

Emphysema refers to any disease process involving an abnormal accumulation of air/gas in the tissues. When used alone, it is usually taken to mean the lung disease, [pulmonary emphysema](#), which forms part of the spectrum of [chronic obstructive pulmonary disease \(COPD\)](#).

- [gastric emphysema](#): includes [emphysematous gastritis](#)
- intestinal emphysema: a synonym for [pneumatosis cystoides intestinalis](#)
- mediastinal emphysema: an uncommon synonym for [pneumomediastinum](#)
- [orbital emphysema](#): gas in the [orbit](#)
- [parotid emphysema](#): gas in the [parotid gland](#)
- [subcutaneous emphysema](#): a more accurate term for surgical emphysema

Many conditions, usually with an infective etiology, are more commonly expressed as an "emphysematous [-itis](#)" form.

- [emphysematous aortitis](#)*
- [emphysematous cholecystitis](#)
- [emphysematous cystitis](#)
- [emphysematous epididymo-orchitis](#)*
- [emphysematous hepatitis](#)*
- [emphysematous myositis](#)*
- [emphysematous esophagitis](#)*
- [emphysematous osteomyelitis](#)*
- [emphysematous pancreatitis](#)
- [emphysematous prostatitis](#)
- [emphysematous pyelitis](#)
- [emphysematous pyelonephritis](#)

Pulmonary emphysema is defined as the "abnormal permanent enlargement of the airspaces distal to the terminal bronchioles accompanied by destruction of the alveolar wall and without obvious fibrosis" ¹. Emphysema is best evaluated on CT, although indirect signs may be noticed on conventional radiography in a proportion of cases. This article focuses on panlobular emphysema, paraseptal emphysema, and especially centrilobular emphysema.

Terminology

Emphysema is one of the entities grouped under the overarching term [chronic obstructive pulmonary disease \(COPD\)](#) and is best thought of primarily as a pathological rather than clinical entity. The Global Initiative for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease (GOLD) is explicit that patients present with COPD, rather than emphysema ¹¹.

Epidemiology

In 2010, approximately 385 million people were affected worldwide by COPD, leading to 3 million deaths annually, with these numbers expected to increase for the foreseeable future¹¹. It is predominantly a disease of middle to late life owing to the cumulative effect of lifelong [tobacco smoking](#) and other environmental risk factors, e.g. air pollution¹.

Historically, it affected more men than women, due to the higher smoking rates in the former, but with increased smoking and environmental risk factor exposure among women, the incidence is now equal between the sexes. Patients with genetic risk factors such as [alpha-1-antitrypsin deficiency](#) may present earlier according to phenotype.

Risk factors

- smoking: by far the most common, ~90% of all cases¹
- [alpha-1-antitrypsin deficiency](#): rare
- intravenous injection of methylphenidate ([Ritalin lung](#)): rare

Clinical presentation

The clinical features of emphysema should be distinguished from the signs and symptoms of [chronic bronchitis](#). Patients with emphysema are hypocapnic and are often referred to as "pink puffers". This compares with the hypercapnia and cyanosis of chronic bronchitis with patients referred to as "blue bloaters". In practice, features of these two syndromes coexist as [chronic obstructive pulmonary disease](#).

Patients typically report dyspnea without significant sputum production.

Signs of emphysema include:

- tachypnea
- absence of cyanosis
- pursed-lip breathing, tripod position
- chest hyperinflation "barrel chest"
- reduced breath sounds
- hyper-resonant to percussion
- [cor pulmonale](#) (late)

Pathology

Emphysema is one of a heterogeneous group of pathological processes forming [chronic obstructive pulmonary disease](#) and is itself a relatively vague term encompassing a number of entities and morphological patterns including:

- morphologic subtypes
 - [centrilobular emphysema](#) (most common¹²)
 - [panlobular emphysema](#)
 - [paraseptal emphysema](#)

- [paracapacital emphysema](#)
 - [localized emphysema](#)
- [idiopathic giant bullous emphysema](#) (or [vanishing lung syndrome](#))
- [congenital lobar emphysema](#)
- [pulmonary interstitial emphysema](#)

The three morphologic subtypes of emphysema are named according to their relationship to the [secondary pulmonary lobule](#).

Centrilobular or centriacinar emphysema¹² is the most common type and affects the proximal respiratory bronchioles, particularly of the upper zones. It has a strong dose-dependent association with smoking³. Rarely, severe centrilobular emphysema can be seen in the bases in patients with [Salla disease](#)⁴.

Panlobular or panacinar emphysema¹² affects the entire secondary pulmonary lobule and is more pronounced in the lower zones, matching areas of maximal blood flow. It is seen particularly in [alpha-1-antitrypsin deficiency](#) (exacerbated by smoking)²⁻⁴, intravenous injection of methylphenidate ([Ritalin lung](#))³ or [Swyer-James syndrome](#)⁴.

Paraseptal or distal acinar emphysema¹² affects the peripheral parts of the [secondary pulmonary lobule](#) and is usually located adjacent to the pleural surfaces (including [pleural fissures](#))³. It is also associated with smoking and can lead to the formation of [subpleural bullae](#) and spontaneous [pneumothorax](#)³.

Radiographic features

Plain radiograph

Except in the case of very advanced disease with bulla formation, chest radiography does not image emphysema directly, but rather implies the diagnosis due to associated features^{2-3,9}:

- **hyperinflation**
 - [flattened hemidiaphragm\(s\)](#): the most reliable sign
 - increased and usually irregular radiolucency of the lungs
 - [increased retrosternal airspace](#)
 - increased anteroposterior diameter of the chest
 - widely spaced ribs
 - sternal bowing
 - [tenting of the diaphragm](#)
 - [saber-sheath trachea](#)
 - blunting of the lateral and posterior costophrenic angles
- **vascular changes**
 - a paucity of blood vessels which are often distorted

- [pulmonary arterial hypertension](#)
 - pruning of peripheral vessels
 - an increased caliber of central arteries
 - [right ventricular enlargement](#)

It should be remembered, however, that the most common plain film appearance of COPD is "normal" and the role of chest radiography is to eliminate other causes of lung symptoms such as infection, bronchiectasis or cancer⁶.

CT

CT is the modality of choice for detecting emphysema; [HRCT chest](#) is particularly effective. It should be noted, however, that there is a relatively poor correlation between autopsy-proven emphysema, [pulmonary function test](#) abnormalities and CT with 20% of pathology-proven cases not being evident on CT and 40% of patients with abnormal CT having normal pulmonary function tests.

CT is able to discriminate between centrilobular, panlobular, and paraseptal emphysema.

Centrilobular emphysema

Centrilobular is by far the most common type encountered and is a common finding in asymptomatic elderly patients. It is predominantly located in the upper zones of each lobe (i.e. apical and posterior segments of the upper lobes, and superior segment of the lower lobes) and has a patchy distribution⁴. It appears as focal lucencies (emphysematous spaces) which measure up to 1 cm in diameter, located centrally within the [secondary pulmonary lobule](#), often with a central or peripheral dot representing the central bronchovascular bundle²⁻⁴.

Panlobular emphysema

Panlobular emphysema is predominantly located in the lower lobes, has a uniform distribution across parts of the [secondary pulmonary lobule](#), which are homogeneously reduced in attenuation²⁻⁴.

Paraseptal emphysema

Paraseptal emphysema is located adjacent to the pleura and septal lines with a peripheral distribution within the [secondary pulmonary lobule](#). The affected lobules are almost always subpleural and demonstrate small focal lucencies up to 10 mm in size.

Any lucency >10 mm should be referred to as [subpleural blebs/bullae](#) (synonymous)³.

In all three subtypes, the emphysematous spaces are not bounded by any visible wall³.

MRI

MRI is in the research phases for the evaluation of lung parenchymal abnormalities like emphysema. Dynamic breathing MRI may have a future role in assessing pulmonary emphysema.⁵

Treatment and prognosis

Unfortunately, once lung tissue is lost, no regrowth occurs. Treatment is therefore supportive and aimed at preserving the remaining lung parenchyma. Interventions include:

- smoking cessation

- oxygen therapy (in chronic hypoxemia)
- symptom and exacerbation control
 - short and long-acting beta-2 agonists
 - inhaled anticholinergics
 - inhaled glucocorticoids
 - antibiotics
- pulmonary rehabilitation

In patients with severe bullous change with resultant compression of remaining normal lung parenchyma, [lung volume reduction therapy](#) may be considered in selected patients.

[Lung transplantation](#) is considered in cases of [alpha-1-antitrypsin deficiency](#).

Prognosis is worse in patients who continue to smoke, are alpha-1-antitrypsin deficient, have [low FEV₁](#) at time of diagnosis, or have other comorbidities (e.g. [heart failure](#), [respiratory failure](#), frequent exacerbations).

Differential diagnosis

- [cystic lung disease](#): all have visible cyst walls
 - [lymphangioleiomyomatosis \(LAM\)](#)
 - [pulmonary Langerhans cell histiocytosis \(LCH\)](#): often co-exists with emphysema
 - [honeycomb lung](#): usually reduced lung volumes

- Topic Importance
- Combined pulmonary fibrosis and emphysema (CPFE) is an underdiagnosed syndrome in which individuals have variable degrees of pulmonary fibrosis and emphysema. Patients with CPFE have high morbidity, including poor exercise tolerance and increased development of comorbidities. CPFE mortality also seems to outpace that of lone emphysema and pulmonary fibrosis. A major limitation to rigorous, large-scale studies of CPFE has been the lack of a precise definition for this syndrome. A 2022 American Thoracic Society/European Respiratory Society/Japanese Respiratory Society/Latin American Thoracic Association Research Statement called attention to fundamental gaps in our understanding of CPFE and highlighted the potential use of quantitative imaging techniques to better define CPFE.
- Review Findings
- Broadly, CPFE has been defined using visual interpretation of chest CT imaging documenting the presence of both emphysema and fibrosis, with varying distributions. When quantitative approaches were involved, varying thresholds of emphysema and fibrosis on imaging have been used across different studies.
- Summary
- This review is structured into 3 primary themes, starting with early imaging studies, then evaluating the use of quantitative methods and imaging-based

thresholds, both in large population studies and single-center cohorts to define CPFE and assess patient outcomes. It concludes by discussing current challenges and how to focus our efforts so that quantitative imaging methods can effectively address the most pressing clinical dilemmas in CPFE.

Literature Search

A literature search was performed in PubMed to identify all peer-reviewed journal articles from January 1, 2000, to December 1, 2023. All studies with the following key words were included: “pulmonary fibrosis,” “emphysema,” and “imaging.” These studies included all subtypes of fibrotic ILDs as they are currently part of the broader CPFE population as highlighted in the 2022 CPFE Research Statement.⁴ Inclusion criteria were consistent with definitions previously published by Wong et al.⁵ Papers

Early Imaging Studies of CPFE as a Clinical Syndrome

Despite case reports, CPFE was not recognized as a syndrome clinically distinct from lone emphysema and pulmonary fibrosis until 2005.⁶ At that time, the fibrotic component was primarily evaluated in patients with idiopathic pulmonary fibrosis (IPF), leading radiologic CPFE on HRCT imaging to be defined as upper lobe predominant emphysema with a usual interstitial pneumonia (UIP) pattern of fibrosis. A subsequent study found that radiologic CPFE correlated with histopathologic emphysema and UIP.

Future Directions

Table 1 summarizes the key CPFE CT imaging studies and their longitudinal outcomes discussed in this review. Although there are extensive imaging-based studies in both ILD and COPD populations, there remain no prospective cohort studies of CPFE. In addition, most imaging-based studies of CPFE, including those assessing key imaging characteristics, lung function decline, survival, and lung cancer outcomes, relied on retrospective, single-center studies without rigorously defined thresholds for

Summary

There is increasing awareness that CPFE is an underdiagnosed clinical syndrome with poor outcomes. The lack of a precise disease definition, beyond a combination of fibrosis and emphysema in a single individual, has hampered multicenter studies and often limited a timely diagnosis. In parallel, exciting advances in novel quantitative CT imaging methodologies provide opportunities to refine the imaging criteria for CPFE and should be a focus of future collaborative research efforts among COPD

Discussion

Spontaneous pneumomediastinum (SPM) and spontaneous emphysema (SE) are rare conditions, where “spontaneous” refers to the escape of air from the lung cavity into other areas, such as subcutaneous tissue, without any traumatic event. These conditions are often self-limiting and may only require supportive care unless there is a serious underlying lung disease or a complication like pneumothorax or secondary bacterial pneumonia.¹ SPM and SE can also arise from activities that increase

Visual case discussion

A 7-year-old boy with no prior medical conditions presented with a five-day history of fever, runny nose, and cough. Following an intense coughing episode, he developed swelling in his neck and chest, accompanied by rapid breathing several hours before hospital admission. There was no history of exposure to sick individuals, hospitalization, or prior use of nebulizers. He had no known contact with tuberculosis patients. Upon arrival at the emergency department, he was alert and conscious but

Abstract

Introduction

[Tonsillectomy](#) is known as one of the safest [otorhinolaryngology](#) surgery procedure. Rarely, it can lead to serious complications. Cervico-facial [emphysema](#) is an exceptional complication of tonsillectomy.

Here we reported a case of post-tonsillectomy emphysema. Our objective was to emphasize the different characteristics of this entity and draw attention to the risk of potentially fatal respiratory complications.

Presentation of case

A 46-year-old healthy woman had a tonsillectomy because of recurrent [tonsillitis](#). Four hours after [extubation](#), she presented a [subcutaneous emphysema](#) under the left mandibular angle, slightly extended to the left cheek and left laterocervical region. An immediate cervicofacial CT scan showed a dissecting cervical emphysema of the left hemiface of moderate abundance that extended to the pre-vascular space of the superior mediastinum. The decision was to keep the patient hospitalized, to avoid forced glottic closure and to put her on prophylactic antibiotics. The further course was uneventful with respiratory state stability and emphysema's disappearance.

Clinical discussion

Cervicofacial emphysema is a very rare but life-threatening tonsillectomy complication that may cause [acute respiratory failure](#). Emphysema's main clinical characteristics are a non-tender cervicofacial swelling and [crepitus](#). Post-tonsillectomy emphysema treatment is usually conservative.

In cases of respiratory failure, it is necessary to secure the airway by [intubation](#) or [tracheostomy](#). An important mediastinal expansion of the emphysema requires a [thoracotomy](#).

Conclusion

Cervicofacial emphysema is an unpredictable complication of tonsillectomy. Its prevention requires per-operative vigilance from both ENT surgeons and anesthetists. Moreover, early diagnosis and management are essential to avoid its potentially fatal consequences.

1. Introduction

Palatine [tonsillectomy](#) is a common surgical procedure. Although rare, its complications can be life-threatening. Cervicofacial emphysema remains an exceptional complication after tonsillectomy. However, as exceptional as it is, it is potentially fatal [1].

Here we reported a case of post-tonsillectomy emphysema and we attempted to emphasize the clinical, paraclinical, and therapeutic characteristics of this unusual complication. And thus, alert the medical community to consider this extremely rare but life-threatening complication.

2. Methods

The work has been reported in line with The SCARE criteria [2].

3. Presentation of case

A 46-year-old woman was hospitalized for treatment of recurrent [tonsillitis](#). She had no co-morbidities. The preoperative physical examination revealed hypertrophied [palatine tonsils](#) (grade 3 of Friedman) with a normal-looking [soft palate](#). The patient had a tonsillectomy under [general anesthesia](#) with [orotracheal intubation](#). [Intubation](#) was atrumatic and uneventful particularly no vomiting or coughing. An extracapsular removal of the [tonsils](#) was performed using a tonsil dissector. [Hemostasis](#) was achieved using bipolar [cautery](#). There were some bilateral adhesions between the tonsils and tonsillar beds. However, there was no remarkable [peroperative bleeding](#) and no peritonsillar bed injury was observed peroperatively. [Extubation](#) was uneventful.

Four hours after extubation, in the [recovery room](#), the patient complained of painless left cervicofacial swelling. The physical examination found [subcutaneous emphysema](#) under the left mandibular angle, slightly extended to the left cheek and left laterocervical region. Oral examination, cardiopulmonary [auscultation](#) and [oxygen saturation](#) were normal with no signs of airway compromise. An immediate cervicofacial CT scan showed dissecting cervicofacial emphysema of moderate abundance that extended to the pre-vascular space of the superior mediastinum ([Fig. 1](#), [Fig. 2](#)). The decision was to keep the patient hospitalized while monitoring her [hemodynamic](#) and respiratory status. We put the patient on prophylactic broad-spectrum antibiotics based on [cefotaxim](#) and [metronidazole](#). Also, we administered [cough suppressants](#) and laxatives to avoid forced glottic closure. We instructed the patient to avoid actions that would increase intrathoracic pressure such as coughing, sneezing with a closed mouth, blowing the nose. A nasogastric tube was inserted for nutrition.

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Fig. 1. CT scan: axial section: left cervical dissecting [emphysema](#)

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Fig. 2. CT: axial section: extension of [emphysema](#) in the superior mediastinum

The further course was uneventful with respiratory state stability and emphysema's progressive resolution. On the seventh postoperative day, the emphysema had fully resolved and the patient was discharged. One week after discharge, the patient was seen at the outpatient department with complete resolution of emphysema and normal post-tonsillectomy status in the pharynx examination. The patient remained well with a one-year follow-up.

4. Discussion

Tonsillectomy is a routine and one of the safest ENT surgery procedure [1]. Although often uneventful, this surgery can present a number of complications. Post-tonsillectomy, primary or

secondary hemorrhage remains the most significant risk. However, other common complications include damage to teeth, otalgia, [odynophagia](#), [throat infection](#) and lingual edema [3]. Moreover, several serious complications have been reported, such as intraoperative [vascular injury](#), Eagle syndrome, [atlantoaxial subluxation](#), cervical [osteomyelitis](#), and taste disorders [4]. One of the rarest complications is cervicofacial emphysema, only 33 cases have been reported [5,6].

Most authors admit that the development of post-tonsillectomy [subcutaneous emphysema](#) is caused by an injury to the pharyngolaryngeal wall [1,3]. Such [iatrogenic damage](#) may be caused either by surgical or anesthetic procedures. In fact, a mucosal tear during traumatic [intubation](#), excessive positive ventilation and excessive [manual ventilation](#) can cause a defect in the pharyngolaryngeal wall [1]. Moreover, patients with recurrent [tonsillitis](#) history usually develop adhesions of the tonsillar tissue to the fossa and underlying muscular layers as part of the healing process [7]. These adhesions increase the risk of injury to the superior constrictor muscle during surgical dissection.

Congenital mucosal dehiscence, such as clefts or [laryngocoeles](#) or bullae may also predispose to development of postoperative surgical emphysema [3].

Thus, air enters through the iatrogenic defect and travels through the cervicofacial planes to the parapharyngeal, retropharyngeal, and [prevertebral spaces](#) [8,9]. Air may descend to the mediastinum through the deep neck spaces causing a [pneumomediastinum](#), or/and to the [pleural space](#) resulting in a [pneumothorax](#) [8].

Post-tonsillectomy emphysema's main clinical characteristic is a non-tender cervicofacial swelling with [crepitus](#) on palpation. If the patient also developed dyspnea, [dysphagia](#), chest and [back pain](#), [cyanosis](#) and [Hamman's sign](#) (crepitus synchronous with systole), pneumomediastinum should be suspected [8]. Chest X-ray readily detects the subcutaneous air. However, a CT scan best detects emphysema extension in the cervicofacial region and mediastinum [5,10].

The management of post-tonsillectomy emphysema is usually *conservative*, as it is a self-limiting pathology with spontaneous resolution. This includes closed airway observation, keeping the patient on an absolute diet and avoidance of any activities that can increase the intrapharyngeal [airway pressure](#) [9]. In this rationale, laxatives, [cough suppressants](#) or [antihistaminic drugs](#) may be prescribed. Most authors agree that the administration of broad-spectrum antibiotic therapy is obligatory to prevent contamination from [oral cavity](#) [5,8].

A cephalosporin-metronidazole combination is the choice of most authors against oral organisms infection. Normobaric oxygen therapy may increase the absorption of nitrogen from air accumulating in the emphysematous cavity in a favorable downward concentration gradient [11].

If there is an evident mucosal rupture with symptoms that are not responding to conservative measures, a careful suture of the damaged [mucosa](#) might be helpful. In cases of respiratory failure, securing the airway by intubation or [tracheostomy](#) may be necessary [3]. An important mediastinal expansion of the emphysema may require a [thoracotomy](#) [3].

In the majority of cases, post-tonsillectomy cervicofacial emphysema regresses spontaneously within a few days [12]. However, a fatal outcome is to be feared. Finally, prevention is crucial. Anesthetic prevention measures mainly boil down to atraumatic intubation, avoiding overinflation of the endotracheal tube's cuff, and suctioning airway with an orogastric tube to avoid laryngeal irritation [3]. As for surgical preventive measures, in cases of adherent [tonsils](#), the surgeon must carry out a meticulous dissection in order to preserve the fascial layers. There should be good communication

between the surgeon and the anesthesiologist to ensure the patient does not wake before completion of surgery [3,13].

Finally, we must point out the lack of data on post-tonsillectomy emphysema in the literature.

Future observational or experimental studies are strongly required.

5. Conclusion

Post-tonsillectomy cervicofacial emphysema is an exceptional complication that cannot be overlooked. Its prevention requires per-operative extreme vigilance from both ENT surgeons and anesthetists. Moreover, early diagnosis and management are essential to avoid its potentially fatal consequences.

Background

Williams-Beuren syndrome (WBS) is a multisystem genetic condition characterized by a submicroscopic deletion on the seventh chromosome (7q11.23), which usually includes the elastin gene.

Research Question

Although the elastin deficiency in WBS can predispose to emphysema, the prevalence of emphysema in WBS is unknown. This narrative review aims to address this gap by estimating the frequency of emphysema (or suggestive features thereof) in patients with WBS, with a special focus on concomitant alpha-1 antitrypsin deficiency.

Study Design and Methods

Literature was reviewed according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.

Results

Of 419 studies identified by the search strategy, 19 eligible studies reported 393 adult patients with WBS. The criteria by which emphysema was assessed varied greatly among the relatively few reports addressing this issue. Chest CT evidence of emphysema was reported in three of 26 patients (11.5%). Physiological evidence of airflow obstruction, although not definitive for emphysema (ie, with asthma not excluded), was present in as many as 38.6%. Considering studies that reported multiorgan clinical manifestations of WBS, irrespective of whether chest CT imaging and/or pulmonary function testing was reported, the frequency of spirometric and imaging signs suggestive of emphysema was 4.3%. Emphysema was not reported in any of the 11 patients with concomitant PI*MZ heterozygous alpha-1 antitrypsin deficiency.

Interpretation

In the context that only few adults with WBS have been fully characterized regarding the occurrence of emphysema, confidently estimating the prevalence of emphysema is difficult. This review shows that the frequency of imaging and pulmonary function test abnormalities suggestive of emphysema seems relatively low in the context that the elastin deficiency of WBS clearly can predispose to emphysema, and that other manifestations of elastin deficiency are present early in life.

Acknowledging the challenges of studying uncommon diseases or syndromes, further systematic study of adults with WBS is needed.

Key Words

alpha-1 antitrypsin

COPD

elastin

emphysema

Williams-Beuren syndrome

Abbreviations

AATD

alpha-1 antitrypsin deficiency

LLN

lower limit of normal

PFT

pulmonary function test

WBS

Williams-Beuren syndrome

Take-home Point

Study Question: What is the risk for emphysema in adults with the elastin gene deletion disorder Williams-Beuren syndrome (WBS)?

Results: Although prevalence estimates vary according to the assessment method used, the overall frequency of imaging and pulmonary function test abnormalities suggestive of emphysema in WBS is generally low, even with concomitant PI*MZ alpha-1 antitrypsin deficiency.

Interpretation: Despite the highly plausible risk conferred by loss of the elastin gene and the protean other manifestations mediated by elastin deficiency in WBS, the reported risk of emphysema in WBS appears to be relatively low. More systematic study is needed to secure confident risk estimates.

Williams-Beuren syndrome (WBS) is a multisystem genetic condition characterized by a submicroscopic deletion on the seventh chromosome (7q11.23), which includes the entire elastin gene in 97% to 98% of cases.¹ WBS has protean clinical manifestations, characteristically including cardiovascular disease (eg, supravalvular aortic stenosis, pulmonic stenosis, hypertension), abnormal body habitus (eg, kyphoscoliosis), sensorineural hearing loss, diverticular disease, diabetes, hypercalcemia, and developmental delays. Although usually resulting from a de novo mutation, instances of autosomal dominant inheritance have been described.²

There is a predisposition to emphysema in individuals with elastin deficiency or abnormality (as in cutis laxa syndrome³). Given that most cases of WBS are characterized by elastin deletion, attention has naturally focused on the association between WBS and emphysema. One case report describes the occurrence of emphysema in a patient with WBS.¹ A 31-year-old never smoking patient with WBS and normal pulmonary function tests (PFTs) (FEV₁ 106% predicted, diffusing capacity for carbon monoxide 100% predicted) was found to have emphysema on quantitative chest CT scan (26% in the

upper and middle lung zones and 23% in the lower lung zone). A second case report describes a boy with a point mutation in the *ELN* gene whose father and grandfather (who smoked) both had emphysema.³

Despite the demonstrable risk that an elastin gene abnormality can predispose to emphysema, the actual magnitude of risk for emphysema in adults with WBS is unclear. Prompted by our recent experience with a 31-year-old patient with WBS who was also heterozygous for alpha-1 antitrypsin deficiency (AATD) (PI*MZ), we undertook a narrative review of the available literature on WBS to characterize the prevalence of emphysema among adults with WBS.

