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【 CASE REPORT 】

The Diagnosis of Exogenous Lipoid Pneumonia Caused by the Silent Aspiration of Vegetable Oil Using a Lipidomic Analysis

Tetsuo Shimizu, Yoshiko Nakagawa, Yuko Iida, Kentaro Hayashi, Yoshihiro Sato, Shuichiro Maruoka, Noriaki Takahashi and Yasuhiro Gon

Abstract:

We herein report a case of refractory exogenous lipoid pneumonia that was successfully attributed to vegetable oil through a lipidomic analysis of bronchoalveolar lavage fluid (BALF). As a 25-year-old woman diagnosed with lipoid pneumonia experienced repeated exacerbations and improvement, we performed a BALF lipidomic analysis. The major lipid components were oleic acid, linoleic acid, and α-linolenic acid, which are constituents of vegetable oil. She stopped consuming any vegetable oil and has since experienced no instances of lipoid pneumonia relapse. A lipidomic analysis appears to be useful for identifying causative lipids, since patients with lipoid pneumonia are sometimes unaware of aspiration episodes.

Key words: exogenous lipoid pneumonia, lipidomic analysis, bronchoalveolar lavage fluid, vegetable oil

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Introduction

Lipoid pneumonia is a type of pneumonia in which lipids are observed in lesions and macrophages that have phagocytized lipids are present in the bronchoalveolar lavage fluid (BALF) (1). Lipoid pneumonia cases are classified as endogenous or exogenous. In patients with endogenous lipoid pneumonia, macrophages phagocytizing cholesterols or cholesterol esters emerge on the peripheral side of the airflow obstruction caused by cancer or foreign matter (2). Idiopathic cases have also been reported, occurring without airway obstruction.

Exogenous lipoid pneumonia is caused by the aspiration or inhalation of lipids (3). Causative lipids include mineral, vegetable, and animal oils and liquid paraffin, which is used as a laxative and is the most common cause, accounting for approximately 75% of cases. Exogenous lipoid pneumonia is a rare disease in Japan because low amounts of liquid paraffin are used. Gastroesophageal reflux and neuropsychiatric diseases have been reported to contribute to the onset of exogenous lipoid pneumonia; however, cases without any underlying disease have also been reported (4, 5).

Depending on the course after onset, exogenous lipoid pneumonia cases are classified into acute and chronic types. In acute cases, the amount of aspiration per episode is large, such as in fire-eater's lung, and symptoms, such as cough, a fever, and dyspnea, emerge a few hours after aspiration (6, 7). Chronic exogenous lipoid pneumonia develops after repeated aspirations of a small amount of lipid, and symptoms are less obvious than in acute cases; indeed, patients with chronic exogenous lipoid pneumonia are often unaware of aspiration episodes (8). Many reports have documented that symptoms caused by foreign matter aspiration are particularly obscure in cases of mineral oil-induced lipoid pneumonia (9). This lack of symptoms is attributed to the suppression of the cough reflex and ciliary motility by mineral oil (10). Therefore, in cases of exogenous lipoid pneumonia, patient interviews alone are insufficient to identify the causal relationship between the aspirated substance and disease onset.

Recently, lipidomic analyses have enabled a comprehensive analysis of lipid components. In patients with lipoid pneumonia, this technique has been used to comprehensively

Division of Respiratory Medicine, Department of Internal Medicine, Nihon University School of Medicine, Japan
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Correspondence to Dr. Tetsuo Shimizu, shimizu.tetsuo@nihon-u.ac.jp

