 | 92 | 103 | 6,38 | 113 |
| DAY+51 | 1,76 | 52 | 1,71 | 52 | 1,59 | 55 | 93,39 | 104 | 6,65 | 116 |
| DAY+78 | 2,08 | 67 | 1,93 | 60 | 1,73 | 61 | 90 | 100 | 6,61 | 117 |

Table 2.

Respiratory function of Patient 1 before and after the sargramostim treatment. BAL = bronchoalveolar lavation, VC = vital capacity, FVC = forced vital capacity, FEV1 = forced expiratory volume in 1 second, PEF = peak expiratory flow.

Figure 1

Click here to download Figure Figure 1. LPI PAS.jpg

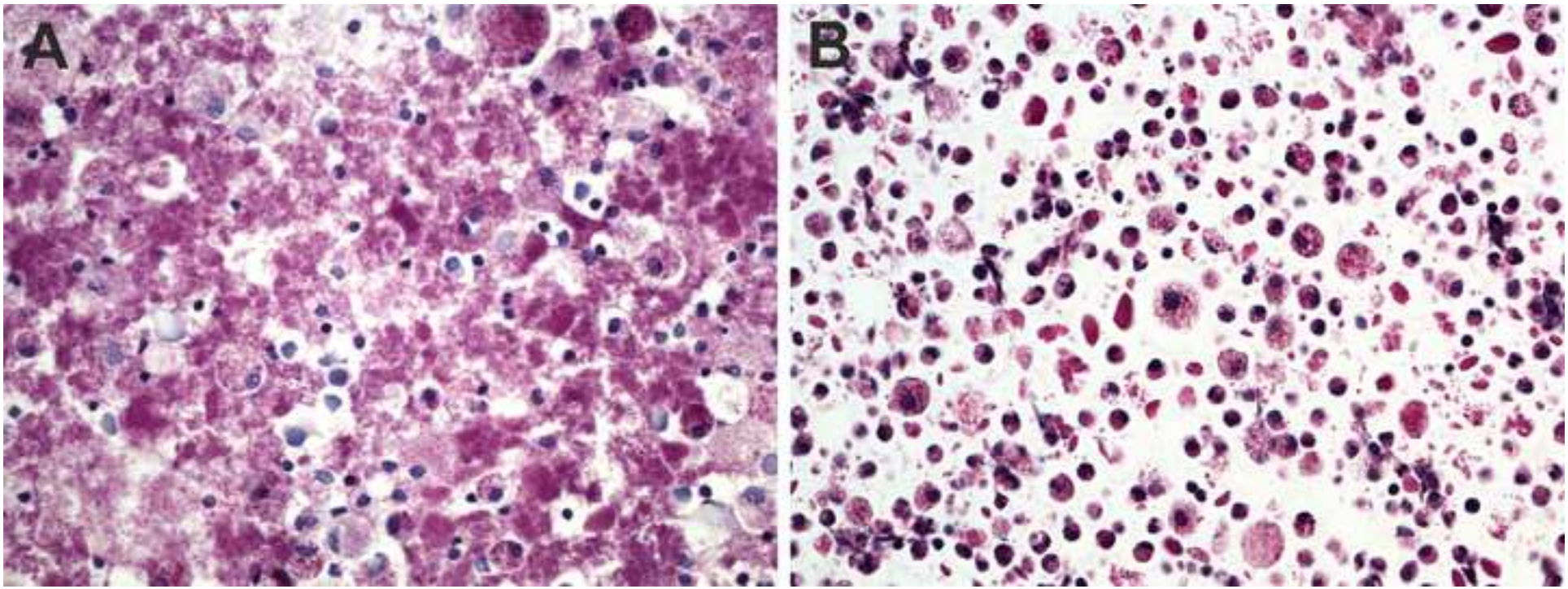

Figure 2.