Study Design and Methods

Data Sources and Searches

In keeping with Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines,⁴ a search strategy to capture the occurrence of emphysema in adults with WBS was developed. As reviewed in [e-Table 1](#), search terms were interrogated in various databases (Ovid MEDLINE, Ovid Embase, Web of Science, and Cochrane Central Register of Controlled Trials databases) through August 4, 2022. A combination of controlled vocabulary and key words was used along with truncation and adjacency operators to search for relevant literature. No date, language, or publication type restrictions were used in the search strategy. References in eligible reports were also reviewed for possible inclusion.

Two of the authors independently reviewed abstracts for final inclusion and, using a Delphi process, reached concordance using Covidence ([Fig 1](#)). Full texts of eligible reports were then reviewed for content.

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Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram for reviewing the occurrence of emphysema in adults with Williams-Beuren syndrome, which included searches of databases and other sources. CENTRAL = Central Register of Controlled Trials.

The patient subsequently described and both of his parents consented to the patient's inclusion in this report.

A Newly Reported Case

Accompanied by his parents, a 31-year-old never smoking male with WBS sought consultation regarding the risk of emphysema posed by having both WBS and AATD. Heterozygous PI*MZ AATD was first diagnosed in childhood. Clinical manifestations of WBS included a history of coarctation of the aorta, status post-repair of ventricular septal defect, patent ductus arteriosus closure, supravalvular pulmonic stenosis status post-repair, heart block with subsequent pacemaker placement, and hypertension. The patient also had Crohn's disease, treated currently with methotrexate and adalimumab. He had no significant occupational exposure and physical examination of the chest was normal, along with room air oxygen saturation of 95%. Report of a CT chest at his home institution 2 years earlier showed no bronchiectasis and no emphysema. Recent PFTs showed no airflow obstruction (FEV₁, 3.19 L [82% predicted]; FEV₁/FVC, 0.76; diffusing capacity

for carbon monoxide, 23.24 mL/min/mm Hg; lower limit of normal [LLN], 23.23 [70% predicted]; and diffusing capacity for carbon monoxide adjusted for alveolar ventilation, 77% predicted).

Results

A total of 419 identified reports were reviewed with 19 satisfying inclusion criteria of describing either the specific pulmonary and/or the multiorgan features of adult patients with WBS. In total, 393 adult patients with WBS ([Fig 1](#); [Table 1](#), [Table 2](#))^{1-5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 were described in these reports, including a single newly reported patient (as previously discussed). The largest available report describing multiorgan clinical manifestations in 205 patients with WBS (including 10 with PI*MZ AATD)⁵ included adults but did not report the individual patients' ages (range, 8-45 years of age). No instance of emphysema was described in that series of 205 patients, which focused on exhaustively categorizing the clinical manifestations of WBS in many organ systems (eg, cardiovascular, GI, musculoskeletal, endocrine).}

Table 1. Summary of Available Series of Adult Patients With Williams-Beuren Syndrome

Study	No. of Patients Reported	Age, Mean Years (Range)	AATD Status	Findings: Pulmonary Function	Findings: Imaging
Morris et al ⁵	205	Mean and individual patient ages not stated (8-45)	19 patients with AATD (10 with PI*MZ, 9 with PI*MS, 0 with PI*ZZ)	NS	NS
Honjo et al ⁸	55	14 (2-30)	NS	NS	No pulmonary abnormalities noted
Kronquist et al ⁹	22 (normal AAT levels in all patients)	29 (18.1-55.2)	NS	Obstruction in 8 patients (36%); air trapping in 10 patients (45.5%)	Small subpleural blebs in 1 patient; heterogeneity (air trapping) noted in 3 patients
Pangallo et al ¹⁰	22	18.9 (11.5 ± 26.3)	Normal	Obstruction in 6 patients (27%); bronchodilator reversibility tested in 5 of 6 patients, and all 5 patients had completely	NS

Study	No. of Patients Reported	Age, Mean Years (Range)	AATD Status	Findings: Pulmonary Function	Findings: Imaging
				reversible obstruction; 2 of 5 patients carried a clinical diagnosis of asthma; patients on antihypertensives had poorer pulmonary function	
Cherniske et al ¹¹	20	38.8 (30-51)	NS	NS	No pulmonary abnormalities noted
Wan et al ¹	17	20 (15-27)	Normal in 1 patient, not stated in others	13 had spirometry, all of which were normal (most patients were not able to sustain expiration > 6 s or to reach a plateau on their volume-time curve); therefore, analyses of FVC and FEV ₁ /FVC were not performed	Only 1 patient underwent chest CT scan, which showed moderate paraseptal emphysema; DLCO and spirometry were normal but RV was 129% predicted
Morris et al ⁶	17	23.5 (17-34)	NS	NS	No pulmonary abnormalities noted
Morris et al ⁷	13	28.5 (17-45)	NS	NS	No pulmonary abnormalities noted
Plissart et al ¹²	11	36.6 (17-66)	NS	NS	No pulmonary abnormalities noted
Kurolap et al ¹³	2	48 (38-58)	NS	NS	No pulmonary abnormalities noted
Soukup et al ¹⁴	1	19	NS	NS	Granulomatous mass

Study	No. of Patients Reported	Age, Mean Years (Range)	AATD Status	Findings: Pulmonary Function	Findings: Imaging
Wojcik et al ¹⁵	1	49	Normal	Severe obstruction (FEV ₁ /FVC, 0.31; FEV ₁ , 21% predicted)	Extensive emphysema and bullous disease
Botnaru et al ¹⁶	1	23	NS	NS	No emphysema seen; abnormalities of segmental arteries—poststenotic aneurysmal dilation
Opoka-Winiarska et al ¹⁷	1	15	NS	NS	No pulmonary abnormalities noted
Stasia et al ¹⁸	1	16	NS	NS	No pulmonary abnormalities noted
Nicolini et al ¹⁹	1	33	NS	NS	Pulmonary arteriovenous malformations
Gilbert-Barness et al ²⁰	1	20	Normal	NS	Pulmonary aspergillosis (concomitant chronic granulomatous disease)
Mulik et al ²¹	1	27	NS	NS	No pulmonary abnormalities noted
Rashid et al ²²	1	49	NS	NS	Atelectasis in right upper lobe, from associated diaphragmatic hernia

AATD = alpha-1 antitrypsin deficiency; DLCO = diffusing capacity for carbon monoxide; NS = not stated.

Table 2. Results of Systematic Review of Emphysema in Adult Patients With Williams-Beuren Syndrome

Characteristic	Value
No. of eligible studies	19
No. of patients	393
No. of patients tested for alpha-1 antitrypsin deficiency	44
No. of patients with alpha-1 antitrypsin deficiency	19
No. of patients with PFTs	57
No. of patients with PFT abnormalities ($\text{FEV}_1/\text{FVC} < \text{LLN}$ or air trapping) (%)	22 (38.6)
No. of patients with persistent obstruction on PFTs ($\text{FEV}_1/\text{FVC} < \text{LLN}$) (%)	10 (17.5)
No. of patients who were tested for bronchodilator response	6
No. of patients who had persistent obstruction after bronchodilator	0
No. of patients who underwent chest CT scan	26 (including current newly reported patient)
No. of patients who had emphysematous changes on CT scan (%)	3 (11.5)
No. of patients with COPD features, by imaging or PFTs (%)	17 (4.3)

LLN = lower limit of normal; PFT = pulmonary function test.

Although reports in this narrative review were included based on clinically characterizing multiple organ involvement in adults with WBS, PFTs were reported in only 57 patients included in three series^{19,10} and an additional case report.¹⁵ PFTs were also performed in the case previously reported. Taken together, evidence of obstruction (defined as $\text{FEV}_1/\text{FVC} < \text{LLN}$ or air trapping) was reported in 38.6% (22 of 57) of these physiologically characterized individuals; however, postbronchodilator FEV_1/FVC results were only available in six cases, of which none were abnormal. Results of CT imaging data were presented in 26 previously reported individuals with WBS and in the previously mentioned case reported. Radiographic evidence of emphysema on chest CT scan was reported in only three of these 26 individuals (11.5%).

In the available cohort in which PFTs and imaging of adults with WBS were most completely characterized, Kronquist et al⁹ reported PFTs in 22 adults with WBS (median age, 25.0 years; interquartile range, 24.3) and results of chest CT scans in 19 individuals. Among these 22 adults, 10 (45.5%) had values of $\text{FEV}_1 < \text{LLN}$ and eight (36.3%) had $\text{FEV}_1/\text{FVC} < \text{LLN}$; however, postbronchodilator results were not reported in any (which precludes excluding asthma). Air trapping manifested as residual volume and/or residual volume/total lung capacity $> 120\%$ was evident in 10 individuals (45.5%). Notably, subpleural blebs were reported in only one of the 19 chest CT scans (5.2%), and parenchymal heterogeneity with focal hyperlucency suggestive of air trapping was evident only in three scans, totaling 18.1% with chest CT findings even suggestive of (though not definitive for in the instance of hyperinflation) emphysema.

Based on these descriptions, recognizing that estimates vary across series based on the inclusion and type of testing performed (eg, from as high as 45.5% for any physiological suggestion [albeit not definitive evidence] of emphysema to 18.1% for any chest CT findings even suggestive of [though not definitive for] emphysema), an inclusive estimate ([Table 2](#)) of the prevalence of chronic airflow obstruction or radiographic emphysema in adults with WBS was 4.3% (17 of 393). If the largest series of 205 patients⁶ is excluded from this calculation ([Table 3](#)), because failure to mention emphysema is not regarded as tantamount to its absence, then the prevalence estimate adjusts to 17 of 188 (9.0%). Another approach to generating an estimate of the burden of emphysema is to pool the series that have reported only PFTs,¹⁰ or only chest CT findings,^{14,16,17,19,22} or as in the newly reported case in this paper, both chest CT scan and PFTs.^{1,9,15} From these series, reporting on a total of 62 patients, evidence of emphysema was seen in 24 patients. Of these, 21 patients had decreased FEV_1/FVC or air trapping, two patients had only CT evidence of emphysema,^{1,9} and one patient had findings suggestive of emphysema on both CT scan and PFTs.¹⁵ This approach leads to an upper limit estimate of the prevalence of emphysema of 38.7%. On this basis, the range of prevalence estimates for findings consistent with (but not confirmatory of) emphysema based on PFT and/or CT scan abnormalities in WBS is 4.3% to 38.7%.

Table 3. Results of Systematic Review of Emphysema in Reported Patients With Williams-Beuren Syndrome, Excluding Patients in Morris et al⁶

Characteristic	Value
No. of eligible studies	18
No. of patients	188
No. of patients tested for alpha-1 antitrypsin deficiency	25
No. of patients with alpha-1 antitrypsin deficiency	0
No. of patients with PFTs	57
No. of patients with PFT abnormalities ($\text{FEV}_1/\text{FVC} < \text{LLN}$ or air trapping) (%)	22 (38.6)

Characteristic	Value
No. of patients with persistent obstruction on PFTs (FEV₁/FVC < LLN) (%)	10 (17.5)
No. of patients who were tested for bronchodilator response	6
No. of patients who had persistent obstruction after bronchodilator	0
No. of patients who underwent chest CT scan	26 (including current newly reported patient)
No. of patients who had emphysematous changes on CT scan (%)	3 (11.5)
No. of patients with PFT/imaging abnormalities suggestive of emphysema (%)	17 (9)

LLN = lower limit of normal; PFT = pulmonary function test.

Regarding the additive risk of having WBS with AATD, in the only prior series⁵ describing the clinical features of 10 patients with both WBS and PI*MZ AATD, although PFTs were not reported and the age of patients was not stated, no mention was made of pulmonary symptoms or obstructive lung disease; rather, the only abnormalities that were significantly overrepresented among those with WBS and AATD included scoliosis and joint dislocation (both $P < .001$). The prevalence of supravalvular aortic stenosis was not higher among those with AATD variants than among patients with WBS with the PI*MM (normal) genotype. The case patient with WBS and PI*MZ AATD had undergone both PFTs and chest CT scan and had no evidence of emphysema.

Discussion

In this narrative review of emphysema risk in adults with WBS, despite the predisposition to emphysema that is posed by loss of the elastin gene, the frequency of PFT and CT scan abnormalities suggestive of emphysema based on available series and experience with the single case patient with concomitant PI*MZ AATD—although highly variable across series based on the type of testing reported—is 4.3% to 38.7%. The precision of this estimate is hampered by the lack of uniformity in testing protocols across series, with the largest series of adults with WBS reporting only clinical observations,⁵ and even series reporting PFTs hampered in their differentiation of COPD vs asthma by lack of postbronchodilator testing.^{9,10} Definitive evidence of emphysema on CT chest scan was reported in only 11.5% of the relatively few adult patients with WBS in whom chest CT scan was performed ([Table 2](#)).

Although PI*MZ AATD might be thought to pose a heightened risk for developing emphysema in WBS based on the decreased level of antiprotease defense associated with lowered alpha-1 antitrypsin levels that compounds the loss of elastin related to WBS, the sparse available experience of 10 such patients,⁶ and the single newly reported patient presented here, suggests otherwise in that no emphysema was reported among this small group of patients. As important qualifiers, neither

smoking status nor pulmonary function results in the 10 patients with PI*MZ reported by Morris et al⁵ was reported; the case patient was a never smoker and more convincingly lacked evidence of emphysema based on chest CT scan and PFTs, which included normal measurements of diffusing capacity.

In the context that this aggregate experience suggests that emphysema is relatively uncommon among reported patients with WBS, including those with PI*MZ AATD, several limitations about the precision of this estimate of emphysema prevalence warrant mention. First, few of the patients (other than 20 reported by Wan et al¹ and Kronquist et al⁹ and the newly described case patient) underwent chest CT scan, so that definitive evidence of emphysema was largely unavailable. To the extent that emphysema may be present in the absence of airflow obstruction, as was the case in the 31-year-old male reported by Wan et al,¹ the prevalence of emphysema may be underestimated in the available series. On the other hand, inclusion of individuals whose PFTs showed obstruction and/or air trapping on PFTs performed without postbronchodilator testing⁹ could lead to overestimation of the emphysema prevalence (eg, by counting patients with asthma). Indeed, five patients in the series by Pangallo et al¹⁰ had airflow obstruction that normalized postbronchodilator; two patients noted a history of asthma. Some of the abnormalities on CT scans (eg, air trapping) and PFTs (spirometric obstruction), which are considered suggestive of emphysema or COPD, can be explained by other etiologies (eg, asthma). In this report, which was prompted by a clinical consultation to comment on emphysema in the patient with PI*MZ AATD and WBS, [Table 1](#), [Table 2](#), [Table 3](#) represent an attempt to estimate the risk of emphysema among individuals with WBS. It is noteworthy that performing full PFTs in patients with WBS may be challenged in instances when learning difficulties preclude optimal technical performance.

A second limitation of this analysis is that we cannot discount the possibility that patients in different series were counted more than once. Such multiple counting would confound the prevalence estimates.

Third, although there is little reason to suspect that the reported adult patients with WBS in these series were ascertained in a manner that would have biased the prevalence of pulmonary abnormalities, such selection bias cannot be absolutely discounted. We are unaware of any population-based screening studies in which patients with WBS are fully characterized.

Fourth, the emphysema prevalence estimate of 4.3% ([Table 2](#)) includes in the denominator of the calculation (393) the 205 patients from the largest clinical series reported.⁶ As noted, this series characterized multiorgan manifestations of WBS yet reported no emphysema. Because the assumption that the absence of mention of emphysema does not discount the possibility that emphysema was present, this prevalence estimate could be challenged, encouraging exclusion of these 205 patients from the prevalence calculation. [Table 3](#) summarizes the available reported frequency of CT scan and PFT abnormalities suggestive of emphysema, while excluding those 205 patients from the case series by Morris et al.⁶ In this manner, the estimate of abnormalities suggesting emphysema in WBS changes to 9.0%, higher than 4.3% but still low.

A final shortcoming in the attempt to estimate the prevalence of emphysema in adults in WBS is the paucity of reports reporting older patients in the literature. Most of the reports describing WBS include young adults, and none included patients > 66 years of age.¹³ Of note, in the largest series of 205 patients described by Morris et al,⁶ almost one-half were < 8 years of age. Overall, the observed variability in prevalence estimates and reported assessment methods (including the paucity of CT imaging in reported series) undermines confidence in the prevalence estimates regarding emphysema in WBS. This limitation underscores the need for more systematic study of emphysema

in patients with WBS. At the same time, this narrative review—to our knowledge, the only one available to date—reinforces the current impression that the prevalence of emphysema in WBS remains lower than these authors might have expected based on the associated deletion of the elastin gene and its pathobiologic consequences.

Interpretation

Despite the demonstrable and plausible risk that elastin gene deletion or abnormality can predispose to emphysema, and in the context of the aforementioned significant uncertainties regarding the precision of the prevalence estimate from this narrative review, this survey of reported adults with WBS and the single newly reported patient with PI*MZ AATD shows that definitive emphysema occurs in a minority of individuals with WBS, at least generally, to young adulthood. This relative infrequency of emphysema occurs even in the face of other potential risk factors (eg, PI*MZ AATD) and other clinical manifestations of WBS that likely reflect the effects of elastin deficiency (eg, supravalvular aortic and pulmonic stenosis, diverticular disease, inguinal and other hernias). That radiographically apparent emphysema may be physiologically silent may encourage physicians to consider chest CT imaging in the assessment of all patients with WBS, but certainly in those with any pulmonary symptoms or additional risk factors (eg, smoking, occupational risk). Furthermore, the sparse experience of now only 11 reported patients with PI*MZ AATD and WBS indicates the need for further identification and systematic study of this special population to more confidently ascertain the pulmonary risk that is associated with these combined and potentially compounding risk factors for emphysema.

Abstract

Cigarette smoke (CS), an indoor environmental pollutant, is a prominent risk factor for emphysema, which is a pathological feature of chronic obstructive pulmonary disease (COPD). Emerging function of circRNAs in immune responses and disease progression shed new light to explore the pathogenesis of emphysema. In this research, we demonstrated, by single-cell [RNA](#) sequencing (scRNASeq), that the ratio of M2 macrophages were increased in lung tissues of humans and mice with smoking-related emphysema. Further, our data showed that circADAMTS6 was associated with cigarette smoke extract (CSE)-induced M2 macrophage polarization. Mechanistically, in macrophages, circADAMTS6 stabilized CAMK2A mRNA via forming a circADAMTS6/IGF2BP2/CAMK2A RNA-protein ternary complex to activate CREB, which drives M2 macrophage polarization and leads to emphysema. In addition, in macrophages of mouse lung tissues, downregulation of circADAMTS6 reversed M2 macrophage polarization, the proteinase/anti-proteinase imbalance, and the [elastin](#) degradation, which protecting against CS-induced emphysema. Moreover, for macrophages and in a model with co-cultured lung organoids, the target of circADAMTS6 restored the growth of lung organoids compared to CSE-treated macrophages. Our results also demonstrated that, for smokers and COPD smokers, elevation of circADAMTS6 negatively correlated with lung function. Overall, this study reveals a novel mechanism for circADAMTS6-driven M2 macrophage polarization in smoking-related emphysema and postulates that circADAMTS6 could serve as a diagnostic and therapeutic marker for smoking-related emphysema.

Graphical abstract

In macrophages, the increase of circADAMTS6 levels induced by cigarette smoke recruits IGF2BP2 to stabilize [CAMK2A](#) mRNA via forming a circADAMTS6/IGF2BP2/CAMK2A RNA-protein [ternary complex](#). Then, elevated [CAMK2A](#) drives M2 macrophage polarization by activating [CREB](#), which leads to an increase of [MMP12](#) levels and a decrease of [TIMP1](#) levels. In the lungs, M2 macrophages

cause a proteinase/anti-proteinase imbalance and the degradation of [elastin](#), which induces destruction of alveolar structures, leading to [emphysema](#).

Keywords

Emphysema

circADAMTS6

Cigarette smoke

M2 macrophage polarization

Abbreviations

CS

cigarette smoke

COPD

chronic obstructive pulmonary disease

scRNAseq

single-cell RNA sequencing

CSE

cigarette smoke extract

AMs

alveolar macrophages

MMP12

Matrix metallopeptidase 12

BALF

bronchoalveolar lavage fluid

ncRNAs

noncoding RNAs

CAMK2A

calcium/calmodulin-dependent protein kinase II alpha

CREB

cAMP responsive element binding protein

SPF

specific pathogen-free

TPM

total particulate matter

PMA

phorbol 12-myristate 13-acetate

GEO

Gene Expression Omnibus

TIMP1

TIMP metalloproteinase inhibitor 1

BMDMs

bone-marrow-derived macrophages

FISH

fluorescence *in situ* hybridization

ECM

Extracellular matrix

ETS

environmental tobacco smoke

SPC⁺

surfactant protein-C-positive

1. Introduction

Cigarette smoke (CS), an indoor environmental pollution, contains more than 7000 chemicals, some of which constitute a serious threat to human health ([Soleimani et al., 2022](#)). Following the 2019 [Global Burden of Disease](#) study, CS affects 1.14 billion people and causes 7.69 million deaths per year ([Collaborators, 2021](#)). CS exposure is the principal environmental risk factor for the occurrence and progression of various systemic ailments, including respiratory, nervous, and cardiovascular diseases ([Ma et al., 2021](#)). [Epigenetic mechanisms](#), including non-coding RNA, [DNA modification](#), and [histone modification](#), exert effects on various diseases associated with smoking ([Gould, 2023](#)). However, the specific molecular mechanisms of the [adverse effects](#) caused by smoking remain to be elucidated.

Chronic obstructive pulmonary disease (COPD) is a heterogeneous respiratory disease and a leading cause of death and disability worldwide, causing a social and economic burden ([Christenson et al., 2022](#)). Emphysema, a principal pathological feature of COPD ([Hisata et al., 2021](#)), is characterized by permanent enlargement of the distal alveolar space, attenuation of alveolar elasticity, and irreversible destruction of alveolar tissue ([Vlahos, 2020](#)). Its main pathology involves inflammation, [oxidative stress](#), a proteinase/anti-proteinase imbalance, and apoptosis of alveolar epithelial cells ([Pasupneti et al., 2020](#)). The underlying molecular mechanisms of emphysema progression remains unclear, but is suggested to associate with imbalance of proteinases response of macrophage polarization.

[Alveolar macrophages](#) (AMs), which are mainly derived from [monocytes](#) and are abundant [immune cells](#), contribute to respiratory [disease progression](#) via polarization into different phenotypes ([Belchamber and Donnelly, 2020](#), [Kulikauskaitė and Wack, 2020](#)). The imbalance of proteinases and their inhibitors secreted by AMs is a cause of CS-induced lung injury ([Lugg et al., 2022](#)). Matrix metallopeptidase 12 (MMP12), a macrophage-derived cytokine, is an extracellular matrix (ECM)-degrading [enzyme](#) that mediates the destruction of alveolar structures ([Doyle et al., 2019](#)). Notably, deficiency of MMP12 protected against the expansion of alveolar space and the development of emphysema in CS-exposed mice ([Hautamaki et al., 1997](#)). In addition, macrophages of the M1 phenotype, which are classically activated, produce [inflammatory cytokines](#) and kill bacteria; those of the M2 phenotype are alternatively activated and participate in [immune regulation](#) and tissue remodeling ([Dong et al., 2022](#)). Meanwhile, the clinical report showed the higher levels of M2-related genes in macrophages isolated from bronchoalveolar lavage fluid (BALF) of smokers and COPD smokers ([Shaykhiev et al., 2009](#)). However, whether M2 macrophage polarization is involved in smoking-related emphysema and the underlying molecular mechanisms need to be explored.

circRNAs, covalently closed single-stranded noncoding RNAs (ncRNAs), are generated by back-splicing from [precursor mRNAs](#) and are highly stable ([Liu et al., 2021](#)). circRNAs exhibit a diverse range of molecular mechanisms and functions in cells, and, in addition to their classical function as sponges for [miRNAs](#), interact with proteins or mRNAs to regulate gene expression ([Misir et al., 2022](#)). In gastric cancer cells, circARID1A enhances the stability of [SLC7A5](#) mRNA by binding to IGF2BP3 to promote the [cell proliferation](#) ([Ma et al., 2022](#)). Diseases related to exposure to environmental chemical pollution are usually accompanied by alterations in specific circRNAs expressions ([Li et al., 2020](#)). Our previous study revealed that circRNAs are involved in CS-induced lung injury by mediating apoptosis and [ferroptosis](#) in alveolar epithelium ([Xia et al., 2023](#), [Zhao et al., 2023](#)). circRNAs also play unique roles in immune response and regulating immune [cell differentiation](#) ([Kumar et al., 2023](#)). However, the role of circRNAs in regulating polarization of macrophage in smoking-related emphysema remains unknown.