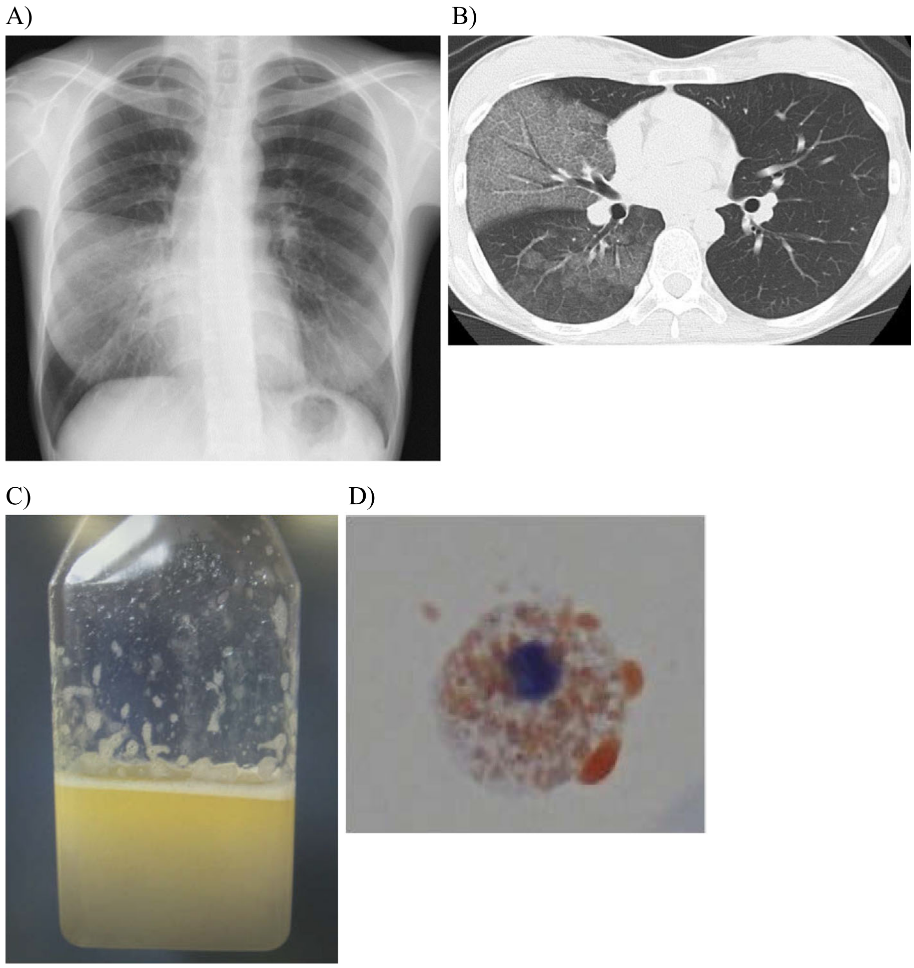

Figure 1. **A) A pretreatment chest X-ray showing a uniform infiltrative shadow in the right middle and inferior lung fields. B) A pretreatment chest CT image showing ground-glass opacities in the right middle and inferior lobes. C) Appearance of the BALF; it was white in colour and separated into oil and aqueous layers after standing. D) Cytological findings of the BALF showing a large number of macrophages that had phagocytosed lipids (Sudan IV staining, ×400 magnification). BALF: bronchoalveolar lavage fluid**

analyze the lipids that have accumulated in the lungs for the purpose of identifying likely aspirated substances and therefore may contribute to accurate diagnoses and the exclusion of other diagnoses.

We herein report a case of exogenous lipoid pneumonia, for which vegetable oil was successfully identified as the causative agent through a lipidomic analysis of the BALF.

Case Report

A 25-year-old woman had experienced a fever and a cough since late December 2015. Additionally, she started complaining of shortness of breath and visited a local doctor. She was diagnosed with mycoplasma pneumonia and hospitalized for 1 week, during which period antibacterial drugs (ceftriaxone sodium hydrate 2 g/day, moxifloxacin hydrochloride 400 mg/day, and clarithromycin 400 mg/day) were administered and her symptoms improved.

However, chest X-ray after discharge showed little improvement in the infiltrative shadow on the right inferior lobe observed when she was hospitalized, and she was referred to our hospital on mid-February 2016. Based on the clinical course, she was suspected of having organizing pneumonia secondary to mycoplasma pneumonia and followed without medication because she did not have symptoms such as a fever or cough. She developed a nocturnal cough beginning in May 2016, and chest X-ray and chest computed tomography (CT) (Fig. 1A, B) revealed an intensified ground-glass opacity in the right middle and inferior lobes; therefore, a bronchoscopy examination was performed.