Click here to download Figure Figure 2. LPI EM macrophage and myelin.jpg

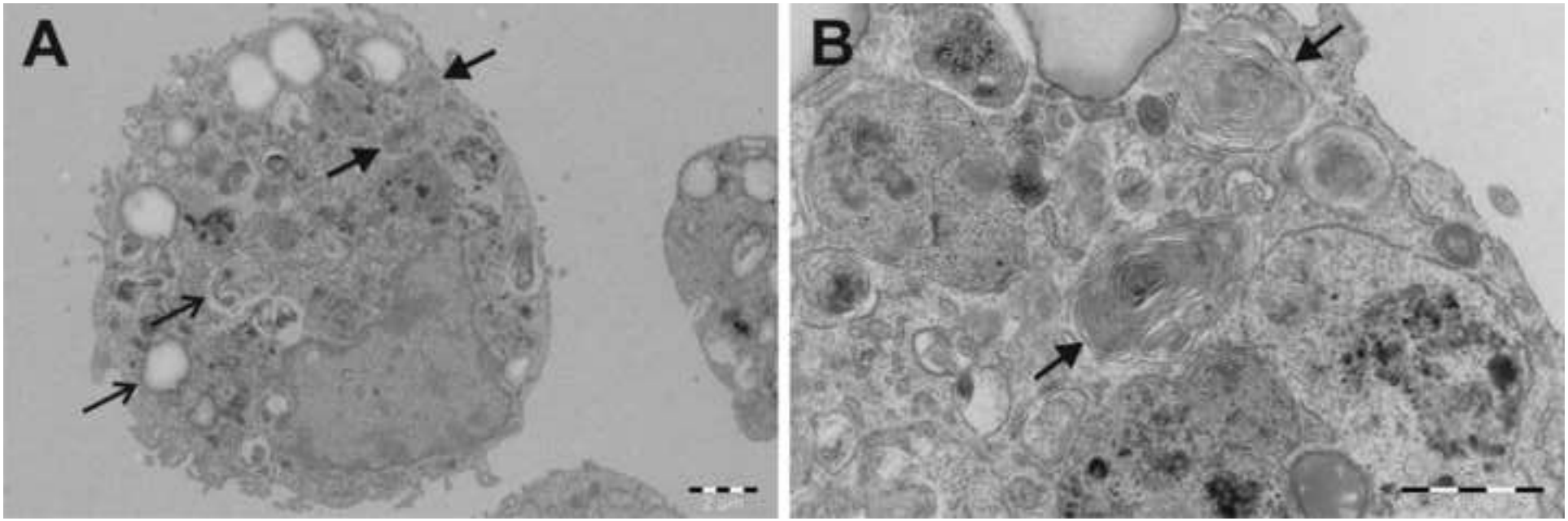

Figure 3.