In the present research, we establish the mechanism for circADAMTS6-driven M2 macrophage polarization in smoking-related emphysema. Mechanically, circADAMTS6 recruits IGF2BP2 to stabilize calcium/calmodulin-dependent protein kinase II alpha (CAMK2A) mRNA via forming a circADAMTS6/IGF2BP2/CAMK2A RNA-protein [ternary complex](#), which activates cAMP responsive element binding protein (CREB), a transcription factor, to promote M2 macrophage polarization. M2 macrophages trigger a proteinase/anti-proteinase imbalance and cause elastin degradation, which contributes to the destruction of the alveolar structure, leading to emphysema. Herein, we demonstrate that circADAMTS6 is involved in CS-induced emphysema via driving M2 macrophage polarization, and circADAMTS6-driven M2 polarization is a potential target for smoking-related emphysema.

2. Methods

2.1. Human samples

Lung tissues of humans used in this study were collected from lobectomies for benign [lung nodules](#) or from lung transplants performed at Wuxi People's Hospital affiliated to Nanjing Medical University (NJMU). The protocol for human research was approved by the ethics committee of NJMU (number: 2021-130). All human samples were obtained with informed consent from human donors and were anonymized before use. Physician diagnoses of COPD followed Global Strategy for Diagnosis, Treatment, and Management of COPD. For qRT-PCR, a cohort of serum samples were

harvested from COPD smokers, Smokers, and Non-smokers. The clinicopathological characteristics for serum donors and for lung tissue donors are collected in [Tables 1](#) and [S1](#).

Table 1. Clinical information for human serum donors.

Empty Cell	Non-smokers	Smokers	COPD smokers
Number	35	28	40
Sex (male)	33 (94.3 %)	27 (96.4 %)	37 (92.5 %)
Age (years)	60.5 ± 5.8	63.6 ± 7.2	65.6 ± 7.4
BMI	25.4 ± 2.8	24.6 ± 3.1	23.3 ± 4.4
Smoking history (pack-years)	—	31.3 ± 15.7	45.9 ± 33.3
GOLD grade, I / II / III / IV	—	—	12/11/10/7
FEV₁ (%) predicted	92.7 ± 14.5	85.5 ± 16.7	58.5 ± 25.2 ^{**,##}
FEV₁/FVC (%)	82.9 ± 7.0	82.7 ± 7.8	52.1 ± 12.3 ^{**,##}

Data are presented as means ± SD.

**

P < 0.01 versus Non-smokers group.

##

P < 0.01 versus Smokers group.

2.2. Mice with CS-induced emphysema

Male BALB/c mice at 6 weeks of age, obtained from the Animal Core Facility of NJUM, were housed under specific pathogen-free (SPF) conditions at the Safety Assessment and Research Center for Drug, Pesticide and Veterinary Drug of Jiangsu Province. The protocols for animals were reviewed and approved by the animal ethics committee of NJUM (IACUC-2209016, IACUC-2209049).

Mice were housed in an SPF room, maintained at a constant temperature (23 ± 3 °C), relative humidity (40 %–70 %), and with successive 12-hr light/dark cycles. Based on the existing research and our previous study, we established the concentration of CS to be used in mice and established mice with CS-induced emphysema. Yoshida et al. induced experimental COPD in mice at 6–8 weeks of age by using a whole-body exposure system, which exposed them to CS (200 mg/m³ total suspended particles) from 3R4F Research Cigarettes five days a week for six months ([Yoshida et al., 2019](#)). Ten mice were exposed to five 3R4F reference cigarettes at 30-min smoke-free intervals four times a day, five days a week for 24 weeks, and lungs of the mice showed expansion of alveolar space and destruction of alveolar walls ([Bracke et al., 2013](#)). Our previous study showed that mice with CS-induced emphysema were exposed to 300 mg/m³ total particulate matter (TPM) in a whole-body exposure system for 60 min twice a day, 4 h apart, 5 days a week for a total of 16 weeks ([Xia et al.,](#)

[2022](#), [Zhao et al., 2023](#)). Further, we converted environmental tobacco smoke (ETS) exposure dose for humans and mice based on published ETS exposure dose conversion models ([Hartog et al., 2019](#), [Phillips, 2017](#)). The CS exposure dose of 0, 200, and 300 mg/m³ in mice is equivalent to none, moderate (0.5–1 pack/day), and severe smoking (>1 pack/day) for humans ([Jang et al., 2012](#)). In the present study, the exposure protocol for a mouse model of emphysema was as previously reported ([Xia et al., 2023](#)). In brief, mice were placed in a whole-body exposure system (Beijing Huironghe Technology CO., Ltd., China) and exposed at 200 or 300 mg/m³ TPM using 3R4F Research Cigarettes from the University of Kentucky, USA. The mice were chronically exposed to CS for 1 hr twice a day, with smoke-free intervals of 4 hr, five days a week for 24 weeks. Age-matched mice kept in a similar environment without exposure to CS served as controls. Finally, with this concentration of CS, there was destruction of the alveolar wall and an increase in the mean linear intercept in the mouse lungs, which was involved in the progression of smoking-related emphysema ([Fig. S1](#)).

2.3. Cell culture

THP-1 cells and RAW264.7 cells were obtained from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences. THP-1 cells were maintained in RPMI-1640 (Life Technologies/Gibco, C11875500BT), and RAW264.7 cells were maintained in Dulbecco's modified Eagle's medium (Life Technologies/Gibco, C11875500BT) containing 10 % fetal bovine serum (FBS, Life Technologies/Gibco, 10099141C) and 0.1 % Penicillin-Streptomycin (Beyotime Institute of Biotechnology, C0222) under 5 % CO₂ at 37 °C. THP-1 [monocytes](#) were treated with 100 µg/mL of phorbol 12-myristate 13-acetate (PMA, Sigma-Aldrich, St. Louis, MO) for 48 h to transform them into macrophage-like cells, THP-Ms. This was followed by removing the PMA by washing and a 24-hr rest period in fresh media prior to treatment. PMA (10 mg/mL) was dissolved in [DMSO](#) to 1 mg/mL, then diluted to 100 µg/mL in RPMI-1640. Aliquots were stored at –80 °C. The THP-1 cells were passaged at a ratio 1:2 every 2 days. The RAW264.7 cells were passaged at a ratio 1:4 every 2 days.

Bone-marrow-derived macrophages (BMDMs) were extracted with the following protocol ([Tang et al., 2022a](#)). First, male BALB/c mice were sacrificed at 12 weeks of age under permission of the animal ethics committee of NJMU. Mice were immersed in [iodophor](#), and femurs and tibias were removed on a clean laboratory bench. All bones were transferred to 75 % ethanol for disinfection and kept in ice-cold sterile PBS. Second, the bones were flushed with ice-cold PBS by suction in a 1-mL syringe until the color was white. The collected cell suspension was filtered through a 0.70-µm pore filter, and the suspension was centrifuged at 1500 rpm for 5 min. Cells were suspended in 1 mL of red blood [cells lysis](#) buffer (Solarbio, R1010) for 5 min, and the cell suspension was centrifuged at 1500 rpm for 5 min. The bone marrow cells were maintained in Dulbecco's modified Eagle's medium (Life Technologies/Gibco, C11875500BT) containing 20 % fetal bovine serum (FBS, Life Technologies/Gibco, 10099141C), 0.1 % Penicillin-Streptomycin (Beyotime, C0222), and 20 ng/ml M-CSF (PeproTech, 315-02) under 5 % CO₂ at 37 °C for 7 days. The purity of the [BMDM culture](#) was validated by flow cytometry; the percentage of F4/80⁺ cells was >90 % ([Fig. S7A](#)).

According to previous research and the association of the exposure dose between humans and cell cultures, we identified the concentration of CS used in cell culture. As previously reported, Fu et al. induced M2 macrophage polarization by treatment with 0.25 %, 0.5 %, 1 %, 2 %, or 4 % CSE ([Fu et al., 2015](#)). Our previous study showed that 1 %, 2 %, and 4 % CSE promotes the progression of Non-Small Cell Lung Cancer by inducing M2 polarization of macrophages *in vitro* ([Cheng et al., 2023](#)). In our study, there was an increased number of M2 macrophages in CSE-treated macrophage cell lines and primary macrophages with a dose–response relationship ([Figs. S7B and 8A](#)). In addition, 2.5 % to 10 % CSE roughly corresponds to exposure from smoking 0.5 to 2 packs of cigarettes per day ([Su et al., 1998](#)). Referring to relevant studies, the concentrations of 0 %, 1 %, 2 %, and 4 % CSE for

macrophages is similar to none, mild (0.5 pack/day), moderate (0.5–1 pack/day), and severe smoking (>1 pack/day) by humans ([Jang et al., 2012](#)). Therefore, we treated macrophages with 0 %, 1 %, 2 %, or 4 % CSE.

2.4. Silencing of circADAMTS6 in macrophages of mouse lung tissue

AAV9-circADAMTS6 [shRNA](#) was purchased from VigeneBio (Shangdong, China). pAV-F4/80-GFP-mir30.shRNA with an inserted nonsense sequence was used as a negative control. We administered 100 μ L of sterile saline containing 5×10^{11} virus particles by a nasal drip once a week for two weeks, initiating treatment after a week of CS exposure. Animals were checked daily, and their weights were compared to those for control mice. The viral load in the lungs of the mice peaked from 2 weeks to 1 month, after which the efficiency of infection was verified by fluorescence.

2.5. Statistical analysis

Statistical analyses were performed by use of GraphPad Prism 9 (Graphpad Software Inc., San Diego, CA) or SPSS 19.2 (SPSS Inc., Chicago, USA). Data were derived for at least three independent experiments and were presented as means \pm SD or SEM. Methods of statistical testing included unpaired Student's t tests, chi-square tests, and one-way analysis of variance with Bonferroni correction. For the human population, the Pearson correlation coefficient was used to assess the correlation between serum circADAMTS6 expression and lung function. The differences were statistically significant when $P < 0.05$ or $P < 0.01$.

Further details of materials and methods are described in [supplementary information](#).

3. Results

3.1. M2 macrophage polarization exists in smoking-related emphysema

Emphysema is a common characteristic of COPD ([Spix et al., 2022](#)). To elucidate the macrophage phenotype in smoking-related emphysema, we analyzed the results of scRNAseq from GSE173896 and GSE168299. These results were validated for both human and mouse lung tissues by immunofluorescent staining and [western blotting](#) ([Fig. 1A](#)). We identified 16 [cell lineages](#), which expressed specific marker genes, from 3 normal and 5 COPD patients by dimensionality reduction and cluster analysis ([Fig. 1B](#) and [1C](#)). Then, we proceeded to recluster the macrophages using the UMAP analysis and formed 7 cell lineages ([Fig. 1D](#) and [1E](#)). The ratio of M2 macrophages was increased in COPD lungs, compared to the control lungs ([Fig. 1F](#)). In addition, we also analyzed scRNAseq from GSE168299 and identified 18 cell lineages on mouse lung tissues treated with or without CS ([Fig. 1G](#) and [1H](#)). Macrophages were reclustered into 3 cell lineages and the ratio of M2 macrophages was elevated in lungs of mice exposed to CS ([Fig. 1I–K](#)). Previous studies have shown that AMs of the M2 phenotype were elevated in BALF of normal smokers and COPD patients, which is consistent with results in CS-exposed COPD mouse models ([Eapen et al., 2017](#), [He et al., 2017](#)). Then, we collected lung tissues from COPD smokers, Smokers, and Non-smokers ([Table S1](#)) and established mice with CS-induced emphysema ([Fig. S1A and S1B](#)). The numbers of [CD68⁺CD206⁺](#) M2 macrophages were elevated for Smokers and COPD smokers ([Fig. 2A](#)). We evaluated the M2 macrophage polarization in the lungs of COPD patients following the GOLD guideline and found that the numbers of [CD68⁺CD206⁺](#) cells were elevated with the development of COPD ([Fig. S2](#)). The protein levels of ARG1 were also elevated ([Fig. 2B](#)). In addition, as determined by RNA-seq, there was elevated expression of M2 genes of AMs from Smokers and COPD smokers as shown by data from two Gene Expression Omnibus (GEO) databases (GSE13896 and 130928) ([Fig. S3](#)). In lung tissues of mice, the numbers of F4/80⁺CD206⁺ M2 macrophages were elevated ([Fig. 2E](#)), as were ARG1 protein

levels (Fig. 2F). These findings demonstrate that M2 macrophages are elevated in smoking-related emphysema.

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Fig. 1. Single-cell [transcriptomic](#) atlas of lung cells and macrophages in lung tissues of human and mice. (A) Schematic chart of workflow. The results of [scRNAseq](#) from GSE173896 and GSE168299 showed expression of M2 macrophage in lung tissues, which were validated for human and mouse lung tissues by immunofluorescent staining and [western blotting](#). (B) Uniform manifold approximation and projection (UMAP) visualizing the distribution of identified 16 lung [cell lineages](#) from 3 normal and 5 [COPD](#) patients (GSE173896). (C) Dot plots showing the expression of marker genes for per-cell cluster. (D) UMAP analysis of macrophages identified 7 distinct clusters. (E) Dot plots showing the expression of marker genes for per-cell cluster. (F) The ratio of different cell lineages in macrophages, colored by cell lineages. (G) Uniform manifold approximation and projection (UMAP) visualizing the distribution of identified 18 lung cell lineages from 4 mice treated without CS and 4 mice treated with CS (GSE168299). (H) Dot plots showing the expression of marker genes for per-cell cluster. (I) UMAP analysis of macrophages identified 3 distinct clusters. (J) Dot plots showing the expression of marker genes for per-cell cluster. (K) The ratio of different cell lineages in macrophages, colored by cell lineages.

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Fig. 2. M2 macrophages, a proteinase/anti-proteinase imbalance and the degradation of [elastin](#) exist in smoking-related [COPD](#) and in mice with CS-induced emphysema. Lung tissues of humans were collected from Non-smokers ($n = 8$), Smokers ($n = 10$), or COPD smokers ($n = 14$). Male BALB/c mice ($n = 6$) at 6 weeks of age were exposed to 0, 200, or 300 mg/m³ [TPM](#) CS for 24 weeks. (A) Representative images (left) of [CD68](#) and CD206 [immunostaining](#) in lung tissues of humans (scale bars, 20 μ m). The numbers of [CD68](#)⁺CD206⁺ cells in lung tissues of human (right). (B) Representative [immunoblots](#) (left), and relative quantitative expressions (right) of [ARG1](#) in lung tissues of human. (C) Representative images (left) of CD206 and [MMP12](#) [immunostaining](#) of lung tissues of humans (scale bars, 20 μ m). The numbers of CD206⁺MMP12⁺ cells in lung tissues of humans (right). (D) Representative immunoblots (left), and relative quantitative expressions (right) of MMP12, [TIMP1](#), and [elastin](#) in lung tissues of humans. (E) Representative images (left) of F4/80 and CD206 immunostaining in lung tissues of mice (scale bars, 20 μ m). The numbers (right) of F4/80⁺CD206⁺ cells in lung tissues of mice. (F) Representative immunoblots (left), and relative quantitative expressions (right) of ARG1 in lung tissues of mice. (G) Representative images (left) of CD206 and MMP12 [immunostaining](#) in lung tissues of mice (scale bars, 20 μ m). The numbers of CD206⁺MMP12⁺ cells in lung tissues of mice (right). (H) Representative immunoblots (left), and relative quantitative expressions (right) of MMP12, [TIMP1](#), and elastin in lung tissues of mice. Data represent means \pm SEM. ** $P < 0.01$, compared with Non-smokers group or compared with 0 mg/m³ TPM CS group.

The imbalance of proteinase/anti-proteinase derived from macrophages is the central mechanism of COPD pathogenesis ([Ishii et al., 2014](#)). We found that, in Smokers and COPD smokers, the numbers of

MMP12⁺ cells in CD206⁺ M2 macrophages, which degrades elastin, was elevated ([Fig. 2C](#)). In the lung tissues of humans, MMP12 protein levels were elevated, but TIMP metallopeptidase inhibitor 1 (TIMP1), which is involved in controlling MMP12 activity ([Spix et al., 2022](#)), protein levels were lower ([Fig. 2D](#)). In addition, as determined with the two GEO databases described above, MMP12 expressions were increased and TIMP1 expressions were decreased in AMs from smokers and COPD smokers ([Fig. S4](#)). The protein levels of elastin decreased in Smokers and COPD smokers ([Fig. 2D](#)). Further, we found the same effects in CS-induced lungs of mice ([Fig. 2G](#) and [2H](#)). Together, these results show that a proteinase/anti-proteinase imbalance and elastin degradation, induced by M2 macrophage polarization, exist in smoking-related emphysema.

3.2. The levels of circADAMTS6 are elevated in CSE-treated macrophages

To explore the role of circRNAs in macrophages, we utilized macrophages (THP-Ms) derived from THP-1 cells and BMDMs. Firstly, we performed circRNA sequencing for THP-Ms exposed to 0 % or 4 % CSE, and found 45 altered circRNAs ($P < 0.05$ and $|\log^2\text{FC}| > 2.0$) compared to controls as shown by a heatmap ([Fig. 3A](#)). A volcano plot ([Fig. 3B](#)) showed the top ten up-regulated circRNAs, which are circ_0007113 (circHERC4), circ_0072688 (circADAMTS6), circ_0001821 (circPVT1), circ_0003836 (circUGGT2), circ_0008338 (circZNF215), circ_0000033 (circCEP85), circ_0006063 (circAPBB1IP), circ_0004851 (circCAPRIN1), circ_0008043 (circPTGR1), and circ_0016374 (circTFEC). Subsequently, the levels of the top ten up-regulated circRNAs in THP-Ms were determined by qRT-PCR ([Fig. 3C](#)) or in BMDMs ([Fig. S5](#)) exposed to 0 %, 1 %, 2 %, or 4 % CSE for 48 h. In CSE-treated macrophages, circADAMTS6 and circAPBB1IP were elevated in a concentration-dependent manner, but circADAMTS6 was most significantly increased ([Fig. 3C](#) and [S5](#)). Based on these results, circADAMTS6 was selected for further validation and for functional experiments.

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Fig. 3. Identification and characterization of circADAMTS6 in CSE-treated THP-Ms. THP-Ms were treated with 0 %, 1 %, 2 %, or 4 % CSE for 48 h. (A) Heatmap showing differentially expressed circRNAs in THP-Ms. (B) Volcano plot showing the top 10 up-regulated circRNAs in THP-Ms. (C) Levels of the top ten up-regulated circRNAs in THP-Ms as measured by qRT-PCR. (D) Schematic representation showing that circADAMTS6 was cyclized by six exons from the ADAMTS6 gene and [Sanger sequencing](#) for the back-splicing junction in THP-Ms (top) and BMDMs (bottom). (E) [Agarose gel electrophoresis](#) (AGE) showing the expression of circADAMTS6 and ADAMTS6 in THP-Ms treated with or without [RNase](#) R. (F) The abundances of circADAMTS6 and ADAMTS6 in THP-Ms were measured by qRT-PCR. (G) The expression of circADAMTS6 in the cytoplasm and nuclei of THP-Ms. (H) The localization of circADAMTS6 in THP-Ms was assessed by [RNA](#) fluorescence *in situ* hybridization (scale bars: 20 μm). Data represent means \pm SD ($n = 3$). * $P < 0.05$, ** $P < 0.01$, compared with 0 % CSE-treated cells or ADAMTS6 mRNA. n.s., no significance.

circADAMTS6 was formed by back-splicing of exon 2–7 of the ADAMTS6 gene in THP-Ms and BMDMs ([Fig. 3D](#)). Divergent primers that amplify circADAMTS6 detected only in cDNA but not in gDNA, and circADAMTS6 amplified from cDNA, were resistant to [RNase](#) R ([Fig. 3E](#)). The expression of circADAMTS6 was more stable than linear ADAMTS6 mRNA ([Fig. 3F](#)). Furthermore, circADAMTS6 was localized principally in the cytoplasm of THP-Ms, as demonstrated by nuclear and cytoplasmic extraction ([Fig. 3G](#)) and fluorescence *in situ* hybridization (FISH) ([Fig. 3H](#)) examinations. Finally, by use of FISH and immunofluorescence assays, we found that, in lung tissues of Smokers and COPD

smokers, the expression of circADAMTS6 was elevated and mainly located in CD68⁺ macrophages ([Fig. S6](#)). Together, these findings show that, in CSE-treated macrophages, the expression of circADAMTS6 is abundant and stable.

3.3. In CSE-treated macrophages, circADAMTS6 is involved in M2 polarization and in a proteinase/anti-proteinase imbalance

To determine the influence of CS on macrophage polarization, we cultured murine BMDMs ([Fig. S7A](#)). For CSE-treated BMDMs, flow cytometry analysis revealed elevated numbers of F4/80⁺CD206⁺ M2 macrophages ([Fig. S7B](#)). Consistent with flow cytometry results, immunofluorescence and immunoblots demonstrated that CD206 and ARG1, markers for M2 macrophages, were elevated ([Fig. S7C and S7D](#)). Immunofluorescence experiments confirmed that levels of MMP12 in CD206⁺ M2 macrophages were high ([Fig. S7E](#)). Further, levels of MMP12 protein and mRNA were elevated, but levels of TIMP1 protein and mRNA were lower ([Fig. S7D and S7F](#)). The levels of elastin degradation activity of CSE-treated BMDMs were high ([Fig. S7G](#)). Further, we found the same effects with CSE-treated THP-Ms ([Fig. S8](#)). These results reveal that elevated M2 polarization, and a proteinase/anti-proteinase imbalance in CSE-treated macrophages.

Next, to clarify the function of circADAMTS6 in M2 macrophage polarization, we down-regulated circADAMTS6 in CSE-treated THP-Ms ([Fig. 4A](#)). Flow cytometry analysis demonstrated that downregulation of circADAMTS6 decreased the CSE-induced elevated numbers of CD11b⁺CD206⁺ M2 macrophages and ARG1 protein levels, but not circAPBB1IP ([Fig. 4B, 4C and Fig. S9](#)). RNA-seq showed that expressions of TGM2 and ADORA3, M2-related genes, were significantly lower, as verified by qRT-PCR ([Fig. S10A-C](#)). For THP-Ms, downregulation of circADAMTS6 reversed the CSE-induced increased MMP12 protein and mRNA levels and decreased TIMP1 protein and mRNA levels ([Fig. 4C, S10D and S10E](#)). For CSE-treated THP-Ms, downregulation of circADAMTS6 blocked the increased levels of elastin degradation activity ([Fig. 4D](#)). We found the same results for CSE-treated RAW264.7 cells, a murine macrophage cell line ([Fig. S11](#)). These results indicate that, in CSE-treated macrophages, circADAMTS6 participates in M2 polarization and in a proteinase/anti-proteinase imbalance.

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Fig. 4. circADAMTS6 is involved in M2 polarization, the proteinase/anti-proteinase imbalance, and activation of the cAMP signaling pathway in CSE-treated THP-Ms. THP-Ms were treated with 4 % CSE for 48 h after transfection with circADAMTS6 siRNA or NC siRNA for 6 h. (A) The levels of circADAMTS6 in THP-Ms were measured by qRT-PCR. (B) Representative flow cytometry plots (top) and quantification (bottom) of CD11b⁺CD206⁺ macrophages in THP-Ms. (C) Representative immunoblots (left), and relative quantitative expressions (right) of ARG1, MMP12, and TIMP1 in THP-Ms. (D) The levels of elastin degradation activity of THP-Ms were measured by fluorescence. (E) Schematic chart of workflow. Total RNA was extracted from THP-Ms treated with 4 % CSE for 48 h following transfection with circADAMTS6 siRNA or NC siRNA and then subjected to RNA sequencing. Heatmap (F) and Volcano plot (G) of differentially expressed genes in RNA sequencing. (H) Chord plot of KEGG functional enrichment analysis of the dysregulated genes. (I) Representative immunoblots (top) and relative quantitative expressions (bottom) of CAMK2A and p-CREB in THP-Ms. Data represent means \pm SD (n = 3). **P < 0.01, compared with 0 % CSE-treated cells. ##P < 0.01, compared with 4 % CSE-treated cells.