A transbronchial lung biopsy (TBLB) showed inflammatory cell infiltration and fibrous thickening with several foam cells; the BALF was white in colour and separated into oil and aqueous layers after incubation (Fig. 1C). The total cell count of the BALF was 270/μL. The cells consisted of macrophages (76.0%), lymphocytes (12.0%), and neutrophils (12.0%). A large number of lipid droplets, positive Sudan IV staining consistent with small vesicles in the cytoplasm of foam cells, and a large number of lipid-

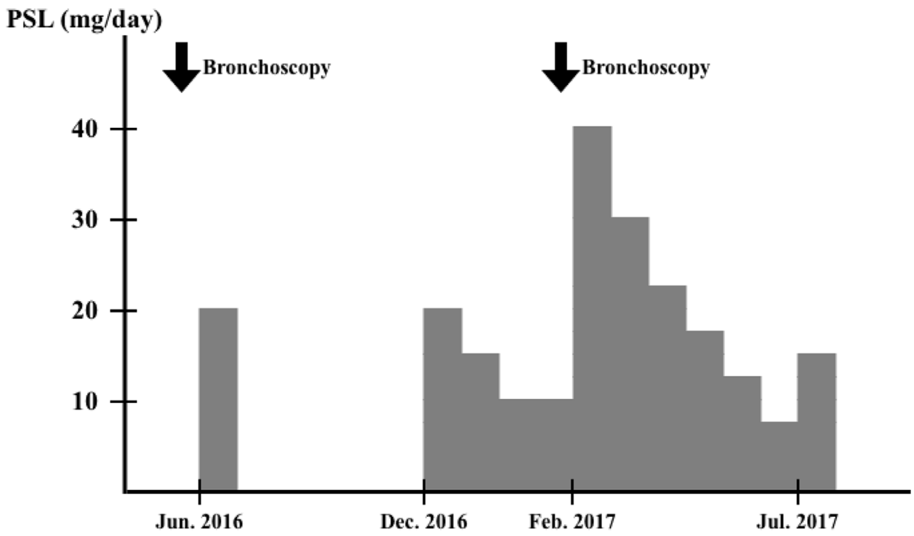

Figure 2. **The clinical course of the present case. Steroid dose escalation and reduction were repeated by exacerbations and improvement in lipoid pneumonia.**

phagocytized macrophages were observed in the BALF (Fig. 1D). Accordingly, the patient was diagnosed with lipoid pneumonia. Based on the clinical course, she was considered to have post-pneumonia endogenous lipoid pneumonia, and treatment with 20 mg/day prednisolone (PSL) was initiated on June 2016.

The chest radiographic findings remained unchanged, although the cough improved. After one month of PSL administration, PSL was discontinued because of oedema of the lower limbs and a sense of abdominal fullness. Approximately two months after discontinuation, her coughing worsened and chest pain emerged. As concomitant bacterial pneumonia was suspected, antibacterial drugs were administered, but the symptoms did not improve. Thereafter, 20 mg/day PSL was resumed, as chest X-ray showed worsening of the infiltrative shadow in the right inferior lobe. As the chest radiographic findings gradually improved, the PSL dose was reduced; chest X-ray then showed worsening of the infiltrative shadow in the right inferior lobe. As a fever and cough also emerged, another bronchoscopy examination was performed in late January 2017.

Another TBLB revealed organizing pneumonia, and BALF findings included many lipid droplets, similar to the previous examination; accordingly, she was diagnosed with relapsed lipoid pneumonia. The PSL dose was increased to 40 mg/day and both symptoms and chest radiographic findings improved. The PSL dose was then gradually reduced to 7 mg/day. A fever and cough emerged again, and chest CT showed the presence of an intensified ground-glass opacity in the right middle and inferior lobes similar to the CT findings at the initial diagnosis and right pleural effusion. The symptoms and chest radiographic findings improved after the PSL dose was increased again to 15 mg/day (Fig. 2).