Click here to download Figure Figure3.jpg

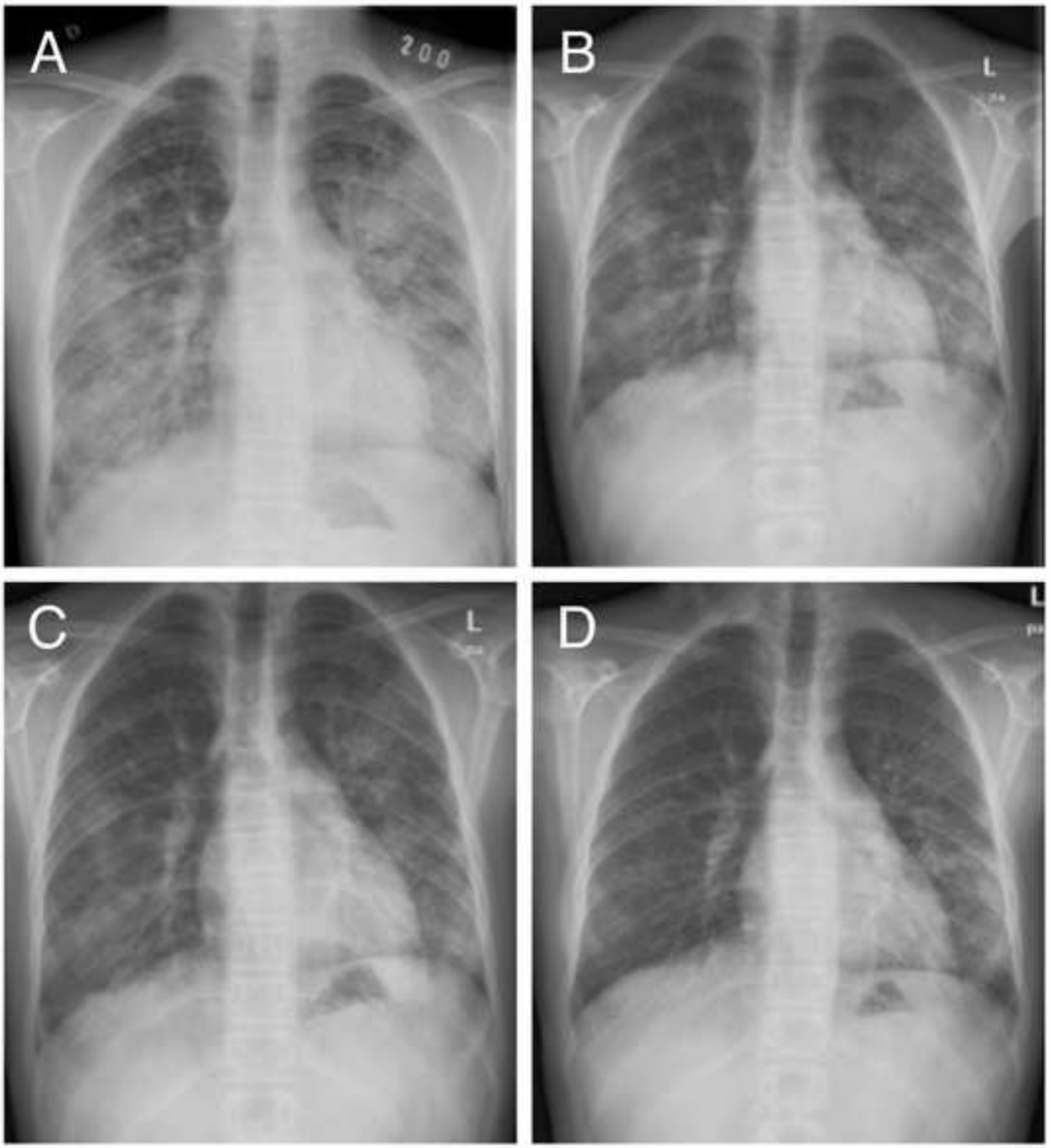

Figure 4.