3.4. circADAMTS6 activates CREB via CAMK2A in CSE-treated macrophages

To elucidate the mechanism of circADAMTS6 in CS-induced M2 macrophage polarization, we performed RNA-seq for CSE-treated THP-Ms transfected with or without circADAMTS6 siRNA ([Fig. 4E](#)). Bioinformatic analysis revealed 751 differentially expressed genes ($P < 0.05$ and $|\log^2\text{FC}| > 1.0$), of which 431 genes were down-regulated and 320 genes were up-regulated, compared to CSE-treated THP-Ms ([Fig. 4F](#) and [4G](#)). KEGG analysis showed that the cAMP [signaling pathway](#) was enriched ([Fig. 4H](#)). [Prostaglandin E2](#) activates the cAMP-CREB signaling pathway to promote M2 macrophage polarization ([Yang et al., 2019](#)). During [bone regeneration](#), adenosine promotes M2 macrophage polarization by activating the cAMP pathway through the A2b [adenosine receptor](#) ([Sun et al., 2024](#)). Our results demonstrated that, for macrophages, downregulation of circADAMTS6 inhibited the CSE-induced activation of CREB ([Fig. 4I](#) and [S12](#)). We assessed the upregulated and downregulated genes by KEGG pathway analysis and found that the cAMP signaling pathway was both upregulated and downregulated after downregulating circADAMTS6 ([Fig. S13](#)). Further, levels of CAMK2A, CAMK2B, NRP1, GLI, [ATP1A2](#), and ATP1B2 were low and levels of AHM, HCAR2, ATP2B1, [PDE4B](#), FOS and NFKBIA were high, compared to those of CSE-treated THP-Ms ([Fig. S14A](#)). These are mediators of the cAMP signaling pathway. We excluded HCAR3, which is not homologous for human and mice ([Kapolka and Isom, 2020](#)). Next, For CSE-treated macrophages, qRT-PCR verification showed that the mRNA levels of *CAMK2A*, *CAMK2B*, and [GLI1](#) were suppressed and the mRNA levels of *PDE4B* were recovered after downregulating circADAMTS6 ([Fig. S14B and S14C](#)). Further, RNA pull-down assays showed that circADAMTS6 interacted with CAMK2A ([Fig. S14D](#)). For CSE-treated macrophages, CAMK2A protein levels decreased with lower levels of circADAMTS6 ([Figs. 4I](#) and [S12](#)). In addition, in THP-Ms, downregulation of CAMK2A blocked CSE-induced activation of CREB ([Fig. S15](#)). These results show that, in CSE-treated macrophages, circADAMTS6 activates CREB via CAMK2A.

3.5. A circADAMTS6/IGF2BP2/CAMK2A RNA-protein ternary complex stabilizes CAMK2A mRNA

We used the RNA [Interactome Database](#) ([Kang et al., 2022](#)) to predict the interaction between circADAMTS6 and the *CAMK2A* mRNA 3'UTR ([Fig. 5A](#)), and RNA pull-down assays confirmed this interaction ([Fig. 5B](#)). Next, for CSE-treated THP-Ms, downregulation of circADAMTS6 inhibited elevation of [luciferase](#) mRNA and the [luciferase](#) activity of CAMK2A-WT ([Fig. 5C](#)). For THP-Ms, knockdown of circADAMTS6 reduced the half-life of *CAMK2A* ([Fig. 5D](#)). We also found that downregulation of circADAMTS6 in CSE-treated THP-Ms did not reduce CAMK2A [protein degradation](#) ([Fig. S16](#)). These results suggest that circADAMTS6 regulates CAMK2A expression by affecting its [mRNA stability](#).

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Fig. 5. Enhancement of circADAMTS6 increases [CAMK2A](#) levels through recruiting IGF2BP2 to stabilize [CAMK2A](#) mRNA in CSE-treated THP-Ms. THP-Ms were treated with 4 % CSE for 48 h. (A) RNA Sequence showing the [binding site](#) between circADAMTS6 and 3'UTR of CAMK2A. (B) [AGE](#) showed that circADAMTS6 was associated with CAMK2A, as determined by an RNA pull-down assay. (C) Relative [luciferase](#) mRNA levels (left) and [luciferase](#) activity (right) of a luciferase reporter gene containing either CAMK2A-WT or CAMK2A-MUT in CSE-treated THP-Ms transfected with circADAMTS6 [siRNA](#) or NC [siRNA](#) for 6 h. (D) The abundances of CAMK2A in THP-Ms transfected with circADAMTS6 siRNA or NC siRNA for 6 h were measured by qRT-PCR. (E) Identification of the

potential proteins pulled down by a circADAMTS6 probe or circADAMTS6 anti-probe in THP-Ms. (F) Representative immunoblots of IGF2BP2 showing its interaction with circADAMTS6, as determined by an RNA pull-down assay. (G) The association of IGF2BP2 with circADAMTS6 as assessed by RIP assays of THP-Ms. (H) circADAMTS6 was co-localized with IGF2BP2 protein in the cytoplasm, as shown by RNA fluorescence *in situ* hybridization. (I) RNA-EMSA assays showing the binding capacity of purified IGF2BP2 with biotin-labeled oligonucleotides containing the CAUC motif from circADAMTS6. (J) The abundance of CAMK2A in THP-Ms transfected with IGF2BP2 siRNA or NC siRNA for 6 h were measured by qRT-PCR. (K) Representative immunoblots of IGF2BP2 and CAMK2A of CSE-treated THP-Ms transfected with IGF2BP2 siRNA or NC siRNA. (L) The association of IGF2BP2 with CAMK2A was determined by RIP assays of THP-Ms transfected with circADAMTS6 siRNA or NC siRNA. Data represent means \pm SD ($n = 3$). ** $P < 0.01$, compared with 0 % CSE-treated, circADAMTS6 siRNA-treated, or IGF2BP2 siRNA-treated cells. ## $P < 0.01$, compared with 4 % CSE-treated cells. n.s., no significant.

circNSUN2, acting via IGF2BP2, an RNA-binding protein, enhances the stability of HMGA2 mRNA (Chen et al., 2019). By use of RNA pull-down assays followed by mass spectrometry analysis, we demonstrated that IGF2BP2 interacted with circADAMTS6 (Fig. 5E and 5F) (Table S2).

RNA immunoprecipitation (RIP) assays confirmed that IGF2BP2 binds to circADAMTS6 (Fig. 5G). Immunofluorescence and FISH assays showed that circADAMTS6 was expressed simultaneously in the cytoplasm with IGF2BP2 (Fig. 5H). catRAPID omics v2.0 (Armaos et al., 2021) was used to predict the propensity of the interaction between circADAMTS6 and IGF2BP2 (Fig. S17). The sequence CAUH (H = A, U, or C) is considered to be the only motif for recognizing IGF2BP2 (Hafner et al., 2010). By performing RNA-EMSA for THP-Ms, we confirmed that circADAMTS6 interacted with IGF2BP2 via the CAUC motif, and that, when the motif was ACAG, circADAMTS6 did not bind to IGF2BP2. Moreover, super-shift experiments showed that IGF2BP2 bound specifically to CAUC (Fig. 5I). These data demonstrate that, in the cytoplasm, circADAMTS6 interacts with IGF2BP2 via the CAUC motif.

We found that, for THP-Ms, knockdown of IGF2BP2 shortened the half-life of CAMK2A (Fig. 5J and S18) and that, after knocking down IGF2BP2, CAMK2A protein levels were lower in CSE-treated THP-Ms (Fig. 5K). In CSE-treated THP-Ms, the protein levels of IGF2BP2 did not change after knocking down circADAMTS6, but the protein levels of CAMK2A were lower (Fig. S19). Furthermore, in RIP assays, downregulation of circADAMTS6 reduced the RNA-protein interaction between IGF2BP2 and CAMK2A (Fig. 5L). These results indicate that circADAMTS6 interacts with IGF2BP2 to enhance the stability of CAMK2A mRNA and increases CAMK2A levels via forming a circADAMTS6/IGF2BP2/CAMK2A RNA-protein ternary complex.

3.6. circADAMTS6 activates CREB via CAMK2A to drive M2 polarization in CSE-treated macrophages

To evaluate the effects of circADAMTS6-mediated activation of cAMP signaling and M2 polarization in macrophages, circADAMTS6 siRNA and CAMK2A pcDNA were transfected into CSE-treated macrophages. We used CAMK2A pcDNA to overexpress CAMK2A in THP-Ms (Fig. S20). For CSE-treated THP-Ms, knockdown of circADAMTS6 prevented the increase of CAMK2A and p-CREB. However, upregulation of CAMK2A reversed this effect (Fig. 6A). The CSE-induced levels of TGM2 and ADORA3 mRNA were reduced by knocking down circADAMTS6, but this influence was reversed after overexpression of CAMK2A (Fig. 6B). Flow cytometry analysis indicated that the CSE-induced increased numbers of CD11b⁺CD206⁺ M2 macrophages were lowered with the downregulation of circADAMTS6, but M2 macrophages were increased after overexpression of CAMK2A (Fig. 6C). In addition, after knocking down circADAMTS6, the protein level of ARG1 was lower in CSE-treated THP-Ms; this influence was reversed after upregulation of CAMK2A (Fig. 6D). Compared with the downregulation of circADAMTS6 alone, co-transfection of circADAMTS6 siRNA and CAMK2A pcDNA

in CSE-treated THP-Ms reversed the decrease in MMP12 protein and mRNA levels and blocked the increase in TIMP1 protein and mRNA levels ([Fig. 6D](#) and [6E](#)). Knockdown of circADAMTS6 reduced the elastin degradation activity of THP-Ms exposed to CSE, and overexpression of CAMK2A increased the elastin degradation activity ([Fig. 6F](#)). Furthermore, there were similar results for RAW264.7 cells ([Fig. S21](#)). These results indicate that, in CSE-treated macrophages, circADAMTS6 activates CREB via CAMK2A to drive M2 polarization and promotes the proteinase/anti-proteinase imbalance and the increased elastin degradation.

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Fig. 6. circADAMTS6 activates [CREB](#) via CAMK2A to drive M2 polarization in CSE-treated THP-Ms. THP-Ms transfected with circADAMTS6 siRNA or CAMK2A pcDNA for 6 h were treated with 0 % or 4 % CSE for 48 h. (A) Representative immunoblots (left) and relative quantitative expressions (right) of CAMK2A and p-CREB in THP-Ms. (B) The levels of TGM2 and ADORA3 in THP-Ms were measured by qRT-PCR. (C) Representative flow cytometry plots (top) and quantification (bottom) of CD11b⁺CD206⁺ macrophages in THP-Ms. (D) Representative immunoblots (left) and relative quantitative expressions (right) of ARG1, MMP12, and TIMP1 in THP-Ms. (E) The levels of MMP12 and TIMP1 in THP-Ms were measured by qRT-PCR. (F) The levels of elastin degradation activity of THP-Ms were determined by fluorescence. Data represent means \pm SD (n = 3). **P < 0.01, compared with 0 % CSE-treated cells. ##P < 0.01, compared with 4 % CSE-treated cells transfected with circADAMTS6 siRNA.

3.7. Target of circADAMTS6 in macrophages prevents against mice with CS-induced emphysema via M2 polarization

To investigate the influence of circADAMTS6 in mice with CS-induced emphysema, we used AAV-circADAMTS6 shRNA to target macrophages in mouse lung tissues ([Fig. 7A](#)). The efficacy of AAV-circADAMTS6 shRNA was examined by [body weights](#), immunofluorescence staining, and qRT-PCR ([Fig. S22](#) and [7B–C](#)). Consistent with the in vitro experiments, the protein levels of CAMK2A and p-CREB were lowered in mouse lung tissues by silencing circADAMTS6 ([Fig. 7D](#)). Immunofluorescence staining showed that, in mouse lung macrophages, silencing of circADAMTS6 blocked the increased numbers of F4/80⁺CD206⁺ M2 macrophages induced by CS ([Fig. 7E](#)). Tgm2 and Adora3 mRNA levels and the ARG1 protein levels were low ([Fig. 7F](#) and [7G](#)). The numbers of MMP12⁺ cells in CD206⁺ M2 macrophages were decreased ([Fig. 7H](#)). Silencing of circADAMTS6 blocked the CS-induced proteinase/anti-proteinase imbalance, which was manifested primarily by blocking the higher protein and mRNA levels of MMP12 and restoring the lower protein and mRNA levels of TIMP1 induced by CS ([Fig. 7G](#) and [7I](#)). Silencing of circADAMTS6 blocked the elastin degradation caused by CS ([Fig. 7G](#)) and the destruction of alveolar walls and enhancement of mean chord lengths ([Fig. 7J](#)). We found that Penh values were elevated in mice with CS-induced emphysema and were lower after downregulation of circADAMTS6 ([Fig. S23](#)). These results demonstrate that, in mice, targeting of circADAMTS6 inhibits the M2 macrophage polarization and protects CS-induced emphysema.

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Fig. 7. Silencing of circADAMTS6 alleviates alveoli destruction through blocking M2 macrophage polarization in mice with CS-induced emphysema. (A) Schematic chart of workflow. Male BALB/c mice at 6 weeks of age treated with AAV-circADAMTS6 shRNA or AAV-NC shRNA were exposed to CS (0 or 300 mg/m³ TPM) for 24 weeks. (B) Representative images of the AAV-circADAMTS6 shRNA and AAV-NC shRNA infection efficiencies in lung tissues of mice (scale bars, 20 µm). (C) In lung tissues of mice, the levels of circADAMTS6 were measured by qRT-PCR. (D) Representative immunoblots (top) and relative quantitative expressions (bottom) of CAMK2A and p-CREB in lung tissues of mice. (E) Representative images (left) of F4/80 and CD206 immunostaining in lung tissues of mice (scale bars, 20 µm). The numbers of F4/80⁺ CD206⁺ cells in lung tissues of mice (right). (F) The levels of Tgm2 and Adora3 in mouse lung tissues as determined by qRT-PCR. (G) Representative immunoblots (top) and relative quantitative expressions (bottom) of ARG1, MMP12, TIMP1, and elastin in lung tissues of mice. (H) Representative images (left) of CD206 and MMP12 immunostaining in mouse lung tissues (scale bars, 20 µm). The numbers of CD206⁺ MMP12⁺ cells in lung tissues of mice (right). (I) The levels of Mmp12 and Timp1 in lung tissues of mice as determined by qRT-PCR. (J) Representative H&E-stained (left) and quantification (right) of mean chord lengths (Lm) in lung tissues of mice (scale bars, 100 µm and 20 µm). Data represent means ± SEM (n = 6). **P < 0.05, compared with the 0 mg/m³ TPM CS group. ##P < 0.01, compared with the 300 mg/m³ TPM CS group.

3.8. circADAMTS6 serves as a potential clinical marker for smoking-related emphysema

Organoids are practical for basic and clinical research, and have potential applications in disease modeling and treatment screening (Tang et al., 2022b). We constructed a co-culture model of macrophages and mice lung organoids following a published method (Wang et al., 2023) (Fig. 8A). We established the lung organ morphology by fluorescence microscopy, and characterized the surfactant protein-C-positive (SPC⁺) alveolar organoids by immunofluorescence staining (Fig. 8B). The growth of organoids co-cultured with CSE-treated RAW264.7 cells was inhibited; this inhibition of growth was reversed by downregulation of circADAMTS6 (Fig. 8C and 8D). In addition, the expressions of SPC⁺ cells were low in organoids co-cultured with CSE-treated RAW264.7 cells, an effect that was reversed by downregulation of circADAMTS6 (Fig. S24). We also collected human serum (Table 1) and the serum levels of circADAMTS6 were elevated in Smokers and COPD smokers (Fig. 8E). They negatively correlated with FEV1/FVC (%) and with predicted FEV1 (%) as determined by Pearson correlation analysis (Fig. 8F). These results demonstrate that circADAMTS6 serves as a potential biomarker for smoking-related emphysema.

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Fig. 8. Circadams6 serves as a potential clinical marker for smoking-related emphysema. Human serum was collected from Non-smokers (n = 35), Smokers (n = 28), or COPD smokers (n = 40). RAW264.7 cells transfected with circADAMTS6 siRNA or NC siRNA for 6 h were treated with 4 % CSE for 48 h. (A) Schematic chart of workflow. Establishment of mouse lung organoids, they were co-cultured with RAW264.7 cells. (B) Representative images of SPC immunostaining in mouse lung organoids (scale bars, 50 µm). (C) Representative images of mouse lung organoids in light microscopy (scale bars, 100 µm). (D) The average diameters of mouse lung organoids. (E) The levels of circADAMTS6 in human serum were measured by qRT-PCR. (F) Pearson correlation analysis of serum circADAMTS6 with FEV1/FVC (%) or predicted FEV1 (%). Data represent means ± SEM. **P < 0.01, compared with mouse lung organoids co-cultured with 0 % CSE-treated RAW264.7 cells or the Non-

smokers group. $^{##}P < 0.01$, compared with mouse lung organoids co-cultured with 4 % CSE-treated RAW264.7 cells.

4. Discussion

Cigarette smoking is a serious public health problem, with approximately 20 % of the global population smoking; about half of them die from smoking-related disease ([Rigotti et al., 2022](#)). CS is a powerful driver of the development of COPD, and studies conducted on cohorts of individuals with COPD have demonstrated that, compared to low CS exposure, high CS exposure exacerbates the decline in lung function ([Kim et al., 2021b](#)). Emphysema, a primary form of COPD, is manifested as the destruction of airway walls leading to the pathological expansion of alveolar space caused by CS ([Fu et al., 2022](#)). Macrophages, as innate [immune cells](#) in the pulmonary system, have the capacity to recognize and process various environmental stimuli, including CS and particulate matter ([Akata and van Eeden, 2020](#)). In the lungs of COPD patients, CS affects the function and phenotype of macrophages ([Kotlyarov, 2023](#)). In the lungs of COPD patients, macrophages of both the M1 and M2 phenotypes are present and are associated with the severity of the disease ([Lee et al., 2021](#)). Here, the results of scRNAseq showed that the ratio of M2 macrophages was increased in the lungs of COPD patients and mice with CS-induced emphysema. M2 macrophages were elevated in lung tissues of Smokers and COPD smokers and in the lung tissues of mice with CS-induced emphysema.

The activation of macrophages is involved in regulating the degradation of elastin, a component of the ECM, and contributes to the pathogenesis of emphysema ([Gharib et al., 2018](#)). In addition, macrophages in emphysema induced by chronic CS exposure are biased toward the M2 phenotype, which, in a chronic inflammatory environment, promotes degradation of the ECM and leads to abnormal tissue repair ([Gharib et al., 2018](#)). However, how the degradation of ECM promoted by M2 macrophages is involved in CS-induced emphysema remains unclear. In bronchial asthma, respiratory syncytial virus-induced M2 polarization of macrophages produces MMP12, which leads to [airway inflammation](#) ([Makino et al., 2021](#)). By degrading elastin, MMP12 causes destruction of the alveolar structure, leading to emphysema ([Lugg et al., 2022](#)). Here, we found, increased MMP12 secreted by M2 macrophages. Decreased TIMP1 and elastin degradation were evident for Smokers and COPD smokers, and for mice with CS-induced emphysema. In addition, for CSE-treated macrophages, there were an increase in MMP12 expression, a decrease in TIMP1 expression, and an increase in elastin degradation. Therefore, these results show that, a proteinase/anti-proteinase imbalance and elastin degradation induced by M2 macrophage polarization are involved in the occurrence and development of smoking-related emphysema.

With the development of bioinformatics and high-throughput sequencing, circRNAs are now established as being widely present in various species and organs ([Chen et al., 2021](#)). They are involved in numerous [cellular processes](#) and are associated with the pathogenesis of various diseases ([He et al., 2021](#)). For mouse lung tissues, CS exposure causes dysregulated expression of circRNAs, which are involved in the progression of COPD ([Chen et al., 2020](#)). circRNAs have various functions in the regulation of differentiation and adaptation of macrophages and [T cells](#) ([Zhang et al., 2020](#)). In our research, circADAMTS6 was elevated and located in CSE-treated macrophages. For these macrophages, knockdown of circADAMTS6 inhibited the M2 polarization, which blocked the imbalance of proteinase/anti-proteinase and the elevation of elastin degradation activity. Consistent with the results for cultured cells, silencing of circADAMTS6 in macrophages of mice with CS-induced emphysema inhibited M2 polarization, which blocked the progression of CS-induced emphysema. Thus, we conclude that circADAMTS6 affects the progression of CS-induced emphysema by modulating the M2 polarization of macrophages.

In the cytoplasm, circRNAs regulate gene expression by forming functional circRNP complexes, acting as sponges for [miRNAs](#), interacting with mRNAs, and/or interacting with proteins ([Liu and Chen, 2022](#)). IGF2BP1-3, a family of highly conserved proteins, regulate the stability, transport, and translation of mRNA through the use of a distinctive co-recognized sequence, CAUH (H = A, U, or C), functioning as RNA-binding proteins ([Hafner et al., 2010](#)). By stabilizing [FGF9](#) mRNA through IGF2BP2, circITGB6 promotes the occurrence and development of [ovarian cancers](#) ([Li et al., 2022](#)). Herein, we demonstrate that circADAMTS6 interacts with IGF2BP2 to form a circADAMTS6/IGF2BP2/CAMK2A RNA-protein ternary complex that enhances stability of CAMK2A mRNA.

In the present study, we elucidated a mechanism by which circADAMTS6 regulates CS-induced M2 polarization in macrophages. We performed RNA-seq in CSE-treated THP-Ms with knockdown of circADAMTS6 and found an enriched cAMP signaling pathway. This pathway acts as a cofactor in macrophage reprogramming and participates in M2 macrophage polarization through the phosphorylation of CREB ([Zhao et al., 2022](#)). After knockdown of circADAMTS6, CAMK2A causes obvious changes in the cAMP signaling pathway; it may have a regulatory relationship. In [microglia](#), CAMK2A activates phosphorylation of CREB to maintain synaptic plasticity ([More et al., 2022](#)). Moreover, phosphorylated CREB contributes to M2 polarization of macrophages ([Jiang et al., 2022](#), [Luan et al., 2015](#)). Our results confirm that circADAMTS6 promotes the activation of CREB via CAMK2A to promote M2 polarization in CSE-treated macrophages.

Lung organoids are used to simulate lung diseases and to provide new therapeutic targets for these diseases ([Liberti and Morrisey, 2021](#)). Compared to two-dimensional disease models, the three-dimensional organoid model better reflects the interaction between cells and the ECM ([Kim et al., 2021a](#)). Therefore, to evaluate the role of M2 macrophages in emphysema, we established a co-culture model of macrophages and lung organoids. After knockdown of circADAMTS6 in CSE-treated RAW264.7 cells, the growth of organoids was restored. We also note that circRNAs are potential targets for disease diagnosis and treatment ([Chen et al., 2022](#)). In our research, we found that, in the serum of Smokers and COPD smokers, the levels of circADAMTS6 were elevated and negatively correlated with lung function. Thus, circADAMTS6 may serve as a clinical biomarker for smoking-related emphysema.

In summary, we provide a newly identified mechanism for circADAMTS6 in CS-induced emphysema. This factor regulates M2 polarization, proteinase/anti-proteinase imbalance, and elastin degradation in CSE-treated macrophages and in a mouse model of CS-induced emphysema. Further, M2 macrophage polarization is involved in the pathogenesis of smoking-related COPD. In addition, elevated circADAMTS6 levels in smoking-related COPD are inversely correlated with lung function, providing a potential marker for COPD diagnosis.

5. Conclusion

Our results demonstrate that polarization of circADAMTS6-driven M2 macrophages has an essential function in CS-induced emphysema. With CS exposure, elevated circADAMTS6 interacts with IGF2BP2 to form a circADAMTS6/IGF2BP2/CAMK2A RNA-protein ternary complex that enhances the stability of CAMK2A mRNA. Subsequently, elevated CAMK2A activates CREB to promote the M2 polarization of macrophages, leading to a proteinase/anti-proteinase imbalance and elastin degradation that triggers emphysema. In addition, in the serum of smokers and COPD smokers, the levels of circADAMTS6 are elevated and negatively correlate with lung function. Therefore, circADAMTS6-driven M2 polarization in macrophages may serve as a diagnostic and/or therapeutic marker for CS-induced emphysema.

Abstract

Purpose

We determine and compare the prevalence, subtypes, severity, and risk factors for emphysema assessed by low-dose CT(LDCT) in Chinese and Dutch general populations.

Methods

This cross-sectional study included LDCT scans of 1143 participants between May and October 2017 from a Chinese Cohort study and 1200 participants with same age range and different smoking status between May and October 2019 from a Dutch population-based study. An experienced radiologist visually assessed the scans for emphysema presence (\geq trace), subtype, and severity. Logistic regression analyses, overall and stratified by smoking status, were performed and adjusted for fume exposure, demographic and smoking data.

Results

The Chinese population had a comparable proportion of women to the Dutch population (54.9 % vs 58.9 %), was older (61.7 ± 6.3 vs 59.8 ± 8.1), included more never smokers (66.4 % vs 38.3 %), had a higher emphysema prevalence ([58.8 % vs 39.7 %], adjusted odds ratio, aOR = 2.06, 95 %CI = 1.68–2.53), and more often had centrilobular emphysema (54.8 % vs 32.8 %, $p < 0.001$), but no differences in emphysema severity. After stratification, only in never smokers an increased odds of emphysema was observed in the Chinese compared to the Dutch (aOR = 2.55, 95 %CI = 1.95–3.35). Never smokers in both populations shared older age (aOR = 1.59, 95 %CI = 1.25–2.02 vs 1.26, 95 %CI = 0.97–1.64) and male sex (aOR = 1.50, 95 %CI = 1.02–2.22 vs 1.93, 95 %CI = 1.26–2.96) as risk factors for emphysema.