Repeated exacerbations and improvement in lipoid pneumonia prompted us to identify the specific oil responsible for lipoid pneumonia in order to achieve successful treatment. The BALF sample collected in the first bronchoscopy examination was subjected to a lipidomic analysis for a comprehensive analysis of the lipid components.

Methods

A bronchoscope (BF-260; Olympus, Tokyo, Japan) was inserted into right B8a, and 150 mL of physiological saline was injected. An 80-mL sample of BALF was manually collected and allowed to separate into two layers, of which only the oil layer was collected. The collected material was sequentially dissolved in methanol and chloroform; the solution was shaken and then ultra-centrifuged at 15,000×*g* for 5 minutes, after which the supernatant was collected (the lipid extract). The lipid extract was shaken successively with a potassium hydroxide ethanol solution, 120 μL of 1 N hydrochloric acid, and hexane, after which the supernatant was collected and used as the analysis sample. Standard solutions were prepared by diluting 67 different fatty acids and an internal standard (fatty acid-18O2) with methanol to a final concentration of 100 ng/mL, which was added to the analysis sample. The analysis sample was then analyzed via liquid chromatography (LC)/mass spectrometry (MS). For LC, a Prominence UFLC XR chromatography system (Shimadzu, Kyoto, Japan) was used with an L-column2 ODS column (inner diameter 2.1 mm×150 mm, particle diameter 2 μm, CERI) at a column temperature of 40°C and a flow rate of 0.3 mL/min. MS was performed with an LTQ Orbitrap XL (Thermo Fisher Scientific, Sunnyvale, USA) in the thermal electrospray ionization mode.

Results

Table shows the fatty acid composition of BALF determined using the lipidomic analysis. Thirteen different fatty acids were detected, with oleic acid being the most abundant (57%), followed by linoleic acid (20%) and α-linolenic acid (7.5%). A chromatogram depicting the peaks for these fatty acids is shown in Fig. 3. Among the detected fatty acids, 9 unsaturated fatty acids accounted for 91.74% of the total fatty acid content, and saturated fatty acids represented a minor component. Based on these results, the main lipid component of the BALF from this patient was deemed to be unsaturated fatty acids.

Table. Results of the BALF Fatty Acid Analysis.

Fatty acid	Molecular formula	Fatty acid composition (%)
Oleic acid	C18: 1n-9	57
Linoleic acid	C18: 2n-6	20
α-Linolenic acid	C18: 3n-3	7.5
Palmitic acid	C16: 0	5.3
cis-Vaccenic acid	C18: 1n-7	3.6
Stearic acid	C18: 0	1.5
γ-Linolenic acid	C18: 3n-6	1.4
11Z-Eicosenoic acid	C20: 1n-9	1.4
Arachidic acid	C20: 0	1.0
Behenic acid	C22: 0	0.44
Rumenic acid	C18: 2(9Z, 11E)	0.37
Palmitoleic acid	C16: 1n-7	0.28
Nervonic acid	C24: 1n-9	0.19

According to the results of the lipidomic analysis, the main lipid components in the BALF were oleic acid, linoleic acid, and α-linolenic acid. As oleic acid, linoleic acid, and α-linolenic acid are typical components of vegetable oil, we again questioned the patient about her eating habits and found that she had been drinking a glass of undiluted vegetable oil in a single gulp with each meal. Accordingly, she was diagnosed with exogenous lipoid pneumonia caused by vegetable oil and instructed to stop drinking vegetable oil. After she stopped, the lipoid pneumonia did not relapse even after the PSL dose was reduced, and the chest X-ray findings also improved.