Click here to download Figure Figure4.jpg

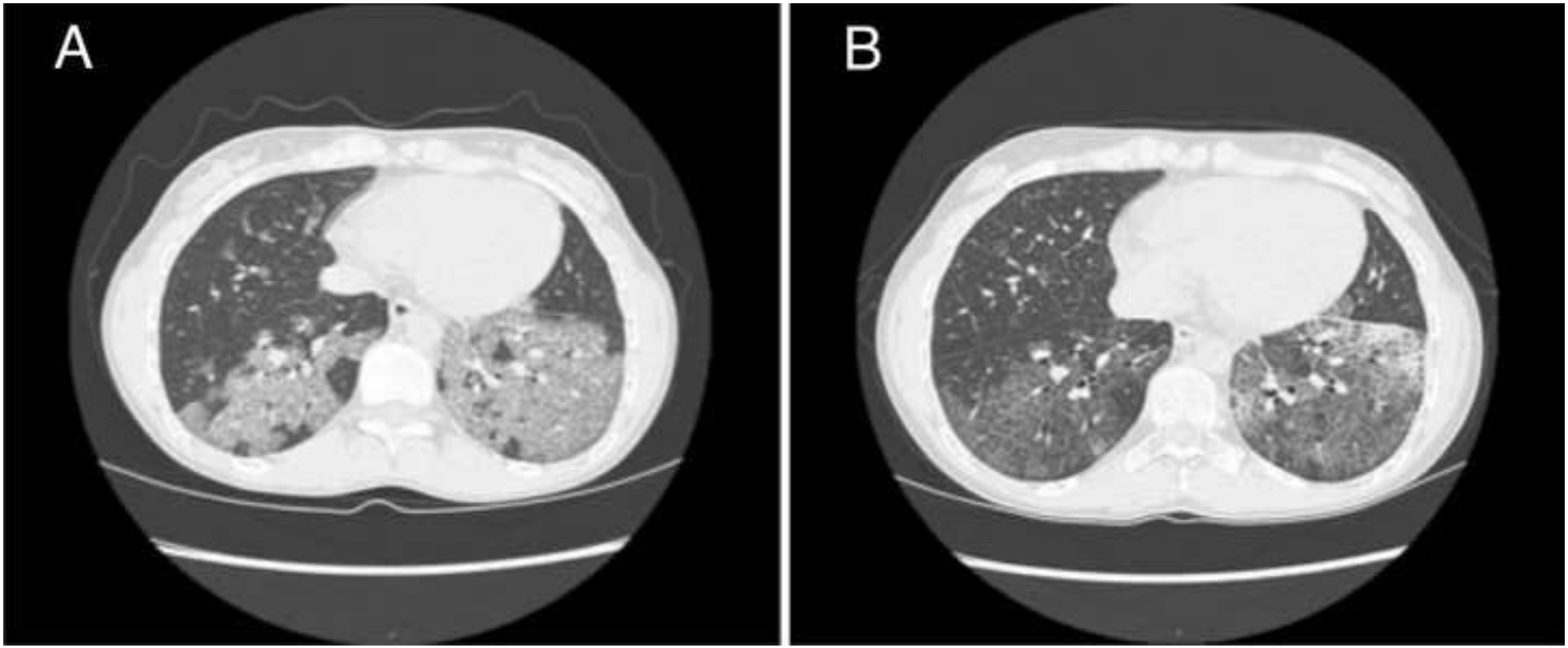

Figure 5.