Conclusions

Only never smokers had a higher prevalence of mainly centrilobular emphysema in the Chinese general population compared to the Dutch after adjusting for confounders, indicating that factors other than smoking, age and sex contribute to presence of CT-defined emphysema.

Keywords

Tomography X-Ray Computed

Pulmonary Emphysema

Asians

European

Prevalence

1. Introduction

Emphysema is a lung condition that manifests as parenchymal destruction [1], [2]. Visual emphysema on CT independently increases the risk of lung cancer and all-cause mortality [3]. Undiagnosed emphysema patients often have impaired health-related [quality of life](#) and increased healthcare use and may have greater mortality of lung cancer [4], [5]. If we are to develop effective national health policies to correct both the underdiagnosis of emphysema and the associated risk of lung cancer, we need to clarify the [epidemiology](#) and causes of CT-defined emphysema [6].

Several studies have reported the CT-defined prevalence of emphysema, with variations from 32.7 % [7] to 66.0 % [8] in high risk population that result from differences in diagnostic strategies (e.g., scanning protocol and evaluation guideline) and risk exposures [9]. To evaluate and compare the prevalence of emphysema between areas, we therefore need studies that use the same diagnostic strategies and assess risk factors in a similar way. However, potential risk factors differ between countries, with notable differences in smoking rates, outdoor air pollution, and cooking-related household air pollution between Asia and Western countries [10]. Much is known about the prevalence and risk factors for lung function-defined [COPD](#) [11], [12], [13]; however, little is known about the prevalence of, and the factors that contribute to, CT-defined emphysema in general populations (e.g., the similarities and differences between Asian and western populations). As part of the Netherlands and China Big 3 diseases (Nelcin-B3) project, which was initiated for the early detection of lung cancer, [COPD](#) and cardiovascular disease [14], an international comparison of the epidemiological features of emphysema and associated risk factors will help to inform strategies for emphysema prevention and therapy development.

The aim is to determine and compare the prevalence, subtypes, and severity of emphysema assessed by low-dose CT (LDCT) between Chinese and Dutch general populations and to explore the related risk factors.

2. Materials and methods

2.1. Study design, study population, and eligibility

This cross-sectional study included a sample of two independent general populations-based prospective cohort including Chinese participants from the Nelcin-B3 study and Dutch participants from the Imaging in Lifelines (ImaLife) study [14], [15]. any smoking status, age 40–74 years. It included a consecutive series of participants with any smoking status, aged 45–74 years who underwent LDCT between May and October 2017 in the Nelcin-B3 study ($n = 1143$). An approximately equivalent number of participants with the same age range and any smoking status in the ImaLife study were also included and these participants underwent the LDCT between May and October 2019 ($n = 1200$, [Fig. 1](#)). We excluded participants if they had [interstitial fibrosis](#), and [pneumothorax](#) which were determined based on the CT scan by the radiologist, and/or if there was incomplete data (i.e., [BMI](#), passive smoking, or pack years) (see [Fig. 1](#)). The Ethics Committee of Biomedicine Research of the Second Military Medical University and of the UMCG approved the Nelcin-B3 study (registration number: NCT03992833) and the ImaLife study (registration number: NL58592.042.16), respectively. Participants in both cohorts provided written [informed consent](#). A detailed description of these two studies can be found in additional file 1: [supplementary material](#).

1. [Download: Download high-res image \(211KB\)](#)
2. [Download: Download full-size image](#)

Fig. 1. Flow-chart of study design. Note: LDCT, low-dose CT.

2.2. Data collection and definitions

In the two prospective cohorts, trained interviewers conducted structured face-to-face interviews using questionnaires. They gathered information about exposure to smoking (i.e., smoking status and passive smoking), demographics (i.e., age, sex, body mass index [BMI], and educational level), and exposure to either cooking fumes or fireplace fumes (see [Table S1](#) for definitions). The educational

level was categorized into low, moderate, and high [16], [17]. BMI was categorized into < 25 and ≥ 25 kg/cm². The cohorts differed slightly in the definitions of smoking status, passive smoking, and cooking/fireplace fume exposure [14], [18]. Passive smoking was defined as exposure to smoke produced by others ≥ 1 day a week for ≥ 15 min indoors in the Chinese cohort and regularly exposed to tobacco smoke from others in the past year in the Dutch cohort. Cooking fumes exposure was defined as exposure to at least moderate smoke during cooking in the Chinese cohort, while fireplace fumes exposure was defined as fireplace use ≥ 1 time/week in the Dutch cohort. For this study, the outcome of interest was visually assessed emphysema on LDCT scan. Participants were classified as having either no emphysema or at least trace emphysema. A detailed description of data collection and definitions can be found in Additional file 1: [supplementary material](#).

2.3. CT scan acquisition

The Chinese study used a 64-detector row CT system (Somatom Definition AS 64, Siemens Healthineers, Germany) for the non-contrast LDCT chest examinations, with the following parameters: 120 kVp, 35 mAs (reference), pitch 1.0 and CTDI vol ≤ 2 mGy. A soft tissue kernel (D45F) was applied to reconstruct the images at 1.0 mm thickness and 0.7 mm increment. All the participants were scanned head first in the [supine position](#) during an inspiratory breath hold.

The Dutch study used a third-generation dual-source CT (SOMATOM Force, Siemens Healthineers, Germany) for the non-contrast LDCT chest examinations, with the following parameters: 120 kVp, 20 mAs (reference), pitch 2.5 and CTDI vol ≤ 1.8 mGy. A soft tissue kernel (Br40) was applied to reconstruct the images at 1.0 mm slice thickness and 0.7 mm increment. The measurement of CT image quality can be found in Additional file 1: [supplementary material](#).

2.4. Visual emphysema assessment

One radiologist (A) with 6 years' experience visually assessed emphysema on Chest CT for all Chinese and Dutch participants, using a standard protocol created by the Fleischner Society [19]. Interobserver agreement was determined based on 100 randomly selected cases in each cohort by a second radiologist (B) with 3 years' experience for the Chinese participants and a third clinical physician (C) with 4 years' experience for the Dutch participants. Before the assessment, all readers received training that had all subtypes of emphysema and was supervised by a senior chest radiologist using a standardized protocol. All readers performed the visual emphysema assessments in version VB30A of Syngo. via. software (Siemens Healthineers, Germany). They used the minimum intensity projection [19], [20] with a 10 mm thickness (WC:-850 HU, WW:400 HU) [21] and multiplanar reconstruction with 1 mm thickness (WC:-750 HU, WW:700 HU) [8]. Emphysema was scored as well-defined or ill-defined low attenuation or lucencies [19]. If present (threshold \geq trace), emphysema was further categorised as one of the two predominant subtypes, centrilobular (CLE), paraseptal (PSE). The predominant subtype was noted by the most severe one in cases of mixed emphysema. CLE was classified as trace (<0.5 %), mild (0.5–5 %), moderate (>5%), confluent and advanced destructive. PSE was classified as mild (<1 cm lucencies) or substantial (mainly > 1 cm lucencies) ([Fig. 3a-3f](#)).

2.5. Statistical analysis

We described continuous variables as means and standard deviations and categorical variables as frequencies and percentages. [Kappa statistics](#) for emphysema and weighted kappa coefficients for CLE and PSE severity were calculated to assess interobserver agreement. To compare emphysema prevalence between the two populations, we performed univariate and multivariable [logistic regression analyses](#) to estimate the odds ratios (ORs) and 95 % confidence intervals (95 % CIs). In the

multivariable analysis, we adjusted for age (per 10-year increase), sex, smoking status (i.e., former/current/never), passive smoking, BMI, educational level, and cooking or fireplace fume exposure. Those were based on previous literature [9], [22]. In addition, we performed analyses stratified by smoking status and by cohorts. Chi-squared tests were conducted to analyse differences in emphysema subtype and severity between the two populations with emphysema. To elucidate the robustness, we performed a sensitivity analysis by repeating the main analysis for the main subtype of emphysema (CLE) by limiting the emphysema threshold to at least trace or at least mild. All analyses were conducted using the SPSS Version 28.0 (IBM Corp.) with an extension of “STATS_WEIGHTED_KAPPA”, treating *p*-values of < 0.05 as statistically significant. As there is limited data available regarding the prevalence of CT-defined emphysema in literature, we could not perform a prior sample size estimation. As an alternative, a post hoc power calculation was performed using G power Version 3.1.9 (Heinrich Heine University Düsseldorf).

3. Results

3.1. Population characteristics

There were 3 % of participants with missing data who were excluded. We included 2,343 participants in this analysis (Fig. 1), comprising 1143 Chinese participants and 1200 Dutch participants, with comparable proportions of women (627 [54.9 %] vs 707 [58.9 %], respectively). Compared with the Dutch, the Chinese population was slightly older (61.7 ± 6.3 vs 59.8 ± 8.1 ,) and included more never smokers (759 [66.4 %] vs 459 [38.3 %]) (Table 1). As shown in Table S2, among the never smokers, the Chinese participants were also older (61.1 ± 6.5 vs 58.1 ± 8.5) and had lower BMI (<25 kg/m², 57.7 % vs 44.2 %). The prevalence of passive smoking exposure was higher in the overall Chinese participants (44.0 % vs 22.6 %) and never smokers (35.4 % vs 15.5 %, Table S2) than in the Dutch. No difference was observed between Chinese and Dutch participants in cooking or fireplace fume exposure (6.7 % vs 6.1 %).

Table 1. Characteristics of participants (overall and those with emphysema \geq trace) in the Chinese and Dutch cohorts.

Empty Cell	Chinese Cohort		Dutch Cohort	
	Participants, n (%)	Participants with emphysema, n (%)	Participants, n (%)	Participants with emphysema, n (%)
Valid Participants	1143 (97.4)	672 (58.8)	1200 (96.7)	476 (39.7)
Cases with missing value	30 (2.6)	17 (2.5)	40 (3.3)	17 (3.6)
Age (Mean \pm SD)	61.7 ± 6.3	62.7 ± 6.1	59.8 ± 8.1	61.0 ± 7.7
Sex				
Women	627 (54.9)	302 (44.9)	707 (58.9)	239 (50.2)

Empty Cell	Chinese Cohort		Dutch Cohort	
	Participants, n (%)	Participants with emphysema, n (%)	Participants, n (%)	Participants with emphysema, n (%)
Men	516 (45.1)	370 (55.1)	493 (41.1)	237 (49.8)
Smoking status				
Never	759 (66.4)	383 (57.0)	459 (38.3)	127 (26.7)
Former	115 (10.1)	84 (12.5)	571 (47.6)	245 (51.5)
Quit years	11.9 ± 10.8	12.4 ± 11.6	20.6 ± 12.2	20.0 ± 12.2
Pack years	22.5 ± 19.2 ^a	23.6 ± 19.9 ^b	10.3 ± 9.8 ^c	12.9 ± 11.5 ^d
Current	269 (23.5)	205 (30.5)	170 (14.2)	104 (21.8)
Pack years	25.2 ± 17.7 ^e	27.2 ± 18.5 ^f	19.9 ± 12.3	22.0 ± 12.8
Passive Smoking				
No	640 (56.0)	361 (53.7)	929 (77.4)	343 (72.1)
Yes	503 (44.0)	311 (46.3)	271 (22.6)	133 (27.9)
Missing	0 (0)	0 (0)	22 (1.8 %)	9 (1.8)
BMI (kg/m²)				
<25	643 (56.3)	398 (59.2)	473 (39.4)	195 (41.0)
≥25	500 (43.7)	274 (40.8)	727 (60.6)	281 (59.0)
Missing	6 (0.5)	2 (0.3)	11 (0.9)	3 (0.6)
Educational Level				
Low	431 (37.7)	278 (41.4)	242 (20.2)	111 (23.3)
Moderate	418 (36.6)	224 (33.3)	615 (51.2)	232 (48.7)
High	294 (25.7)	170 (25.3)	343 (28.6)	133 (27.9)

Empty Cell	Chinese Cohort		Dutch Cohort	
	Participants, n (%)	Participants with emphysema, n (%)	Participants, n (%)	Participants with emphysema, n (%)
Cooking or Fireplace Fume				
No	1066 (93.3)	723 (95.3)	1127 (93.9)	432 (94.1)
Yes	77 (6.7)	36 (4.7)	73 (6.1)	27 (5.9)
Missing	4 (0.3)	2 (0.3)	6 (0.5)	4 (0.8 %)

BMI, body mass index; SD, standard deviation. ^a Missing in 12 cases; ^b Missing in 7 cases; ^c Missing in 2 cases; ^d Missing in 1 case; ^e Missing in 8 cases; ^f Missing in 6 cases; Cases with missing value were included in the analysis only in [Table 1](#).

3.2. CT image quality and interobserver agreement

The result of image quality was shown in the results part of the supplement. Agreement between readers when assessing emphysema was good in both the Chinese ($\kappa = 0.76$; 95 % CI = 0.63–0.89) and the Dutch participants ($\kappa = 0.87$, 95 % CI = 0.76–0.97). Similarly, the agreement was good for the severity of CLE ($\kappa_w = 0.77$; 95 % CI = 0.67–0.88) and PSE ($\kappa_w = 0.77$; 95 % CI = 0.58–0.96) in the Chinese participants, and was comparable for the severity of CLE ($\kappa_w = 0.87$; 95 % CI = 0.78–0.96) and PSE ($\kappa_w = 0.84$; 95 % CI = 0.66–1.00) in the Dutch participants.

3.3. Prevalence, subtype, and severity of emphysema

Emphysema (at least trace) was present in 672 (58.8 %) Chinese and in 476 (39.7 %) Dutch participants. The prevalence of trace, mild, and moderate, confluent-advanced CLE in the Chinese population was 38.2 %, 11.5 %, 2.8 %, and 2.2 %, respectively ([Table S3](#)); by contrast, the prevalence was lower in the Dutch population for the severity levels (24.0 %, 5.8 %, 1.9 % and 1.0 %, respectively; overall $p < 0.001$). The prevalence of emphysema (trace or above) in Chinese current, former and never smokers was 76.2 %, 73.0 % and 50.5 %, respectively; the corresponding prevalence in Dutch participants was 61.2 %, 42.9 % and 27.7 %, respectively ([Table S2](#)). When limiting the emphysema threshold to mild or above, the presence of emphysema in Chinese never and ever smokers was 12 % and 39 %, respectively; the corresponding prevalence in Dutch participants was 6 % and 22 %, respectively. CLE was the most common subtype in participants with emphysema in each cohort (93.2 % and 82.6 %, respectively), followed by PSE (6.8 % and 17.4 %, respectively). Among those with emphysema, the proportion of CLE was higher in the Chinese than in the Dutch participants (93.2 % vs 82.6 %, $p < 0.001$) and the severities of CLE or PSE were comparable ([Table 2](#)). When limiting the emphysema threshold to at least mild, emphysema prevalence (20.6 % vs 15.7 %, $p = 0.002$), and the proportion of CLE (80.4 % vs 55.9 %, $p < 0.001$) in the Chinese was still significantly higher than in the Dutch but no difference was observed for the distribution of severity of CLE or PSE ([Table S4](#)).

Table 2. Presence, distribution of subtype and severity of emphysema (\geq trace) in participants with emphysema in the Chinese and Dutch cohorts.

Empty Cell	Chinese Cohort n = 672, n (%)	Dutch Cohort n = 476, n (%)	p value
Predominant subtype of emphysema			<0.001 ^a
CLE	626 (93.2)	393 (82.6)	
PSE	46 (6.8)	83 (17.4)	
Severity of CLE			0.47 ^a
Trace	437 (69.8)	288 (73.3)	
Mild	132 (21.1)	70 (17.8)	
Moderate	32 (5.1)	23 (5.9)	
Confluent-Advanced	25 (4.0)	12 (3.1)	
Severity of PSE			>0.99 ^b
Mild	44 (95.7)	79 (95.2)	
Substantial	2 (4.3)	4 (4.8)	

CLE, [centrilobular emphysema](#); PSE, paraseptal emphysema; Confluent-Advanced, confluent or advanced destructive. ^a Based on Chi-square testing; ^b Based on Fisher's Exact Testing.

3.4. Risk factors for CT-defined emphysema

3.4.1. Chinese versus Dutch cohort

Participants in the Chinese cohort had two-fold increased odds of emphysema after adjusting for covariates, with an adjusted OR of 2.06 (95 % CI = 1.68–2.53) compared to the Dutch cohort ([Table 3](#)). After stratification by smoking status, this was only observed in never smokers (2.55, 95 %CI = 1.95–3.35; $p < 0.001$), and not in current smokers (aOR = 1.10; 95 % CI = 0.63–1.90; $p = 0.75$) or former smokers (aOR = 1.58; 95 % CI = 0.93–2.52; $p = 0.09$) ([Table 4](#)). Meanwhile, the Chinese participants also had higher odds for CLE ([Table S5A, S5B](#)) than the Dutch, and after stratification by smoking status, still only Chinese never smokers had the increased odds ([Table S6A, S6B](#)) regardless of using the threshold “at least trace” or “at least mild” for emphysema.

Table 3. Associations between participant characteristics and emphysema (\geq trace).

Characteristics	OR	95 %CI	p value	Adjusted OR	95 %CI	p value
Cohort						
Dutch	1			1		

Characteristics	OR	95 %CI	p value	Adjusted OR	95 %CI	p value
Chinese	2.17	1.84–2.56	<0.001	2.06	1.68–2.53	<0.001
Age (per 10 years increase)	1.63	1.45–1.83	<0.001	1.46	1.29–1.66	<0.001
Sex						
Women	1			1		1
Men	2.21	1.87–2.62	<0.001	1.59	1.32–1.93	<0.001
Smoking status			<0.001			<0.001
Never	1					
Former	1.28	1.06–1.54	0.010	1.58	1.26–1.99	<0.001
Current	3.30	2.61–4.17	<0.001	2.78	2.13–3.64	<0.001
Passive smoking						
No	1			1		
Yes	1.65	1.39–1.97	<0.001	1.18	0.97–1.44	0.10
BMI (kg/m ²)			<.0001			<0.001
<25	1			1		
≥25	0.73	0.62–0.86	0.001	0.73	0.61–0.87	<0.001
Educational level			<0.001			0.13
Low	1			1		
Moderate	0.58	0.47–0.70	<0.001	0.80	0.65–0.99	0.044
High	0.66	0.53–0.82	<0.001	0.88	0.69–1.12	0.29
Cooking or fireplace fume						
No	1			1		
Yes	1.56	1.12–2.19	0.009	1.31	0.91–1.89	0.15

95% CI, 95% confidence interval; BMI, body mass index; OR, odds ratio.

Table 4. Multivariable associations between participant characteristics and emphysema (\geq trace), stratified by smoking status.

Variables	Current Smokers (n = 439)		Former smokers (n=686)		Former smokers (n=686)	
	Adjusted OR (95 %CI)	p value	Adjusted OR (95 %CI)	p value	Adjusted OR (95 %CI)	p value
Cohort						
Dutch	1		1		1	
Chinese	1.10 (0.63– 1.90)	0.75	1.58 (0.93– 2.52)	0.09	2.55 (1.95– 3.35)	<0.001
Age (per 10 years increase)	1.70 (1.20– 2.39)	0.003	1.57 (1.22– 2.01)	<0.001	1.38 (1.16– 1.64)	<0.001
Sex						
Women	1		1		1	
Men	1.12 (0.64– 1.96)	0.69	1.96 (1.38– 2.79)	<0.001	1.71 (1.29– 2.27)	<0.001
Passive smoking						
No	1		1		1	
Yes	0.83 (0.52– 1.31)	0.42	1.54 (1.05– 2.27)	0.03	1.09 (0.83– 1.42)	0.55
Quit smoking years	–		0.99 (0.97– 1.00)	0.14	–	
Pack years	1.04 (1.02– 1.06)	<0.001	–		–	
BMI (kg/m²)						
<25	1		1		1	

Variables	Current Smokers (n = 439)		Former smokers (n=686)		Former smokers (n=686)	
	Adjusted OR (95 %CI)	p value	Adjusted OR (95 %CI)	p value	Adjusted OR (95 %CI)	p value
Empty Cell						
≥25	0.53 (0.33– 0.84)	0.007	0.73 (0.51– 0.98)	0.068	0.75 (0.60– 0.96)	0.02
Educational level		0.13		0.21		0.93
Low	1		1		1	
Moderate	0.65 (0.39– 1.10)	0.11	0.70 (0.47– 1.05)	0.08	0.95 (0.68– 1.23)	0.73
High	1.09 (0.58– 2.05)	0.79	0.75 (0.47– 1.18)	0.21	0.95 (0.69– 1.32)	0.76
Cooking or fireplace fume exposure						
No	1		1		1	
Yes	1.06 (0.50– 2.25)	0.88	1.42 (0.74– 2.70)	0.31	1.42 (0.82– 2.46)	0.21

95%CI, 95% confidence interval; BMI, body mass index; OR, odds ratio. Pack years or quit smoking years was adjusted among current and former smokers, respectively.

3.4.2. Chinese and Dutch cohort

Overall, when combining participants from both cohorts, participants with emphysema were typically older (aOR = 1.46 per 10 years of age increase, 95 % CI = 1.29–1.66), male (aOR = 1.59; 95 % CI = 1.32–1.93), and current smokers (aOR = 2.78; 95 % CI = 2.13–3.64) or former smokers (aOR = 1.58; 95 % CI = 1.26–1.99) compared to participants without emphysema; they also had lower BMI (aOR = 0.73; 95 % CI = 0.61–0.87 for BMI $\geq 25 \text{ kg/m}^2$). We found no evidence for an association with emphysema for cooking or fireplace fume (aOR = 1.31; 95 % CI = 0.91–1.89, $p = 0.15$), passive smoking (aOR = 1.18; 95 % CI = 0.97–1.44, $p = 0.10$) or educational level (Overall $p = 0.13$; [Table 3](#)). When limiting the emphysema threshold to mild or above ([Table S5B](#)), the risk factors associated with CLE remained the same.

3.4.3. Never smokers by cohort

After stratifying never smokers by national cohort, increasing age (aOR = 1.59; 95 % CI = 1.25–2.02 vs 1.26; 95 % CI = 0.97–1.64 [$p = 0.08$, per 10 year increase]) and male sex (aOR = 1.50; 95 % CI = 1.02–2.22 vs 1.93, 95 % CI = 1.26–2.96) were associated with increased odds of emphysema with comparable magnitudes in the Chinese and Dutch participants ([Fig. 2](#)). The aOR was increased for cooking/fireplace fumes exposure in both cohorts in never smokers, but this was not significant.

Likewise, passive smoking was not associated with emphysema in never smokers in any of the two populations despite the high passive smoking prevalence in the Chinese (35.4 % vs 15.5 %). When stratifying all participants by national cohort ([Table S7](#)), the risk factors associated with emphysema remained the same with comparable magnitudes in all the Chinese and Dutch participants.

1. [Download: Download high-res image \(432KB\)](#)
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Fig. 2. Multivariable [logistic regression analysis](#) of risk factors for [emphysema](#) (\geq trace) in never smokers, stratified by national cohort. Note: [BMI](#), body mass index; NA, not applicable; OR, odds ratio.

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Fig. 3. Examples CT images of [emphysema](#) classification. a. Trace CLE shows minimal centrilobular lucencies, occupying < 0.5 % of upper lung zone. b. Mild CLE shows scattered centrilobular lucencies, separated by large regions of normal lung, involving an estimated 0.5 %-5% of upper lung zone. [Fig. 3c](#). Moderate CLE shows many well-defined centrilobular lucencies that occupy more than 5 % of upper lung zone. [Fig. 3d](#). Confluent CLE shows coalescent centrilobular or lobular lucencies, including multiple regions of lucencies that span several secondary pulmonary lobules. 3e. Advanced destructive [emphysema](#) shows hyperexpansion of secondary pulmonary lobules with distortion of pulmonary architecture. PSE is also seen along the pleural margin of right and left lungs. [Fig. 3f](#). Mild PSE shows subpleural foci of low attenuation (mainly < 1 cm) separated by intact interlobular septa along the mediastinum. CLE is also seen upper lobes of both lungs. [Fig. 3g](#). Substantial PSE shows numerous large (mainly > 1 cm) juxta pleural cysts like lucencies or bullae along the chest wall and mediastinal pleural margins. The images shown here use the minimum intensity projection with 10 mm thickness (WW:400HU, WC: -850HU).

We included 1143 Chinese participants and 1200 Dutch participants. In a post hoc power analysis, the power to detect a difference in emphysema prevalence (at least mild) between the two countries is 0.88 when two tails and an alpha value of 0.05 were applied.