Discussion

In the present case study, the patient was initially suspected of having endogenous lipoid pneumonia, as she was generally a healthy young woman with no episodes of aspiration. Steroid treatment was initiated; however, the pneumonia repeatedly improved and relapsed, so the BALF was

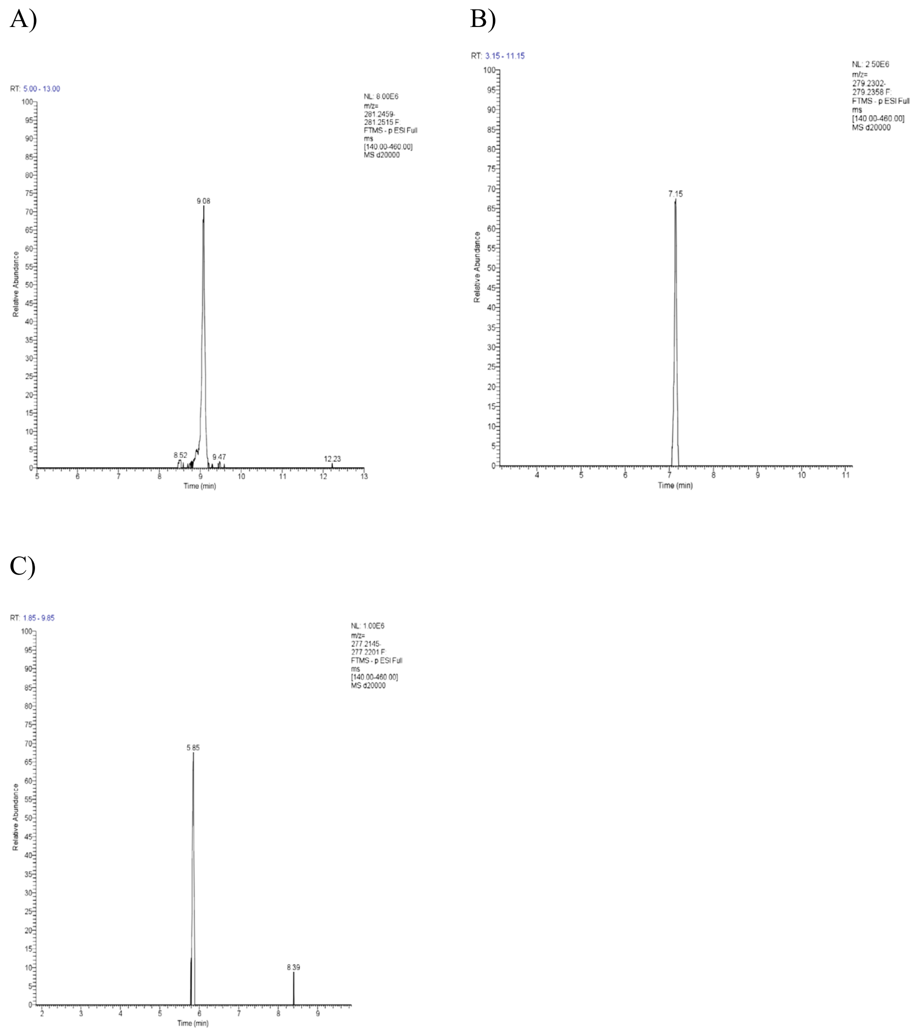

Figure 3. Chromatograms from the lipidomic analysis showing fatty acid peaks. A) Oleic acid, B) linoleic acid, and C) α-linolenic acid.

subjected to a lipidomic analysis in order to identify the lipid responsible for the lipoid pneumonia. Using conventional gas chromatography combined with MS, the lipidomic analysis enabled a comprehensive and quantitative metabolomic analysis of lipids (11).

Lipidomic analyses have recently attracted attention as a comprehensive analytical tool for lipid metabolism (12), as lipids are involved in various diseases as chemical mediators. In the present case study, the results of the lipidomic analysis revealed that the main fatty acids in BALF were oleic acid, linoleic acid, and α-linolenic acid. Oleic acid is an abundant unsaturated fatty acid in vegetable oil, is produced in the body through the action of fatty acid synthase, and is abundant in breast milk. Linoleic acid is an ω-6 fatty acid that is produced from oleic acid by the action of Δ12-fatty acid desaturase, and α-linolenic acid is an ω-3 fatty acid produced from linoleic acid by the action of Δ15-fatty acid desaturase. Since animals do not express Δ12- and Δ15-fatty acid desaturases, humans are unable to synthesize linoleic acid and α-linolenic acid. Therefore, the patient was diagnosed with exogenous lipoid pneumonia, as no endogenous lipids, such as cholesterols or cholesterol esters, were found while essential fatty acids, such as linoleic acid and α-linolenic acid, were detected in the BALF. Oleic acid, linoleic acid, and α-linolenic acid are main the fatty acids present in vegetable oil, and the patient presumably aspirated vegetable oil, which she had habitually been drinking undiluted in a single gulp at meals.