Click here to download Figure Figure5.jpg

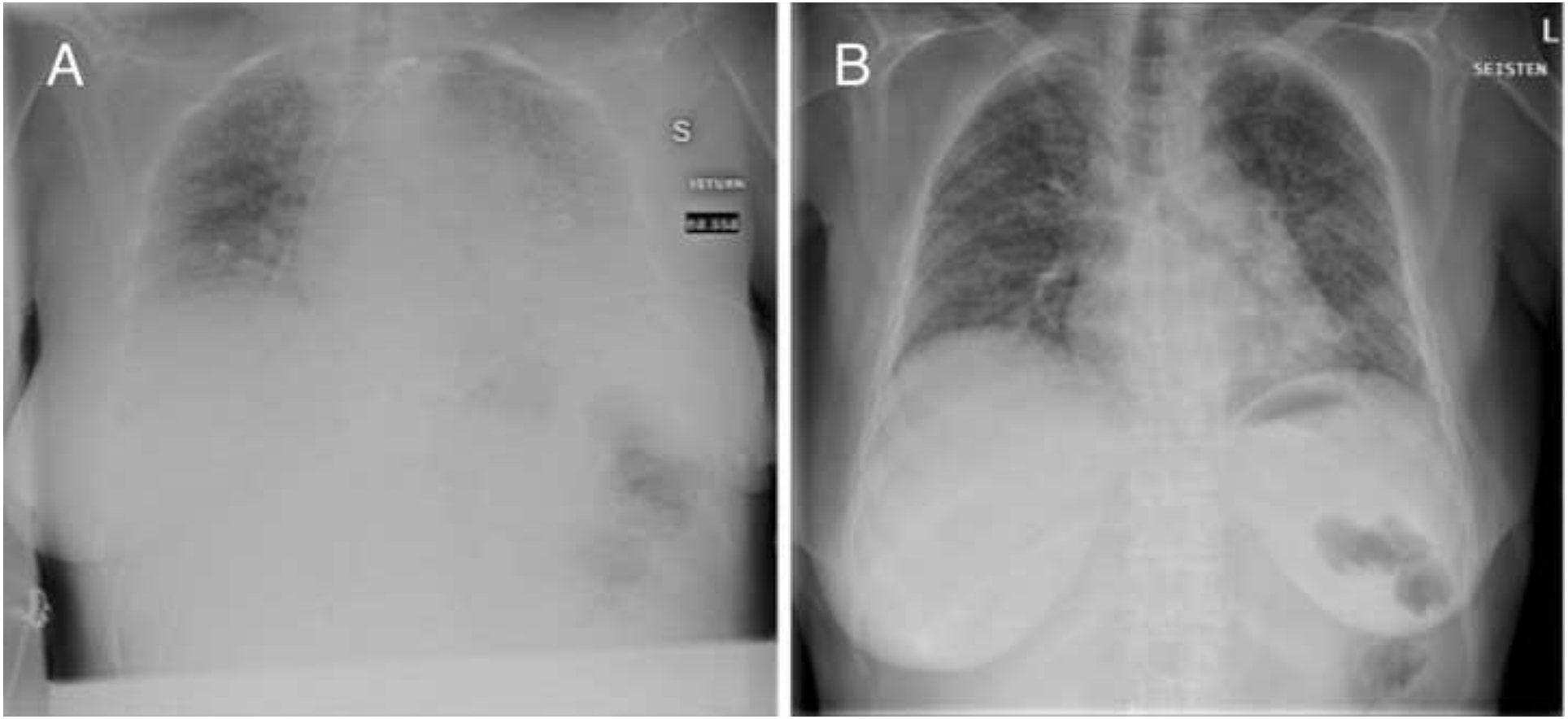

Figure legends

Conflict of Interest Disclosure Form (signed and scanned)

JIMD | JIMD Reports

Springer
the language of science

## Form for Disclosure of Potential Conflict of Interest

**Mandatory Submission Form for Manuscript No. _____**

The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. All authors are responsible for the accuracy and completeness of the submitted information. The corresponding author is requested to sign and submit the scanned form together with the revised manuscript through Editorial Manager.

**1. The Work Under Consideration for Publication**

Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc...)?

This includes:

- Grants
- Consulting fee or honorarium
- Support for travel to meetings the study or other purposes
- Fees for participation in review activities such as data monitoring boards, statistical analysis, end point committees, and the like
- Payment for writing or reviewing the manuscript
- Provision of writing assistance, medicines, equipment, or administrative support.

☐ **No, financial relationships that present a potential conflict of interest**

☑ **Yes, the following financial relationships are present (explain on separate sheet).**

**2. Relevant financial activities outside the submitted work**

Does any of the submitting author have financial relationships (regardless of amount of compensation) with entities in the bio-medical arena that could be perceived to influence, or that give the appearance of potentially influencing, what you wrote in the submitted work. This includes:

- Board Membership
- Consultancy
- Employment
- Expert testimony
- Grants/grants pending
- Payment for lectures including service on speaker bureaus
- Payment for manuscript preparation
- Patents (planned, pending of issued)
- Roaylties
- Payment for development of educational presentations
- Stock/stock options
- Travel/accomodatios/meeting expenses unrelated to activities listed

☑ **No, financial relationships that present a potential conflict of interest**

☐ **Yes, the following financial relationships are present (explain below)**

**3. Other relationships**

Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?

☑ **No other relationships that present a potential conflict of interest**

☐ **Yes, the following relationships/conditions/circumstances are present (explain below)**

Disclosure of Potential Conflict of Interest 10th June 2016

Manuscript:
Tanner et al. Inhaled sargramostim induces resolution of PAP in LPI

During the conduct of this study, Dr. Johanna Kurko received grants from the Päivikki and Sakari Sohlberg Foundation and the Magnus Ehrnrooth Foundation.

If any commercial interests/arrangements are described above, I understand that the staff of the Journal will keep this information confidential but communicate it to the handling Communicating Editor and – if necessary – to the reviewers. I also understand that if the manuscript is accepted, the Editors will discuss with me the manner in which such information is to be communicated to the reader. I hereby grant permission for any such information to be included with publication of the manuscript in the *Journal of Inherited Metabolic Disease*.

Author | Signature, Date

Laura Tanner, MD, PhD | 10th June 2016

Please scan the completed and signed form and then submit it as an electronic file along with your revised manuscript. No manuscripts will be accepted for publication without this form.

Springer

http://www.springer.com/10545