4. Discussion

In this study of general populations, the Chinese and Dutch population had a similar socio-demographics and smoking distribution as compared to other previously reported Chinese and Dutch cohorts respectively [\[23\]](#), [\[24\]](#), [\[25\]](#), [\[26\]](#), we found that the Chinese had a higher prevalence of visual emphysema on LDCT than the Dutch. However, this was only seen in never smokers. Among never smokers, increasing age, and male sex were associated with the presence of emphysema in both cohorts, but fumes exposure and passive smoking were not. Finally, although the CLE subtype was more common in the Chinese than in the Dutch population, the severity of CLE and PSE were comparable.

In total, 12 % of the Chinese never smokers had at least mild emphysema, which is consistent with the prevalence reported in Canadian never smokers (11 %) [\[27\]](#); Whereas in Chinese ever smokers, 39 % had at least mild emphysema, and this is consistent with the rate in the [COPD](#) Gene study in a

US population (42 %) [28], [29], while 22 % of our Dutch ever smokers had at least mild emphysema. This lower prevalence could be explained by the lower pack years, and fewer men in the Dutch population. The prevalence of emphysema and each emphysema severity level in Chinese was higher than in the Dutch which was associated with older age, more men, greater current smoking rate, pack years and lower BMI in the Chinese population.

However, after adjusting for multiple confounders, the Chinese had a two-fold increased odds for emphysema compared with the Dutch. The increased threshold for emphysema definition had only a minor impact on the higher odds for the Chinese population (\geq trace CLE: aOR = 2.19; 95 % CI = 1.77–2.70; \geq mild CLE: aOR = 1.58; 95 % CI = 1.15–2.17). When stratified by smoking status, only Chinese never smokers had an increased odds compared to the Dutch. We therefore hypothesised that other unmeasured risk factors must account for the difference in emphysema prevalence between the two populations. A well-recognised difference is the higher outdoor air pollution in northern China than in the Netherlands (mean [particulate matter](#) 2.5: 95 vs 16 $\mu\text{g}/\text{m}^3$) [30], [31]. Previous studies have shown that air pollution not only contributes to a higher incidence of emphysema but also becomes an increasingly major risk for emphysema in low-to middle-income countries [32], [33]. Another explanation might be related to [genetic risk factors](#) such as the glutathione S-transferase theta 1 which is a [diagnostic marker](#) of [COPD](#) [34] and shows a high association with increased COPD susceptibility only in Asians but not in Caucasian populations [35]. Contrary to never smokers, we observed no difference in emphysema prevalence in smokers between the two populations. Likely, this is caused by the overwhelming effect of smoking on emphysema prevalence, which covers any effect of other risk factors that could have resulted in a small difference between these two populations. Our study showed that older age, male sex, smoking status, and low BMI in the overall population were associated with emphysema on LDCT. This is consistent with earlier reports that these are risk factors for emphysema or COPD [36], [37]. We did not detect a significant association between passive smoking exposure and emphysema in either the overall combined cohorts or the stratified cohorts. A previous study reported that passive smoking was associated with increased odds of COPD (OR = 1.18; 95 % CI = 1.01–1.39) only when the exposure duration is at least 20 h/week [38]. Passive smoking in our Chinese cohort was defined as positive only when the exposure duration was \geq 15 mins/week. The lower cut-off applying for exposure may have led to the nonsignificant result. Though insignificant probably due to the lack of power, we observed a higher odds for cooking/fireplace fume exposure and emphysema in our study (OR = 1.31; 95 % CI = 0.91–1.89 in all participants; OR = 1.42; 95 % CI = 0.82–2.46 in never smokers). Previous findings showed that poor ventilation in the kitchen is associated with COPD (OR = 1.28; 95 % CI = 1.14–1.43) [26]. Furthermore, among the Chinese and Dutch never smokers, increasing age and male sex were associated with an increased odds of emphysema, consistent with the finding for COPD risk among Korean never smokers [39]. Meanwhile, Age and sex two risk factors were present at similar effect sizes for emphysema in the Chinese and the Dutch population. The ORs of males for emphysema in the Chinese never smokers was 1.50 (95 % CI = 1.02–2.22), which is comparable to the odds of 1.40 (95 % CI = 1.21–1.63) for COPD reported in a Chinese large-scale and population-based study [40].

Importantly, current smokers in our study had 2.5-fold increased odds of emphysema, reminding us of the importance of smoking in emphysema formation and supporting the necessity of [smoking cessation](#). Chest CT is more sensitive than [pulmonary function test](#) in detecting emphysema and underdiagnose of emphysema was common in [primary healthcare](#) [41]. Our findings show that CT screening for lung cancer provides an additional opportunity for emphysema detection. Meanwhile, it reminds clinicians of the need to consider screening older, male participants with low BMI, which could decrease the chance of emphysema underdiagnosis. A recent [systematic review](#) and *meta-analysis* showed that CT-defined emphysema was associated with a higher odds ratio (OR, 2.3) of

lung cancer, and this association increased with emphysema severity (OR, 2.5–4.5 for trace to severe emphysema) [3]. Given the high prevalence of CT-defined emphysema in the general population, we will evaluate the association of emphysema and lung cancer in future studies, especially among never smokers. At least trace emphysema was set as a threshold for the presence of emphysema based on the Fleischner Society guideline. Considering the impact of image noise on the assessment of trace emphysema, at least mild emphysema was also set as another threshold. The results were similar, which demonstrated the stability of our conclusion. For the CT image quality, the HU deviation and HU standard deviation of air in our two cohorts are slightly higher than the requirements in the phantom (≤ 6 HU for HU deviation and ≤ 20 HU for HU standard deviation) [42]. However, these available requirements for lung density are applicable for quantitative CT assessments of emphysema, and our visual assessment of emphysema is less sensitive to image noise than quantitative assessment [8]. Therefore, we expect limited impact on our results.

Our study has some limitations. First, only one radiologist performed the emphysema assessment; however, the interobserver agreements with two other readers were good to very good, which helps to mitigate this concern. Second, we might have an unfair comparison between the two cohorts. On the one hand, we collected some characteristics (i.e., passive smoking, fireplace fume exposure) for Dutch participants several years before the CT scan, making it possible that some will have shifted from the smoking status group. On the other hand, the definition of variables like smoking status and passive smoking differed slightly between the Chinese and Dutch cohorts. We expect that a small proportion of misclassification of participants has a limited impact on the effect estimation for emphysema risk. Third, occupational exposures were not available in both cohorts, while this might be a confounding factor for emphysema that needed to be adjusted for. Considering that ORs of occupational exposure for emphysema reported in a Chinese Biobank and Dutch Lifeline [cohort studies](#) are comparable [43], [44], it might have a limited impact on our results. Fourth, though we adjusted for the most relevant confounders based on previous studies, there might be other unmeasured cohort-related differences that were not included in the model. Further research is needed to fully understand the high prevalence of CT-defined emphysema in Chinese never smokers. Fifth, our study included a consecutive series of participants both in Chinese and Dutch single-center cohorts and thus it is uncertain to what extent this is representative of the whole Chinese and Dutch general population. It may limit the general applicability of results and conclusions. Sixth, the number of participants with ADE of CLE was relatively small ($n = 8$), so we merged it with confluent severity. Seventh, air pollution data was not available in these two cohorts. However, the data from published studies was used to investigate the potential reason for the emphysema prevalence difference between the two cohorts.

5. Conclusions

In conclusion, the Chinese have a higher prevalence of CT-defined emphysema than the Dutch in a sample of a general population, with higher odds of emphysema only among Chinese never smokers. These findings underscore that factors other than smoking, age, and sex play a key role in emphysema formation, with outdoor air pollution being a hypothetical candidate. Considering the potentially important role of non-smoking factors in emphysema formation, future studies should now focus on elucidating other risk factors, like air pollution, that could contribute to the high prevalence of CT-defined emphysema in Chinese never smokers to help prevent the disease.

Abstract

[Pneumomediastinum](#) denotes the presence of gas within the mediastinum and generally occurs by leakage of air from an aerated viscus that traverses or abuts the mediastinal plane. The Macklin

effect has been described in several veterinary studies and describes gas tracking along the perivascular interstitium following alveolar rupture causing interstitial [emphysema](#), pneumomediastinum and subsequently cervical [subcutaneous emphysema](#). This retrospective case series describes incidental spontaneous pulmonary interstitial emphysema, pneumomediastinum and cervical subcutaneous emphysema secondary to the Macklin effect in [dogs](#) with no related clinical signs. Twelve dogs were identified from the author's institution, of which 75 % were Sighthounds (Greyhounds, Whippets or Lurchers). Pulmonary interstitial emphysema had a predominantly paravascular distribution, although in some cases a parabronchial distribution was also identified. We conclude that incidental pulmonary interstitial emphysema, pneumomediastinum and secondary cervical subcutaneous emphysema can be incidental, presumed secondary to the Macklin effect and that Sighthound breeds may be overrepresented.

Introduction

[Pneumomediastinum](#) denotes the presence of gas within the mediastinum and generally occurs by leakage of air from an aerated viscus that traverses or abuts the mediastinal plane (oesophagus, laryngotracheal or alveolar) ([Russel et al., 2018](#)). It can be classified as spontaneous (where no inciting cause is identified) or secondary. The pathophysiological basis underlying pneumomediastinum secondary to alveolar rupture was first demonstrated experimentally by Macklin and Macklin in 1944, the so called 'Macklin Effect' ([Macklin and Macklin, 1944](#)). It is postulated to occur secondary to gas tracking along the perivascular interstitium following alveolar rupture and multiple secondary inciting causes are described ([CO, 1964](#), [Macia et al., 2007](#), [Carzolio-Trujillo et al., 2016](#), [Colin Gc et al., 2012](#), [Sakai et al., 2006](#), [Wintermark and Schynder, 2001](#)).

The Macklin effect has been described in several veterinary studies ([Agut et al., 2015](#); [Broek, 1986](#); [Jones et al., 1975](#); [Kuo et al., 2021](#); [Thomas and Syring, 2013](#); [Weissenbacher-Lang et al., 2017](#); [Bertolini et al., 2009](#)). [Bertolini et al. \(2009\)](#) and [Agut et al. \(2015\)](#) describe pulmonary interstitial [emphysema](#) (PIE) secondary to severe [respiratory disease](#). Other secondary causes have also been reported in [dogs](#), the most frequently reported being [thoracic trauma](#) ([van den Broek, 1986](#)). However, in the same study, at least 4 cases presented with pneumomediastinum of unknown etiology ([van den Broek, 1986](#)). A small case series by [Jones et al. \(1975\)](#) describes spontaneous pneumomediastinum in the racing [greyhound](#) in the [absence](#) of [respiratory obstruction](#), evidence of primary respiratory disease or any condition that would cause reduced capillary pressure.

Anecdotally we have observed spontaneous interstitial emphysema (IE) and pneumomediastinum, presumed secondary to a Macklin effect in Sighthounds and other dogs with no clinical signs during thoracic CT examination, providing support to the findings of [Jones et al. \(1975\)](#). The aim of this retrospective, descriptive case series is to report the clinical and CT imaging findings of spontaneous PIE and pneumomediastinum in a population of dogs at our institution.

Methods

This study was a retrospective, single center, case series. Ethical approval was granted from the University of Liverpool (RETH 000765 and RETH1438).The database at the Small Animal Teaching Hospital, University of Liverpool (Neston, UK) was searched for [dogs](#) with [pneumomediastinum](#). Inclusion criteria were a thoracic CT examination and a final diagnosis of presumed spontaneous pneumomediastinum. Patients with any secondary cause for pneumomediastinum were excluded. For all included dogs, the following data was recorded; Breed, gender, neuter status, age, weight, presenting clinical signs, physical examination and hematology and biochemistry. Anaesthesia

records were assessed to determine if the patient was sedated or anesthetized and if the patient was manually ventilated.

All images were acquired between July 2016 and April 2023 with an 80-slice CT scanner (Aquilon Prime 80; Toshiba Medical Systems) using a standard institution protocol (Typical parameters: helical acquisition, 1.0 mm slice thickness, 120 KVp, 50mAs, spiral pitch factor 0.825, convolution kernel FC51). All patients were placed in sternal recumbency and were either sedated or anesthetized at the discretion of the attending anesthesia clinician.

Two board-certified (DipECVDI) imaging specialists reviewed the CT images independently using both lung and soft tissue reconstructions using proprietary imaging viewing software (Carestream, Philips) for the following features: Evidence of pneumomediastinum, cervical extension (free gas adjacent to the trachea, oesophagus or carotid sheath structures or in the subcutaneous tissues), evidence of PIE, distribution of interstitial emphysema (paravascular or parabronchial), anatomical distribution and evidence of any other pulmonary pathology. If any discrepancy between readers was noted, final classification was made by consensus.

Results

A total of 12 dogs met the inclusion criteria. 9/12 (75 %) were classified as Sighthounds (Greyhound, Whippet or Lurcher). The most common breed was the Whippet 4/12(33 %), followed by the Greyhound 3/12 (25 %) and with one each of the following breeds: Dalmatian, Alaskan Malamute, Boxer and Lurcher. 10/12 (83 %) of the cases were male and 2/12 cases were female (17 %). 50 % of the male patients were neutered and 100 % of the female patients were neutered. The mean age was 86 months. The median weight 21.4 kg.

Clinical presentation varied among patients with the most common presenting complaint, pyrexia of unknown origin in 4/12 (33 %), followed by repeat staging or re-staging for neoplasia in 3/12 (25 %). One case each presented for weight loss, lethargy, exercise intolerance, vomiting/melena and acute onset of collapse.

10/12 of the cases (83 %) were sedated for CT with 2/12 (17 %) examined under general anesthesia. No case received IPPV during CT examination.

Imaging findings

In 12/12 (100 %) cases PIE was noted. In 9/12 (75 %) of these cases, gas extended into the mediastinum (pneumomediastinum). Four out of 12 cases had cervical extension.

In 12/12 (100 %) of cases the distribution of PIE was paravascular (Fig. 1). In only 4/12 cases (16.7 %) was parabronchial PIE identified (Fig. 1).

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Fig. 1. Pre-contrast computed tomography images in lung reconstruction from a dog demonstrating evidence of cervical emphysema (Panel A – black arrowhead), parabronchial (Panel B – white arrow) and paravascular (Panel B – white arrowhead) pulmonary interstitial emphysema in the right caudal lung lobe.

No case was identified with other pulmonary pathology, evidence of tracheal or [oesophageal injury](#). None of the patients had clinical signs associated with pneumomediastinum.

Discussion

In this case series, we have presented 12 cases of presumed PIE and pneumomediastinum with no radiological evidence of an inciting cause. In this series, Sighthounds were overrepresented making up 75 % of the included cases. In humans, spontaneous pneumomediastinum is reported to occur more frequently in young, previously healthy, tall, thin, males (14:3 compared to females) ([Carzolio-Trujillo et al., 2016](#)). Many [risk factors](#) for pneumomediastinum are reported, including blunt trauma, acute asthma, [respiratory infection](#), [childbirth](#), positive pressure ventilation, Valsalva maneuvers, [physical activity](#), smoking, cocaine consumption and others. However, in 51 % of cases the triggering factor remains unknown ([Carzolio-Trujillo et al., 2016](#)). Of these risk factors, physical activity is the only parallel that can be feasibly extrapolated to this case series. In humans, this causes an acute rise in interalveolar pressure to greater than 40 mmHg, producing a pressure gradient between the alveoli and the adjacent interstitium and has been associated with diving, basketball, football and volleyball ([Carzolio-Trujillo et al., 2016](#); [Mondello et al., 2007](#)).

The findings of this study also support the anatomical distribution of gas within the interstitium reported by Macklin and Macklin in their original study ([Macklin and Macklin, 1944](#)). In that study, small bubbles of gas were observed to be present in the perivascular sheaths surrounding small pulmonic blood vessels, increasing in size toward the hilum and into the mediastinum. The interstitial tissue surrounding the [bronchioles](#) was spared. The predilection for a paravascular distribution of PIE is due to the differences in pressures between alveoli, adjacent airways and [pulmonary vasculature](#) during conditions of increased [alveolar pressure](#) ([Macklin and Macklin, 1944](#)). The similar distribution observed in this study might support a similar pathomechanism, whereby increase in alveolar pressure occurs suddenly and acutely as in Macklins original experimental study ([Macklin and Macklin, 1944](#)).

While pneumomediastinum has been reported to progress to [pneumothorax](#), as was present in the original study by Macklin, no cases in this series presented with [pneumothorax](#).

This study is limited by the lack of histological confirmation of an [absence](#) of respiratory pathology, however the absence of imaging features and referable clinical signs in this population makes this unlikely. The study is also limited by a small sample size and further work should investigate whether the overrepresentation in Sighthounds persists within a larger population not limited to referral only cases.

To conclude, we have reported spontaneous PIE, pneumomediastinum and cervical [subcutaneous emphysema](#) presumed secondary to a Macklin effect in a small population of dogs, in which Sighthound breeds were overrepresented (75 %). Spontaneous pulmonary interstitial emphysema had a primarily paravascular distribution.

Purpose

Latent TGF- β binding protein 4 (LTBP4) is involved in the production of elastin fibers and has been implicated in LTBP4-related cutis laxa and its complication, emphysema-like changes. Various factors have been implicated in the pathogenesis of emphysema, including elastic degeneration, inflammation, cellular senescence, mitochondrial dysfunction, and decreased angiogenesis in the lungs. We investigated the association between LTBP4 and emphysema using human lung fibroblasts with silenced LTBP4 genes.

Methods

Cell contraction, elastin expression, cellular senescence, inflammation, anti-inflammatory factors, and mitochondrial function were compared between the LTBP4 small interfering RNA (siRNA) and control siRNA.

Results

Under the suppression of LTBP4, significant changes were observed in the following: decreased cell contractility, decreased elastin expression, increased expression of the p16 gene involved in cellular senescence, increased TNF α , decreased GSTM3 and SOD, decreased mitochondrial membrane potential, and decreased VEGF expression. Furthermore, the decreased cell contractility and increased GSTM3 expression observed under LTBP4 suppression were restored by the addition of N-acetyl-L-cysteine or recombinant LTBP4.

Conclusion

The decreased elastin expression, cellular senescence, inflammation, decreased antioxidant activity, mitochondrial dysfunction, and decreased VEGF expression under reduced LTBP4 expression may all be involved in the destruction of the alveolar wall in emphysema. Smoking is the most common cause of emphysema; however, genetic factors related to LTBP4 expression and other factors may also contribute to its pathogenesis.

Abbreviations

ACTA2

actin alpha 2, smooth muscle

ACTR2

actin related protein 2

ACVRL1

activin A receptor like type 1

ALDOC

aldolase, fructose-bisphosphate C

ANXA4

annexin A4

BROX

bro1 domain and caax motif containing

CAV2

caveolin 2

CBR1

carbonyl reductase 1

COL12A1

collagen type XII alpha 1
COPD
chronic obstructive respiratory diseaseCTSC, cathepsin C
CYB5B
cytochrome b5 type B
DAPI
4',6-diamidino-2-phenylindole
DDAH1
dimethylarginine dimethyl aminohydrolase 1
ECM
extracellular matrix
EVA1A
eva-1 homolog A
FABP5
fatty acid binding protein 5
FLOT2
flotillin 2
FMNL2
formin Like 2
GAPDH
glyceraldehyde-3-phosphate dehydrogenase
GNA11
G-protein subunit alpha-11
GSTM3
glutathione S-transferase M3
Hel
human embryonic lung
KRIT2
kinetoplast ribosomal tpr proteins 2
KRT13
keratin 13

LRP1

LDL receptor related protein 1

LTBP4

latent TGF- β binding protein 4

MMP12

matrix metalloproteinase 12

MPZL1

myelin protein zero like 1

NAC

N-acetyl-L-cysteine

NAP1L1

nucleosome assembly protein 1 like 1

PALLD

palladin, cytoskeletal associated protein

PARK7

parkinsonism associated deglycase

PFN2

profilin 2

PGRMC2

progesterone receptor membrane component 2

POTEF

POTE ankyrin domain family member F

PTGS1

prostaglandin-endoperoxide synthase 1

RAB6A

ras-related protein rab-6A

rLTBP4

recombinant latent TGF- β binding protein 4

RPLP2

ribosomal protein lateral stalk subunit P2

RPS6

ribosomal protein S6

SA- β

senescence-associated β

SERPINE2

serpin family e member 2

siRNA

small interfering RNA

SIRPA

signal regulatory protein alpha

SOD1

superoxide dismutase 1

SPEG

striated muscle enriched protein kinase

TEK

tek receptor tyrosine kinase

TIMP1

tissue inhibitor of metalloproteinase-1

TNFAIP2

tumor necrosis factor- alpha induced protein 2

TNF- α

tumor necrosis factor- α

TSPAN6

tetraspanin 6

VASP

vasodilator stimulated phosphoprotein

VEGF

vascular endothelial growth factor

ZYX

zyxin

1. Introduction

Emphysema is a condition in which the gas exchange function of the lungs is impaired by irreversible destruction of the alveolar structures, mainly due to smoking ([Morris et al., 2006](#)). In emphysema, matrix metalloproteinases (MMPs) released from inflammatory cells such as macrophages and neutrophils contribute to the degradation of the extracellular matrix (ECM). This process leads to the destruction and heterogeneity of the alveolar wall structure ([Suki et al., 2013](#); [Taraseviciene-Stewart et al., 2008](#)). Although smoking is the primary cause of emphysema, tobacco sensitivity, genetic factors, chronic inflammation, vascular endothelial growth factor (VEGF), and vitamin D receptors have also been implicated in its pathogenesis ([Ishii et al., 2017](#); [Shifren et al., 2007](#); [Taraseviciene-Stewart et al., 2008](#)).

The ECM is consisting of elastic fibers, primarily elastin. The alveolar interstitium is composed of ECM and interstitial cells, and elastic fibers maintain the flexibility of the alveolar walls ([Mecham et al., 2018](#)). Elastin fibers are formed by the binding of elastin polymers to microfibers ([Dabovic et al., 2015](#)). Latent TGF- β binding proteins (LTBPs) include four subtypes known to regulate transforming growth factor-beta (TGF- β) activity ([Dabovic et al., 2015](#)). Specifically, LTBP4 is also involved in the production of elastin fibers ([Dabovic et al., 2015](#)). Noda et al. reported that the elastin production of LTBP4 is independent from TGF- β ([Noda et al., 2013](#)). In LTBP4 knockout mice, elastin fiber production is impaired ([Dabovic et al., 2015](#)), and emphysema-like changes, alveolar space enlargement, and decreased connective tissue in the lobules are observed in the lung tissue ([Sterner-Kock et al., 2002](#); [Mutlu-Albayrak et al., 2020](#)).

LTBP4-related cutis laxa is a connective tissue disorder involving LTBP4. It is characterized by flabby skin, deep wrinkles, and emphysema-like changes ([Nutle-Albayrak et al. 2020](#)). The underlying cause of LTBP4-related cutis laxa is thought to involve impaired elastin fiber production due to the decreased expression of LTBP4 ([Su et al., 2021](#)). In the United States, LTBP4-related cutis laxa is an autosomal recessive genetic disorder diagnosed through the assessment of family history and the identification of mutations in the *LTBP4* gene ([Callewaert et al., 2016](#)). LTBP4 may be involved in the etiology of emphysema-like changes, a complication of LTBP4-related cutis laxa ([Callewaert et al., 2016](#)).

However, the association between LTBP4 expression and emphysema remains unclear. Finlay et al. reported a disruption of the elastin microstructure in a rat model of emphysema and the lung tissue from patients with chronic obstructive respiratory disease (COPD) ([Finlay et al., 1996](#)). Basic studies suggest that LTBP4 is involved in the production of elastin fibers that maintain lung structure and function ([Noda et al., 2013](#)), as well as in lung tissue regeneration and repair ([Heidler et al., 2013](#)). Furthermore, SNPs in LTBP4 have been linked to exercise tolerance in patients with emphysema ([Hersh et al., 2006](#)). Thus, LTBP4, involved in the production of elastic fibers, may play a role in various pathological conditions associated with emphysema. In addition to elastic fiber degeneration ([Finlay et al., 1996](#)), various factors have been implicated in the pathogenesis of emphysema, including inflammation ([Garcia-Rio et al., 2010](#)), cellular senescence ([Karasch et al., 2008](#)), mitochondrial dysfunction ([Karim et al., 2022](#)), and decreased angiogenesis ([Kanazawa et al., 2003](#)). The disruption of the alveolar wall structure in emphysema is due to the degradation of ECM, the main component of which is elastin ([Finlay et al., 1996](#)). Pulmonary fibroblasts play a central role in synthesizing and maintaining the alveolar ECM ([Plantier et al., 2007](#)). Given these context, we aimed to investigate the association between LTBP4 and the pathogenesis of emphysema using human lung fibroblasts with suppressed *LTBP4* gene expression.