Liquid paraffin, which is a type of mineral oil, has been reported to cause minimal irritation to the airway and is prone to aspiration (13). Mineral oil has been suggested to be prone to silent aspiration since it suppresses the cough reflex (14). In addition, mineral oil reduces the motility of tracheal cilia and is difficult to remove from the airway once it is aspirated (15). A case of exogenous lipoid pneumonia caused by regular nasal irrigation with sesame oil, a type of vegetable oil, has been reported (16); the patient in the reported case experienced minimal symptoms, suggesting that vegetable oil may also inhibit the cough reflex, similar to mineral oil. In India, a custom of nasal irrigation with vegetable oil for promoting health and controlling hygiene presumably associated with ancient Ayurveda influenced by yoga has been reported. Vegetable oil is used for nasal irrigation partially because it is minimally irritative to the nasal mucosa, whereas water is irritative to the nasal mucosa due to its low osmolality. Similarities exist between the nasal mucosa and lower airway mucosa, and vegetable oil may be minimally irritative to the lower airway mucosa and nasal mucosa. Accordingly, vegetable oil aspiration may be less likely to induce the cough reflex and thus results in silent aspiration.

In patients with exogenous lipoid pneumonia, the extent of pulmonary tissue damage varies depending on the specific oil and amount that is aspirated or inhaled. Animal oils, such as milk and lard, cause the most severe pulmonary damage. Animal oil is hydrolyzed in the lungs to generate large quantities of free fatty acids, which cause inflammation by activating lymphocytes and inducing connective tissue proliferation and necrotic changes (17). Vegetable oil causes the second most severe type of inflammation next to animal oil, and the inflammation caused by mineral oil is less severe than that caused by animal oil or vegetable oil. Steroid treatment has been reported to be effective for exogenous lipoid pneumonia, presumably because steroids suppress the activated lymphocytes that are locally induced by lipids (18, 19). Low-viscosity lipids are prone to aspiration in large quantities and are more likely to cause severe pulmonary damage than others (20). In the present case, the patient had no episodes of aspiration; therefore, she was likely to have experienced silent aspiration of a small amount of oil. Nonetheless, she experienced associated symptoms, including fever, cough, and chest pain, presumably because the causative lipid was vegetable oil, which tends to cause inflammation. The symptoms and chest radiographic findings improved after steroid treatment.

In the present case, steroid treatment was performed for lipoid pneumonia, and both the symptoms and chest radiographic and CT findings improved. However, the lipoid pneumonia relapsed as the steroid dose was reduced. The relapses likely occurred because the causative agent, of vegetable oil, was still being consumed during the treatment. To our knowledge, no studies have performed a lipidomic analysis to identify the lipids responsible for lipoid pneumonia, but some have used infrared spectroscopy (IR) to identify the lipids responsible (21, 22). IR is difficult to use for a qualitative analysis of a mixture of various lipids or an accurate quantitative analysis of individual lipids in a mixture. Given the diverse causes of lipoid pneumonia, a lipidomic analysis that is capable of a comprehensive and quantitative analysis of the lipids responsible for lipoid pneumonia is most useful. Once lipoid pneumonia is diagnosed, the proactive identification of the causative lipid is important for successful treatment, and if an exogenous lipid is determined to have caused the disease, the patient must be instructed to stop consuming this lipid.

The authors state that they have no Conflict of Interest (COI).