2. Materials and methods

2.1. Cell maintenance and treatments

Human embryo lung (HEL299) cells were obtained from KAC Company (Kyoto, Japan) and cultured in Eagle's Minimum Essential Medium (EMEM) with Earle's Balanced Salts (w/NaHCO₃ w/o Glutamine) supplemented with 200 mM glutamine, 100 mM sodium pyruvate (Nap), 100X non-essential amino acid (NEAA), 10 % fetal bovine serum (FBS), 100 IU/ml penicillin and 100 ug/ml streptomycin, at 37 °C humidifying incubator in 5 % CO₂ air. Cell culture medium was changed every three days. For stimulating elastin growing, cells were maintained for up to 14 days at 37 °C humidifying incubator. For investigation of the effects of antioxidants and anti-inflammation, cells were treated with 10 mM N-Acetylcysteine (NAC) or 0.5 nM recombinant human LTBP4 (rLTBP4) for 4 h at 37 °C. Data are representative of three independent experiments. The study using human cell samples was performed in adherence with the Declaration of Helsinki. The protocol was performed in accordance with the University of Tokyo Ethics Committee and institutional guidelines.

2.2. RNA isolation and reverse transcription-PCR analysis

Total RNA was isolated from lung tissues using the RNeasy Mini Kit (Qiagen). For real-time PCR, RNA was extracted from cell lines, lungs, and tracheas, after which cDNA was generated from 1.0 µg of total RNA using the Super Script First Strand cDNA Synthesis Kit (Invitrogen). Real-time PCR was conducted using the QPCR SYBR Green Mix (Bio-Rad, Hercules, CA, USA) on an AB 7300 Real time PCR system (AB Applied Biosystems, Singapore). PCR primers for LTBP4, Elastin, p-16, tumor necrosis factor-α (TNF-α), MMP-12, tissue inhibitor of metalloproteinase-1 (TIMP-1), glutathione S-transferase M3 (GSTM3), superoxide dismutase 1 (SOD1), Mitofusin2 and VEGF are shown in [Table 1](#). Results were plotted as relative expression levels compared with control. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the control. Data were analyzed by the comparative cycle threshold (C_t) method and normalized to GAPDH levels in each sample.

Table 1. PCR primers.

Target gene	Sequence	
LTBP4		
	Forward	GTCTCCAACGAGAGCCAGAG
	Reverse	CACTCTGTGTAGGTGGCCTG
Elastin		
	Forward	GTCGCAGGTGTCCCTAGTGT
	Reverse	GGTCCCCACTCCGTACTTG
TIMP-1		
	Forward	TGCTGATGACATACGTGGCA
	Reverse	AGGATTGGCAAGCGTTGG
SOD1		

Target gene	Sequence	
GSTM3	Forward	AGGGCATCATCAATTCGAG
	Reverse	TGCCTCTCTTCATCCTTG
VEGF		
Mitofusin2	Forward	GGAGGCAAGGGACGGAGA
	Reverse	TTCCGAGCCTCGAGGACTAG
TNF- α		
p-16	Forward	CTACCTCCACCATGCCAAGT
	Reverse	TCTCTCCTATGTGCTGGCCT
LTBP4: latent TGF β binding protein 4, TIMP-1: tissue inhibitor of metalloproteinase-1, SOD1: superoxide dismutase 1, GSTM3: glutathione S-transferase M3, VEGF: vascular endothelial growth factor, TNF- α : tumor necrosis factor- α		
LTBP4	Forward	CTCTCGATGCAACTCTATCGTC
	Reverse	TCCTGTACGTGTCTTCAAGGAA
TIMP-1	Forward	CCTGCCCAATCCCTTATT
	Reverse	CCCTAAGCCCCAATTCTCT
SOD1	Forward	CCCACCGCACCGAACATAGTTA
	Reverse	ACCAGCGTGTCCAGGAAG

LTBP4: latent TGF β binding protein 4, TIMP-1: tissue inhibitor of metalloproteinase-1, SOD1: superoxide dismutase 1, GSTM3: glutathione S-transferase M3, VEGF: vascular endothelial growth factor, TNF- α : tumor necrosis factor- α

2.3. LTBP4 silencing

The silencing of LTBP4 in human lung-derived fibroblasts was performed using HEL299 cells. RNA-lipid complexes were prepared with diluted silencer to select LTBP4 small interfering RNA (siRNA) and silencer to select negative control siRNA. For Lipofectamine RNAiMAX, the culture medium was replaced with Opti-MEM (1 x). Both reagent and siRNA were diluted in a 1:1 ratio in serum-free Opti-MEM and incubated.

2.4. Cell contraction assay

HEL299 cells were utilized to assess the cell contractile activity as a wound healing model in four groups: control siRNA, LTBP4 siRNA, LTBP4 siRNA+NAC, and LTBP4 siRNA + rLTBP4. A Collagen-based Cell Contraction Assay, CBA-201 (Cell Biolabs, Inc.), was used.

2.5. Elastin and LTBP4 expression

The number of cell nuclei, elastin and LTBP4 expression levels in the control siRNA and LTBP4 siRNA were compared using fluorescent staining. The GAPDH-normalized LTBP4 and elastin mRNA levels were compared between the LTBP4 siRNA and control siRNA. The cells were sequentially labeled with Alexa Fluor 568- and Alexa Fluor 488-conjugated primary and secondary antibodies. To visualize the nuclei, the cells were counterstained with 4',6-diamidino-2-phenylindole (DAPI) (1:5,000; Bio-Rad). The samples were analyzed using a Keyence BZ-9000 series (BioRevo) fluorescence microscope.

2.6. Cellular senescence

The fluorescence intensity after senescence-associated β gal staining (SA- β) was compared between the LTBP4 siRNA and control siRNA. The mRNA expression of p16 normalized to GAPDH was also compared between the LTBP4 siRNA and control siRNA. SA- β gal staining was performed using the Cellular Senescence Detection Kit (SPiDER- β Gal, SG03, Dojindo). The cells were observed under a fluorescence microscope (excitation: 488 nm, emission: 500–600 nm).

2.7. Inflammatory proteins

The GAPDH-normalized TNF α , TIMP1, and MMP12 mRNA expression levels were compared between the LTBP4 siRNA and control siRNA.

2.8. Proteomics analysis

Among proteins directly involved in oxidation-reduction, those whose expression was more than 2-fold or less than 1/2 under LTBP4 suppression were identified by proteomic analysis. To evaluate the effects of the expression of various proteins associated with reduced LTBP4 expression, exosomes were extracted, and proteins were identified. The supernatant of cells transfected with siRNA was percolated. Then, the extracellular vesicle (EV) solution was purified and prepared. Mass spectrometry was performed using nano-liquid chromatography with tandem mass spectrometry (LC-MS/MS). The samples were loaded onto an Aurora Series emitter column using nano-high performance liquid chromatography. The eluted peptides were analyzed using a Q Exactive Plus mass spectrometer (Thermo Scientific). Nano-LC-MS/MS spectra were obtained using the Mascot Server (version 2.5.1). The spectral data were submitted to SWISS-PROT of Human (Taxonomy ID: 9606). Statistical analysis of the protein spectra was performed using Scaffold software (Proteosome Software, USA). Significant differences were detected using a Student's *t*-test at a *p* value of <0.05. Only proteins with >99.0 % probability were considered.

2.9. Antioxidants

To assess the effect of LTBP4 on antioxidants, the mRNA expression of GSTM3 and SOD1 standardized with GAPDH was compared among the control siRNA, LTBP4 siRNA, LTBP4 siRNA + NAC, and LTBP4 siRNA + rLTBP4.

2.10. Mitochondrial function

Fluorescent staining was performed to assess the mitochondrial status. Mito ViewTM dye was used for staining, and observations were made through fluorescence microscopy. The mRNA expression of

mitofusin 2 and VEGF, normalized to that of GAPDH, was compared between the LTBP4 and control siRNA. The mitochondrial membrane potential was also compared between the LTBP4 siRNA and control siRNA according to the fluorescence intensity. Mitochondrial membrane potential was assessed using a JC-1 MitoMP Detection Kit. The cells were washed, an imaging buffer solution was added, and the cells were observed under a fluorescence microscope.

2.11. Ethical statement

This study was conducted in accordance with the Ethical Guidelines for Medical and Health Research Involving Human Subjects established by the Ministry of Health, Labor, and Welfare in Japan.

2.12. Statistical analysis

All data were expressed as mean \pm standard error (SE). Comparisons between two groups were made by an unpaired t test. ANOVA with Bonferroni's correction was used for comparisons among multiple groups. Statistical analysis was conducted using the SPSS version 15.0 software package for Windows (SPSS). Statistical significance was assumed at $p < 0.05$.

3. Results

3.1. Cell contraction assay

LTBP4 siRNA caused significantly less collagen gel contraction than control siRNA ($p < 0.05$) ([Fig. 1](#)). Both LTBP4 siRNA + NAC and LTBP4 siRNA + rLTBP4 showed significantly greater contraction than LTBP4 siRNA.

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Fig. 1. Suppression of LTBP4 and cell contraction.

The bars represent the mean, and the circles represent individual data. Area ratio of HEL299 indicates contractile activity of cells in a wound healing model. LTBP4 siRNA showed significantly less collagen gel contraction than control siRNA, LTBP4 siRNA + NAC, or LTBP4 siRNA + rLTBP4. Each group $n = 3$, * $p < 0.05$, NAC: N-acetyl-L-cysteine, LTBP4: latent TGF β binding protein 4, rLTBP4: recombinant latent TGF- β binding protein 4, siRNA: small interfering RNA.

3.2. Elastin and LTBP4 expression

In fluorescent staining, although there is no difference in the number of cell nuclei, LTBT4 siRNA showed lower LTBP4 and elastin expression than the control siRNA ([Fig. 2a](#)). LTBT4 siRNA showed significantly lower expression of LTBP4 ($p < 0.001$) and elastin ($p < 0.005$) mRNA than the control siRNA ([Fig. 2b](#) and c).

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Fig. 2. Suppression of LTBP4 and elastin expression.

In figures b and c, each group n = 3, The bars represent the mean, and the circles represent individual data. ** $p < 0.01$, *** $p < 0.001$, Fluorescence intensity indicates the amount of expression. [Fig. 2a](#) shows that LTBP4 siRNA expresses less elastin and BTBP4 compared to controls siRNA, although there is no difference in the number of cell nuclei. DAPI: 4',6-diamidino-2-phenylindole, GAPDH: glyceraldehyde-3-phosphate dehydrogenase, LTBP4: latent TGF- β binding protein 4, siRNA: small interfering RNA.

3.3. Cellular senescence

In SA- β gal staining, LTBP4 siRNA demonstrated stronger fluorescence intensity indicating more cellular senescence compared with the control siRNA ([Fig. 3a](#) and b). LTBP4 siRNA showed a significantly higher expression level of P16 gene, which is associated with cellular senescence, than the control siRNA ([Fig. 3c](#)).

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Fig. 3. Suppression of LTBP4 and cellular senescence.

In figures b and c, each group n = 3, The bars represent the mean, and the circles represent individual data. * $p < 0.05$, *** $p < 0.001$, Fluorescence intensity in SA- β gal staining indicates cellular senescence. Increased expression of the p16 gene indicates cellular senescence. GAPDH: glyceraldehyde-3-phosphate dehydrogenase, LTBP4: latent TGF β binding protein 4, SA- β : senescence-associated β , siRNA: small interfering RNA.

3.4. Inflammatory proteins

LTBP4 siRNA had significantly higher mRNA expression levels of TNF α ($p < 0.05$), TIMP1 ($p < 0.05$), and MMP12 ($p < 0.05$) compared with the control siRNA ([Fig. 4a-c](#)).

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Fig. 4. Suppression of LTBP4 and TNF α , TIMP, and MMP12.

Each group n = 3, The bars represent the mean, and the circles represent individual data. * $p < 0.05$, Increased expression of TNF α , TIMP1 and MMP12 suggests a response to inflammation. GAPDH: glyceraldehyde-3-phosphate dehydrogenase, LTBP4: latent TGF- β binding protein 4, MMP12: matrix metalloproteinase 12, siRNA: small interfering RNA, TIMP1: tissue inhibitor of metalloproteinase-1.

3.5. Antioxidants

Proteomics analysis showed that there are 14 candidate proteins whose expression increases more than 2-fold under conditions where LTBP4 expression was suppressed. ([Table 2](#)). Similarly, there were 29 proteins whose expression decreased by half or less, including GSTM3, involved in the metabolism of toxic substances, and SOD1, responsible for decomposing reactive oxygen species (ROS). We focused on GSTM3 and SOD3, which are involved in the removal of ROS. LTBP4 siRNA showed significantly lower GSTM3 expression than the control ($p < 0.001$), while LTBP4 siRNA+ NAC showed significantly higher GSTM3 expression than the LTBP4 siRNA ($p < 0.005$) ([Fig. 5a](#)). LTBP4 siRNA

showed significantly lower SOD1 expression than the control siRNA ($p < 0.05$) (Fig. 5b). LTBP4 siRNA showed significantly lower GSTM3 expression than the control siRNA ($p < 0.001$), while LTBP4 siRNA+rLTBP4 siRNA showed significantly higher GSTM3 expression than the LTBP4 siRNA alone ($p < 0.005$) (Fig. 5c). LTBP4 siRNA showed significantly lower expression of SOD1 than the control siRNA ($p < 0.05$) (Fig. 5d).

Table 2. Exosome comprehensive protein analysis in decreased LTBP4 expression.

Proteins with expression increased more than 2-fold

Cluster 1

COL12A1, DDAH1, DDAH2, EVA1A, KRT13

Cluster 2

ANXA4, GNA11, RAB6A

Cluster 3

ACTA2, FMNL2, FMNL3, POTEF, TNFAIP2, ZYX

Proteins with expression reduced to less than 1/2

Cluster 1

ALDOC, CAV2, CBR1, CTSC, FABP5, FLOT2, GSTM3, NAP1L1, PARK7, PGRMC2, PTGS1, RPLP2, RPS6, SOD1, TSPAN6,

Cluster 2

ACTR2, ACVRL1, BROX, CYB5B, KRIT2, PALLD, PFN2, SPEG, TEK, VASP

Cluster 3

LRP1, MPZL1, SERPINE2, SIRPA

LTBP4: latent TGF β binding protein 4, COL12A1: collagen type XII alpha 1, DDAH1: dimethylarginine dimethyl aminohydrolase 1, EVA1A: eva-1 homolog A, KRT13: keratin 13, ANXA4: annexin A4, GNA11: G-protein subunit alpha-11, RAB6A: ras-related protein rab-6A, ACTA2: actin alpha 2, smooth muscle, FMNL2: formin Like 2, POTEF: POTE ankyrin domain family member F, TNFAIP2: tumor necrosis factor- alpha induced protein 2, ZYX: zyxin, ALDOC: aldolase, fructose-bisphosphate C, CAV2: caveolin 2, CBR1: carbonyl reductase 1, CTSC: cathepsin C, FABP5: fatty acid binding protein 5, FLOT2: flotillin 2, GSTM3: glutathione S-transferase mu 3, NAP1L1: nucleosome assembly protein 1 like 1, PARK7: parkinsonism associated deglycase, PGRMC2: progesterone receptor membrane component 2, PTGS1: prostaglandin-endoperoxide synthase 1, RPLP2: ribosomal protein lateral stalk subunit P2, RPS6: ribosomal protein S6, SOD1: superoxide dismutase 1, TSPAN6: tetraspanin 6, ACTR2: actin related protein 2, ACVRL1: activin A receptor like type 1, BROX: bro1 domain and caax motif

containing, CYB5B: cytochrome b5 type B, KRIT2: kinetoplast ribosomal tpr proteins 2, PALLD: palladin, cytoskeletal associated protein, PFN2: profilin 2, SPEG: striated muscle enriched protein kinase, TEK: tek receptor tyrosine kinase, VASP: vasodilator stimulated phosphoprotein, LRP1: LDL receptor related protein 1, MPZL1: myelin protein zero like 1, SERPINE2: serpin family e member 2, SIRPA: signal regulatory protein alpha

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Fig. 5. Suppression of LTBP4 and GSTM3 and SOD1.

Each group n = 3, The bars represent the mean, and the circles represent individual data. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, GSTM3 has a role in regulating oxidative stress, and decreased expression of GSTM3 suggests oxidative stress. SOD1 is an enzyme that removes reactive oxygen species, and decreased SOD1 expression suggests oxidative stress. GSTM3: glutathione s-transferase M3, LTBP4: latent TGF β binding protein 4, NAC: N-acetyl-L-cysteine, rLTBP4: recombinant latent TGF- β binding protein 4, siRNA: small interfering RNA, SOD1: super oxide dismutase 1.

3.6. Mitochondrial function

LTBP4 siRNA exhibited a significant reduction in mitofusin 2 expression, a key player in mitochondrial fusion, than the control siRNA ($p < 0.005$) ([Fig. 6a](#)). Additionally, LTBP4 siRNA demonstrated a significantly lower VEGF expression than the control siRNA ($p < 0.001$) ([Fig. 6b](#)). Furthermore, LTBP4 siRNA showed a significantly lower mitochondrial membrane potential than the control siRNA ($p < 0.05$) ([Fig. 7](#)).

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Fig. 6. Suppression of LTBP4 and mitochondrial function and VEGF.

Each group n = 3, The bars represent the mean, and the circles represent individual data. ** $p < 0.01$, *** $p < 0.001$, Mitofusin 2 is involved in mitochondrial fusion and VEGF is involved in angiogenesis. GAPDH: glyceraldehyde-3-phosphate dehydrogenase, LTBP4: latent TGF β binding protein 4, siRNA: small interfering RNA, VEGF: vascular endothelial growth factor.

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Fig. 7. Suppression of LTBP4 and mitochondrial membrane potential.

Each group n = 3, The bars represent the mean, and the circles represent individual data. * $p < 0.05$, High ratio of fluorescence intensity (Red/Green) indicates high mitochondrial membrane potential. LTBP4: latent TGF- β binding protein 4, siRNA: small interfering RNA.

4. Discussion

This study is the first to investigate the involvement of reduced LTBP4 expression in the pathogenesis of emphysema using human lung fibroblasts. In the collagen gel contraction assay, cells exhibiting reduced LTBP4 expression showed significantly less cell contraction than the controls. Fibroblasts play a crucial role in tissues by contracting the repair site during wound healing ([Chitturi et al., 2015](#)). As TGF- β is involved in cell contraction, differentiation, proliferation, and ECM production ([Ma et al., 2017](#)), the results of this study may be attributed to the inhibition of TGF- β function by suppressing LTBP4 expression. This result suggests that decreased LTBP4 expression could potentially impact wound healing in response to damage to the alveolar septum caused by the degradation of the ECM in emphysema.

Fibroblasts with suppressed LTBP4 expression produced less elastin than controls. Bultmann-Mellin et al. reported that the lungs of LTBP4 knockout mice lacked normal elastin fibers ([Bultmann-Mellin et al., 2017](#)), and our results are consistent with their report. These results further demonstrate the importance of LTBP4 in the production of elastin fibers. In addition, the expression levels of TNF α , an inflammatory marker; TIMP1, which is involved in cell necrosis; and MMP12, which is involved in the degradation of ECM, increased owing to the suppression of LTBP4 expression. Shifren et al. reported increased abundance of macrophages, enlarged air spaces, and decreased elastic recoil in the lung tissues of mice with reduced elastin expression ([Shifren et al., 2007](#)). In emphysema, elastase, secreted by inflammatory cells like neutrophils and macrophages, degrades the EMC, particularly elastin ([Mecham et al., 2018](#)). In addition, elastin fragments produced by elastin fiber degradation can accumulate in inflammatory cells, further accelerating elastin degradation ([Mecham et al., 2018](#)). The decreased expression of elastin and increased expression of TNF α , TIMP1, and MMP12 under suppressed LTBP4 expression shown in this study are consistent with the tissue inflammation and decreased elastic recoil observed in emphysema.

The expression of the antioxidants SOD1 ([Di et al., 2018](#)) and GSTM3 ([Wang et al., 2020](#)), which are involved in the metabolism of peroxides, decreased when LTBP4 expression was suppressed. Furthermore, when the LTBP4 expression was suppressed, the expression of mitofusin 2, which is involved in mitochondrial fusion, and the mitochondrial membrane potential decreased. Mitochondria produce ROS and are also affected by ROS ([Liu et al., 2020](#)). Under oxidative stress, damaged and healthy mitochondria are separated by fusion and fission, and the damaged mitochondria are degraded by mitophagy to maintain mitochondrial function ([Liu et al., 2020](#)). However, persistent oxidative stress can impair mitophagy and damage mitochondria, causing mitochondrial dysfunction and cellular senescence ([Liu et al., 2020](#)). The results of this study indicate that the suppression of LTBP4 expression may result in decreased mitochondrial antioxidant activity and dysfunction. Increased mitochondrial ROS, decreased SOD, and decreased mitochondrial membrane potential have been documented in lung biopsies from individuals diagnosed with COPD ([Mumby et al., 2022](#)), and our results are consistent with these findings in COPD. The mitochondria are also involved in angiogenesis by regulating cellular metabolism and vascular endothelial cell proliferation ([Reichard et al., 2019](#)). In response to hypoxia, the mitochondria produce ROS, which induces the transcription of the VEGFA gene and results in the production of VEGF ([Reichard et al., 2019](#)). Our study also showed that VEGF production was decreased under LTBP4 suppression. Bultmann-Mellin et al. also reported decreased expression of VEGF in LTBP4 knockout mice ([Bultmann-Mellin et al., 2017](#)), which was consistent with our findings. Decreased VEGF levels in the sputum have been reported in patients with emphysema compared with healthy individuals ([Kanazawa et al., 2003](#)). Considering these factors, the decreased expression of VEGF observed in the present study may be due to mitochondrial dysfunction.

The results of this study and our hypothesis regarding the relationship between destruction of alveolar wall structure in emphysema are summarized in [Fig. 8](#). It is possible that a decrease in elastin production impairs ECM repair and maintenance, a decrease in cell contractility impairs wound healing in injured alveoli, an increase in inflammatory cytokines accelerates ECM degradation, and a decrease in antioxidants leads to cell senescence and reduced angiogenesis, each of which may contribute to the disruption of alveolar wall structure.

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Fig. 8. Putative link between LTBP4 suppression and alveolar wall destruction in emphysema ECM: extracellular matrix, LTBP4: latent TGF- β binding protein 4, VEGF: vascular endothelial growth factor, GSTM3: glutathione s-transferase M3, SOD1: super oxide dismutase 1.

5. Limitations

There are several limitations in this study. This study only used human lung fibroblasts. Fibroblasts are important for the maintenance and repair of the ECM ([Leslie et al., 2021](#)), but emphysema is a complex disease involving various types of cells. Thus, to further verify the results of this study, experiments using human epithelial cells and immune cells, *in vivo* experiments using animal models, and experiments using human lung tissue are required. In this study, the effects of restoring LTBP4 on elastin production, cellular senescence, TNF α , TIMP1, MMP12, Mitofusin2, and VEGF were not evaluated. TGF- β has various functions, and the mutual roles of TGF- β and LTBP4 have not been fully elucidated. It will be important to investigate the effect of LTBP4 on elastin production in the absence of TGF- β in the future. This study evaluated the effects of LTBP4 suppression using mRNA as an indicator, and did not evaluate protein itself using Western blotting or other techniques.

6. Conclusion

Smoking is the most common cause of emphysema; however, genetic factors related to LTBP4 expression and other factors may contribute to its pathogenesis. Although further studies are needed, exploring techniques to regulate LTBP4 may contribute to future advances in the treatment of emphysema.

Abstract

To retrieve, analyze, and extract evidence related to subcutaneous emphysema in patients undergoing laparoscopic surgery systematically, and provide evidence-based recommendations for reducing its incidence. By browsing the websites of the National Institute for Health and Clinical Excellence, the International Guideline Collaboration Network, the National Guideline Library of the United States, the Registered Nurses Association of Ontario, the Scottish Intercollegiate Guideline Network, the Clinical Practice Guidelines website of the Canadian Medical Association, UpToDate, Web of Science, PubMed, OVID, Cochrane Library, Embase, Chinese Biomedical Database, CNKI, VIP, and Wanfang Database, relevant literatures, guidelines, systematic reviews, evidence summaries, expert consensus, randomized controlled trials, etc. about subcutaneous emphysema in patients undergoing laparoscopic surgery were retrieved. All searches were limited to articles published between 1st January 2010 to 1st August 2023. 2245 articles were identified in total, 10 articles were included after exclude literature that does not meet the standards, including 3 clinical decision-making articles, 2 review papers, and 5 randomized controlled trials. Evidence summarization was

conducted from 5 aspects: influencing factors, prevention, establishment and management of pneumoperitoneum, intraoperative monitoring, and intervention methods, 15 pieces of best evidences were summarized. Clinical staffs should transform and apply the evidence-based practices to decrease the incidence of subcutaneous emphysema and enhance the quality of life for patients.