References

1. Silverman JF, Turner RC, West RL, Dillard TA. Bronchoalveolar lavage in the diagnosis of lipoid pneumonia. Diagn Cytopathol **5**: 3-8, 1989.
2. Wright BA, Jeffrey PH. Lipoid pneumonia. Semin Respir Infect **5**: 314-321, 1990.
3. Spickard A 3rd, Hirschmann JV. Exogenous lipoid pneumonia. Arch Intern Med **154**: 686-692, 1994.
4. Gondouin A, Manzoni P, Ranfaing E, et al. Exogenous lipid pneumonia: a retrospective multicentre study of 44 cases in France. Eur Respir J **9**: 1463-1469, 1996.
5. Marchiori E, Zanetti G, Mano CM, Hochhegger B. Exogenous lipoid pneumonia. Clinical and radiological manifestations. Respir Med **105**: 659-666, 2011.
6. Beermann B, Christensson T, Möller P, Stillström A. Lipoid pneu-

monia: an occupational hazard of fire eaters. Br Med J **289**: 1728-1729, 1984.
7. Brander PE, Taskinen E, Stenius-Aarniala B. Fire-eater's lung. Eur Respir J **5**: 112-114, 1992.
8. Baron SE, Haramati LB, Rivera VT. Radiological and clinical findings in acute and chronic exogenous lipoid pneumonia. J Thorac Imaging **18**: 217-224, 2003.
9. Ohwada A, Yoshioka Y, Shimanuki Y, et al. Exogenous lipoid pneumonia following ingestion of liquid paraffin. Intern Med **41**: 483-486, 2002.
10. Brown AC, Slocum PC, Putthoff SL, Wallace WE, Foresman BH. Exogenous lipoid pneumonia due to nasal application of petroleum jelly. Chest **105**: 968-969, 1994.
11. Ozaki H, Nakano Y, Sakamaki H, Yamanaka H, Nakai M. Basic eluent for rapid and comprehensive analysis of fatty acid isomers using reversed-phase high performance liquid chromatography/Fourier transform mass spectrometry. J Chromatogr A **1585**: 113-120, 2019.
12. Wheelock CE, Goss VM, Balgoma D, et al.; U-BIOPRED Study Group. Application of 'omics technologies to biomarker discovery in inflammatory lung diseases. Eur Respir J **42**: 802-825, 2013.
13. Simmons A, Rouf E, Whittle J. Not your typical pneumonia: a case of exogenous lipoid pneumonia. J Gen Intern Med **22**: 1613-1616, 2007.
14. Franquet T, Giménez A, Rosón N, Torrubia S, Sabaté JM, Pérez C. Aspiration diseases: findings, pitfalls, and differential diagnosis. Radiographics **20**: 673-685, 2000.
15. Bandla HP, Davis SH, Hopkins NE. Lipoid pneumonia: a silent complication of mineral oil aspiration. Pediatrics **103**: E19, 1999.
16. Kuroyama M, Kagawa H, Kitada S, Maekura R, Mori M, Hirano H. Exogenous lipoid pneumonia caused by repeated sesame oil pulling: a report of two cases. BMC Pulm Med **15**: 135, 2015.
17. Asnis DS, Saltzman HP, Melchert A. Shark oil pneumonia. An overlooked entity. Chest **103**: 976-977, 1993.
18. Chin NK, Hui KP, Sinniah R, Chan TB. Idiopathic lipoid pneumonia in an adult treated with prednisolone. Chest **105**: 956-957, 1994.
19. Ayvazian LF, Steward DS, Merkel CG, Frederick WW. Diffuse lipoid pneumonitis successfully treated with prednisone. Am J Med **43**: 930-934, 1967.
20. Gentina T, Tillie-Leblond I, Birolleau S, et al. Fire-eater's lung: seventeen cases and a review of the literature. Medicine **80**: 291-297, 2001.
21. Corrin B, Crocker PR, Hood BJ, Levison DA, Parkes WR. Paraffinoma confirmed by infrared spectrophotometry. Thorax **42**: 389-390, 1987.
22. Levade T, Salvayre R, Dongay G, et al. Chemical analysis of the bronchoalveolar washing fluid in the diagnosis of liquid paraffin pneumonia. J Clin Chem Clin Biochem **25**: 45-48, 1987.