1. Introduction

Laparoscopic surgery, also known as minimally invasive surgery, has indeed revolutionized many medical procedures by offering several advantages over traditional open surgery. Laparoscopic surgery results in less trauma leading to less pain and discomfort. Laparoscopic procedures involve making smaller incisions, contributing to a decreased risk of bleeding or postoperative infections. Patients undergoing laparoscopic surgery typically experience a faster recovery time compared to open surgery, and they may return to regular daily activities earlier.¹ This reduced the overall cost of healthcare and freeing up hospital resources. Laparoscopic surgery has become one of the preferred methods in the field of surgery, compared with robot-assisted technology.² Almost all gynecological and general surgery can be performed under laparoscopy.

In laparoscopic surgery, pneumoperitoneum is established firstly by insufflation of carbon dioxide (CO₂) into the abdominal cavity to create a space for the surgeon to proceed the actual procedure. Both laparoscopic approach and the establishment of pneumoperitoneum can lead to complications.³ Complications of laparoscopic surgery mainly include: vascular and gastrointestinal injury,⁴ urinary tract injury,⁵ nervous system injury,⁶ surgical site infection,⁷ shoulder pain,⁸ and subcutaneous emphysema (SCE).⁹

SCE occurs when air becomes trapped in the subcutaneous tissue beneath the skin. SCE is a common complication of laparoscopic surgery, with an incidence of 24.9 %.¹⁰ Although mild SCE is generally well tolerated, and the body absorbs the carbon dioxide over time, it still can cause discomfort. Severe SCE may impact the patient's respiratory function, leading to increased respiratory distress, compromised gas exchange, and potential respiratory failure and cause circulatory and metabolic disturbances, resulting in a decompensated, unstable state, especially in elderly patients with the coexistence of cardiac, pulmonary, or renal disorders.¹¹ Majority of the literature studies regarding subcutaneous emphysema in laparoscopic surgery are reported as case studies, focusing on specific intervention methods, rather than extensive systematic studies or systematic treatment plan at present. Therefore, this study summarized the preventive measures of laparoscopic SCE through evidence-based practices, and provided preventive measures and treatment strategies for laparoscopic SCE.

2. Material and methods

2.1. Retrieval strategy

According to the "6S" evidence model, relevant literatures, guidelines, systematic reviews, evidence summaries, expert consensus, randomized controlled trials, etc. about subcutaneous emphysema in patients undergoing laparoscopic surgery were retrieved from the websites of the National Institute for Health and Clinical Excellence, the International Guideline Collaboration Network, the National Guideline Library of the United States, the Registered Nurses Association of Ontario, the Scottish Intercollegiate Guideline Network, the Clinical Practice Guidelines website of the Canadian Medical Association, UpToDate, Web of Science, PubMed, OVID, Cochrane Library, Embase, Medi-Touch, Chinese Biomedical Database, CNKI, VIP, and Wanfang Database,. The search period was from 1st January 2010 to 1st August 2023. The search strategy utilizes PubMed as an exemplar:
(("Laparoscopes"[Mesh]) OR (((((laparoscope[Title/Abstract]) OR (laparoscopy[Title/Abstract])) OR

(laparoscopic[Title/Abstract])) OR (peritoneoscope[Title/Abstract])) OR
(Celiostope[Title/Abstract])))) AND ((Subcutaneous Emphysema"[Mesh]) OR
((pneumoderma[Title/Abstract]) OR (cutaneous emphysema[Title/Abstract])) OR (Emphysema,
Subcutaneous[Title/Abstract]))).

2.2. Literature inclusion and exclusion criteria

According to the PIPOST approach to form the questions for this evidence-based care. Inclusion criteria: ① P(population) represents patients undergoing laparoscopic surgery, ② I (intervention) is strategies for managing intraoperative SCE; ③ P(professionals) refers to health care workers, including doctors, nurses, and other medical professionals. ④ O(outcome): the evaluation, the occurrence and extent, symptom management, nursing interventions and prevention of subcutaneous emphysema following laparoscopy surgery. ⑤ Setting: refers to the hospital. ⑥ T (types of evidence) means guidelines, summary of evidence, expert consensus, review, meta-analysis, and randomized controlled trial. Exclusion criteria: incomplete information, conference report, case report, and literature with low quality evaluation.

2.3. Literature screening and criteria for literature quality evaluation

The randomized controlled trial (RCT) was evaluated according to the Cochrane literature quality assessment tool, including sequence generation, allocation concealment, blinding of the participants, blinding of the investigators, incomplete outcome data, selective outcome reporting and other bias. The assessment results were typically categorized as low bias (indicating high quality), high bias (indicating low quality), or unclear (lack of relevant information or uncertainty of bias). The clinical decision-making was identified as high-quality evidence.

2.4. Principle of evidence extraction

The literature being studied included reviews, RCTs, and clinical decision-making. Two trained nursing investigators independently reviewed and integrated preliminary evidence. In cases there is a conflict between different sources of evidence, higher quality evidence and the latest published authoritative literature will be preferred. If there is any disagreement, involvement of a third researcher for evaluation are crucial to finally reach an agreement. The included evidence were graded by using the Australian JBI Evidence-based Health Care Centre 2014 Evidence pre-grading system, with 1 being the highest and 5 the lowest level.¹²

3. Results

3.1. General characteristics of the included literature

Through preliminary retrieval, a total of 2245 articles were obtained. After screening, 10 articles were ultimately included, including 3 clinical decision-making articles, 2 reviews, and 5 RCTs. The basic characteristics of the included articles are detailed in [Table 1](#).

Table 1. Basic characteristics of included articles (n = 10).

Study	year	type	source	Literature content/theme
Lee et al ¹³	2011	RCT	PubMed	Does intraabdominal pressure affect development of SCE at gynecologic laparoscopy?

Study	year	type	source	Literature content/theme
Ren et al ¹⁴	2012	RCT	VIP	Effect of one-lung ventilation on occurrence of SCE in patients undergoing retroperitoneal laparoscopic urologic surgery
Xiao et al ¹⁵	2012	Review	CNKI	Progress on nursing intervention of patients with SCE caused by carbon dioxide pneumoperitoneum
Feng et al ¹⁶	2014	RCT	VIP	Effect of pressurized chest strap on preventing SCE during laparoscopic surgery
Ott et al ⁹	2014	Review	Medline	SCE-beyond the pneumoperitoneum
Wang et al ¹⁷	2020	RCT	VIP	The effect of an inflatable compression device in preventing SCE during gynecological laparoscopic surgery
Girish et al ¹⁸	2022	Clinical decision-making	UpToDate	Anesthesia for laparoscopic and abdominal robotic surgery in adults
Qian et al ¹⁹	2023	RCT	CNKI	Effects of Continuous Exhaust Ventilation on Prevention of SCE after Retroperitoneal Laparoscopy
Aurora et al ²⁰	2023	Clinical decision-making	UpToDate	Complications of laparoscopic surgery
Aurora et al ²¹	2023	Clinical decision-making	UpToDate	Abdominal access techniques used in laparoscopic surgery

3.2. Quality evaluation results of the RCTs

Five RCTs were included in this study, and the evaluation result is shown in [Table 2](#). The overall quality of study design was high.

Table 2. RCT quality assessed by using Cochrane literature quality assessment tool.

study	sequence generation	allocation concealment	blinding of the participants	blinding of the investigators	incomplete outcome data	selective outcome reporting	other bias
Lee et al ¹³	low	low	low	unclear	low	low	low
Ren et al ¹⁴	low	low	unclear	unclear	low	low	low
Feng et al ¹⁶	low	low	unclear	unclear	low	low	unclear
Wang et al ¹⁷	low	high	unclear	unclear	low	low	high
Qian et al ¹⁹	low	low	unclear	unclear	low	low	low

3.3. Evidence summary and description

The contents extracted from the included studies specifically focusing on five aspects: influencing factors, prevention management, pneumoperitoneum establishment management, intraoperative monitoring, and intervention methods. Altogether 15 evidence-based strategies had been summarized in preventing the occurrence of subcutaneous emphysema in laparoscopic surgery, as shown in [Table 3](#).

Table 3. Summary of evidence-based strategies to prevent the occurrence of subcutaneous emphysema in laparoscopic surgery.

Evidence type	Literature content/theme	level
Influencing Factors	1.Complexity of surgery, surgeon experience, weight and subcutaneous fat thickness, patient comorbidities, CO ₂ flow and pressure, Veress needle placement, the number of trocars, and poor cardiopulmonary reserve ^{9,13,15,18,20}	5b
Prevention	2. Proper Veress needle placement. The needle entry failure, and port leakage or detachment should be prevented in the initial abdominal pathway ^{18,20,21}	5b
	3. Reducing the number of times a pneumoperitoneum needle enters the abdomen ^{9,15}	3b
	4. The specific intra-abdominal CO ₂ pressure used can vary based on the type of surgery, ranging from 12 to 15 mmHg, however the lower pressures are also feasible ^{13,15,21}	5b

Evidence type	Literature content/theme	level
Establishment and Management of Pneumoperitoneum	5. Monitoring insufflator settings for pressure, flow rate, and volume of gas with alarm settings ⁹	3b
	6. The rate of CO ₂ gas flow is initially set low and then gradually increased to avoid needle/port displacement or occlusion ^{15,21}	5b
	7. Once the desired intra-abdominal space is secured for the surgical procedure, gas flow should be discontinued, even if it is below the pressure set point ⁹	3b
	8. It is not necessary to increase additional costs for heating or humidifying CO ₂ in laparoscopic surgery ²¹	5b
Intraoperative Monitoring	9. Observing the skin condition of surgical and puncture sites, as well as monitoring vital signs ¹⁵	3b
	10. If minor SCE occurred, and no obvious effect on the patient, it can be temporarily left untreated. However, close observation is necessary, and the surgery should be completed as soon as possible ¹⁵	3b
	11. Once a patient experienced severe SE with increased end-tidal CO ₂ during surgery, the CO ₂ insufflation should be stopped immediately, and the doctor should be notified; assist the surgeon puncturing multiple points in areas with obvious emphysema, squeezing the puncture sites to exhaust gas or placing a negative pressure absorbing balls to take residual gas and fluid, and the surgery should be ended or switched to open surgery ^{9,15,18}	3b
	12. There may be an increased risk of airway compromise after extubation when crepitus or swelling found in the head, neck, or upper chest ¹⁸	5b
Intervention Methods	13. Continuous exhaust ventilation is recommended on prevention of SCE ¹⁹	1c
	14. One-lung ventilation is recommended to prevent SCE ¹⁴	1c
	15. Pressurized chest strap is recommended on preventing SCE ^{16,17}	1c

4. Discussion

4.1. Influencing factors of subcutaneous emphysema

The rate of SCE may go up with one or more of these risk factors. For instance, complexity of surgery, surgeon inexperience, weight and subcutaneous fat thickness, patient comorbidities, physiological changes related to abdominal insufflation, and impaired cardiopulmonary reserve may not be suitable for abdominal inflation.^{9,13,15,18,20} The experience of surgeons and the type of surgeries they have encountered lead to have different ways of dealing with various laparoscopic surgeries.²² For lean patients, lack of subcutaneous fat tissue, have a weaker barrier against CO₂ gas, making them more susceptible to subcutaneous emphysema.^{13,15} The occurrence of SCE is closely related to intra-abdominal pressure, CO₂ flow rate, trocar needle placement, the number of trocars, and the frequency of punctures.¹⁵ Some studies have shown that high CO₂ flow and pressure causes rapid dispersion of CO₂ in a short period of time, with rapid subcutaneous absorption, and increased incidence of emphysema.^{13,15,20} During long surgical procedures, frequent replacement of surgical instruments and continuous CO₂ pneumoperitoneum are also important factors leading to the formation of SCE.^{15,18} Repeat the puncture repeatedly and improper cannula placement, easily cause CO₂ escapes from the puncture hole, leading to subcutaneous emphysema. Therefore, during the operation, vigilance, a cautious attitude, appropriate intra-abdominal pressure, and CO₂ flow rate and usage of CO₂ gas should be recorded, trocars placed at suitable for the planned surgery, and the number of punctures should be minimized.^{9,18,21}

4.2. Prevention

Laparoscopic surgery is generally associated with less trauma and a reduced stress response compared to traditional open surgery, but the physiological responses of patients can vary due to factors such as the duration and difficulty of surgeries, comorbidities, and surgical complications. Therefore, we should focus on those medical conditions that may affect a patient's response to physiologic changes associated with laparoscopic surgery. The peritoneal cavity needs to be accessed before any laparoscopic surgery. Creation of pneumoperitoneum is the first step during laparoscopic surgery. Then, a port will be placed for the laparoscope and this is followed by the placement of additional ports for various laparoscopic instruments. The access techniques to the peritoneal cavity, choice of access technique, placement locations, and port placement for single-incision laparoscopic surgery can be carried out according to the methods proposed by clinical decision-making.^{18,21} Much attention should be paid to the details of operations, such as needle insertion, set up monitoring for insufflator pressure, flow, and gas volume alarms.⁹ The setting of intra-abdominal CO₂ pressure may depend on the type of surgery, with generally ranging from 12 to 15 mmHg. Lower pressures are also feasible, which contributing to relief pain for postoperative patients.^{13,15,21} The rate of CO₂ gas flow should be set low initially and it is gradually increased to achieve the desired intra-abdominal pressure. If the intra-abdominal pressure rapidly increases to the target pressure during the insufflation of CO₂, it can be indicative of potential issues such as needle displacement or port occlusion.^{15,21}

4.3. Establishment and management of pneumoperitoneum

CO₂ is the ideal gas for pneumoperitoneum insufflation during laparoscopic surgery as CO₂ is nontoxic, colorless, readily soluble in the blood, easily expelled from the body or expired through the lungs, and nonflammable.⁹ The degree of subcutaneous emphysema was effected by the intra-abdominal CO₂ pressure, CO₂ pressure and gas flow should be set low and it is gradually increased to avoid needle/port displacement or blockage during the initial phase of insufflation.¹⁵ Once a sufficient intra-abdominal space is secured for the surgical procedure, gas flow should be discontinued, even if it is below the pressure set point, and the surgery should be completed with

the lowest possible intra-abdominal pressure and minimal flow.⁹ CO₂ gas can be administered cold or heated, with or without humidification. Compared with cold gas, heated gas led to only a minimal, insignificant rise, and without any meaningful improvement in patient's outcomes. Thus, the extra cost of heating and/or humidifying gas used in laparoscopy cannot be justified.²³ Therefore, it is not necessary to increase additional costs for heating or humidifying CO₂ in laparoscopic surgery.²¹

4.4. Intraoperative monitoring

During the surgery, healthcare professionals should closely observe the skin condition of the surgical and puncture sites, monitor vital signs, and maintain an appropriate intra-abdominal pressure to ensure patient safety.¹⁵ If minor SCE occurred and there was no immediate apparent effect on the patient, it can be temporarily left untreated. However, close observation is necessary, and the surgery should be completed as soon as possible.¹⁵ If crepitus or swelling is found, readjustment of ports, reduction of insufflation pressure, or conversion to open surgery may be required.¹⁸ Once severe SCE happened, with increased end tidal CO₂ during surgery, CO₂ insufflation should be stopped, and the doctor should be notified immediately; assist the surgeon puncturing multiple points in areas with obvious emphysema, squeezing the puncture sites to exhaust gas or placing a negative pressure absorbing balls to take residual gas and fluid, and the surgery should be ended as soon as possible or switched to open surgery. One should know that there may be an increased risk of airway compromise if crepitus or swelling found in the head, neck, or upper chest. CO₂ insufflation should be stopped immediately, and further precautions should be taken to prevent complications of lung and respiratory disease.^{9,15,18,24} A postoperative chest radiograph should be performed to rule out capnothorax, and the patient should be observed in the post-anesthesia care unit for several hours, until SCE subsides and vital signs return to normal.¹⁸

4.5. Intervention methods

Appropriate intraperitoneal pressure is essential to maintain a clear field of vision for surgical operations. When the intra-abdominal pressure is too high, it will cause CO₂ retention. The higher the pressure, the larger the range of SCE, and the higher chance to have adverse reactions. The continuous exhaust method that maintains stable pneumoperitoneum pressure can shorten the surgical time, and reduce the incidence of SCE in patients after surgery.¹⁹ During retroperitoneal laparoscopic surgery, compared to bilateral lung ventilation, non-surgical unilateral lung ventilation can reduce CO₂ absorption, lower the degree of SCE, and reduce the occurrence of SCE.¹⁴ The use of compression devices can maintain a relatively constant intra-abdominal pressure in the operating space of the abdominal cavity by controlling intra-abdominal pressure, to prevent SCE.^{16,17} Using an inflatable compression device, fixed it on the patient's chest before the surgery begins, and provides a consistent pressure to the patient at the beginning of pneumoperitoneum to increase tissue density, and eliminates potential natural gaps, so slowing the speed of CO₂ diffusion under the subcutaneous and reducing the CO₂ absorption by the human body.^{16,17]} However, the use of inflation and compression devices, chest belts, how exhaust, and pressure settings requires more research to optimize their implementation and minimize adverse reactions.

5. Conclusion

This study summarizes the best evidence on preventing and treating SCE in laparoscopic surgery. It is suggested that medical resources, patient preferences, and the expertise of healthcare professionals should be taken into account when incorporating evidence-based practices into clinical care.

At present, most researches were case studies on SCE after laparoscopic surgery, there is a lack of high-quality studies such as guidelines, and randomized controlled trials (RCTs). There is no clear-cut

guidelines or standardized protocols regarding prevention and management of SCE after laparoscopic surgery. We have only conducted preliminary integration of evidence. Further research is crucial to advance the understanding and management of SCE after laparoscopic surgery, so as to provide research evidence for the unification of clinical procedures in the future.

Abstract

In patients with pulmonary emphysema and mild to moderate airflow limitation, one does not expect the features marked exertional dyspnea and hypoxemia as well as a profound decrease in diffusing capacity of the lung for carbon monoxide (DLCO). Here we describe this phenotype and its prognosis. From our database, we retrospectively selected cases associating emphysema, exertional breathlessness, O₂ requirement at least upon exercise, forced expiratory volume in 1 sec (FEV₁) ≥ 50% predicted, and DLCO ≤ 50% predicted, without associated combined pulmonary fibrosis and emphysema, right-to-left shunt, or severe pulmonary hypertension Over a 12-year period, we identified 16 patients with emphysema and the above presentation. At the initial evaluation, the median age was 62 years (interquartile range 53.8–68.9). The median FEV₁ and DLCO% predicted and mean pulmonary artery pressure were 86 (65–95)%, 38 (31–41)%, and 20 (17–25) mm Hg, respectively. On room air, the median arterial partial pressure of oxygen and partial pressure of carbon dioxide in arterial blood were 63.5 (55.8–69) mm Hg and 34.5 (31–36) mm Hg with increased median alveolar-arterial oxygen difference (46 [39–51] mm Hg). After the initial evaluation, the respiratory condition worsened in 13 of 14 (92.8%) patients with one or more re-evaluations (median follow-up 2.6 [0.9–5.8] years). In 12, lung transplantation was considered. Four patients died after 5.8, 5.7, 7.1, and 0.8 years of follow-up, respectively. We describe an underrecognized phenotype of pulmonary emphysema featuring a particular profile characterized by marked exertional dyspnea, impaired pulmonary gas exchange with low DLCO and marked oxygen desaturation at least on exercise but with mild or moderate airway obstruction.

Introduction

Pulmonary emphysema is an anatomical entity characterized by abnormal and permanent enlargement of the airspaces distal to the terminal bronchioles without obvious fibrosis. Emphysema may be present without airflow obstruction but, along with small airways disease, is a usual histological pattern of chronic obstructive pulmonary disease (COPD), contributing to the chronic airflow limitation that characterizes COPD. Marked exertional dyspnea, hypoxemia, and decreased diffusing capacity of the lung for carbon monoxide (DLCO) are common features of COPD especially if airflow limitation is severe. However, these are not expected in a patient presenting emphysema not fitting the definition of COPD or with mild to moderate airflow limitation. Besides this usual profile, COPD with severe pulmonary hypertension (PH) (severe PH-COPD) and combined pulmonary fibrosis and emphysema (CPFE) are two known entities that may feature the association of pulmonary emphysema, marked exertional dyspnea and hypoxemia, and low DLCO but moderate airway obstruction [1], [2], [3], [4], [5], [6], [7], [8], [9].

One of the topics of our lung center is the management of advanced forms of lung diseases such as COPD and lung fibrosis. Many symptomatic patients with emphysema are referred to our center for consideration of lung transplantation (LT) or lung volume reduction or for exploring the mechanism of unexplained hypoxemia. Therefore, the current practice at our center is to perform a thorough cardiopulmonary evaluation in COPD patients as part of pre-LT or pre-lung volume reduction workup or for diagnostic purposes. This extensive evaluation of some selected COPD patients has allowed us to identify another subset of patients presenting the above-mentioned clinical and functional characteristics.

Here, we report a series of 16 patients with emphysema without severe PH and or associated pulmonary fibrosis who presented the particular pattern of marked exertional dyspnea and hypoxemia as well as low DLCO but with mild to moderate airflow limitation.

The study was carried out in Hospital Bichat-Claude Bernard, a university tertiary care center located in Paris. It was approved by the ethics committee of the institutional review board of the French language society for respiratory medicine (*Société de Pneumologie de Langue Française. Comité d'évaluation des protocoles de recherche observationnelle 2021–044*).

From our database, we retrospectively selected the files for all patients presenting this particular profile over the last years. Cases were included if all the following criteria were met at the time of the initial evaluation: 1) marked exertional dyspnea of at least grade 2 on the modified Medical Research Council (mMRC) dyspnea scale; 2) presence of pulmonary emphysema on chest CT, defined as focal areas or regions of low attenuation, usually without visible walls [10]; 3) no evidence of a pattern suggesting CPFE on chest CT after a review by an experienced thoracic radiologist (MPD); 4) requirement of long-term oxygen therapy, at least with exercise; 5) DLCO value $\leq 50\%$ predicted; 6) absence of severe PH-COPD on right-heart catheterization; 7) absence of intracardiac or intrapulmonary shunt documented on contrast-enhanced echocardiography; 8) absence of documented extra-pulmonary fixation on lung perfusion scan; and 9) pre-bronchodilator forced expiratory volume in 1 sec (FEV₁) $\geq 50\%$ predicted with or without airflow limitation defined by fixed FEV₁/forced vital capacity (FVC) ratio < 0.70 . We selected only the files for patients who had a thorough evaluation at diagnosis, including at least pulmonary function tests, DLCO test, blood gas analysis on room air, 6-min walk test, thoracic CT, contrast-enhanced echocardiography, lung perfusion scan, and right-heart catheterization.

For each selected file, the medical record, chest CT, contrast-enhanced echocardiography, lung scan, and right-heart catheterization results were analyzed. When several tests had been performed, we used the results of tests performed as near as possible to the initial evaluation.

Clinical data included smoking history, cursus laboris, level of dyspnea evaluated by the mMRC scale, number and severity of exacerbations, body mass index, presence of comorbidities, and modalities of long-term oxygen therapy (at rest and exercise or exercise only, oxygen flow rate).

High-resolution CT scans of the chest were analyzed by an experienced thoracic radiologist (MPD), with quantitative analysis performed for all patients and with procubitus position analysis in case of suspected gravitational abnormalities. Emphysema was defined according to the Fleischner Society guidelines by the presence of $> 6\%$ of pixels < -950 Hounsfield units (HU) on quantitative CT and/or visual identification of emphysema [11]. The following data were retrieved: type of pulmonary emphysema (centrilobular, panlobular, and paraseptal), subtype of centrilobular emphysema (trace, moderate, confluent, advanced destructive), presence of bullae > 2 cm in diameter (bullae defined by avascular low-attenuation areas > 1 cm in diameter with a thin but perceptible wall) [11], quantification of the percentage of emphysema, and presence of associated opacities such as minimal lung interstitial abnormalities (ground-glass opacities, reticulations). Moreover, we retrieved the results of CT pulmonary angiography, performed for all cases.

Pulmonary function tests (PFTs) were performed according to guidelines used at the time of inclusion [12], [13], [14]. Only blood gas values obtained on room air at the initial evaluation were considered. The alveolar-arterial oxygen difference [$P(A-a)O_2$] was estimated as the difference between the alveolar partial pressure of oxygen (PAO_2) and the arterial partial pressure of oxygen (PaO_2). PAO_2 was calculated with the following simplified equation: PAO_2 (in mmHg) = $150 - PaCO_2 / 0.8$. For

assessing the single-breath DLCO in our laboratory, the American Thoracic Society/European Respiratory Society guidelines were followed [15,16]. The 6-min walk test was performed according to the American Thoracic Society recommendations [17].

Right-heart catheterization was performed at rest, distant from an exacerbation. We retrieved the values for mean pulmonary arterial pressure (mPAP), systolic pulmonary arterial pressure (sPAP), diastolic pulmonary arterial pressure, pulmonary arterial occlusion pressure (PAOP), cardiac output (CO), cardiac index (CI), and pulmonary vascular resistance (PVR). Severe PH-COPD, an exclusion criterion, was defined by mPAP \geq 35 mm Hg or \geq 25 mm Hg with low CI ($< 2\text{ l/min/m}^2$) [18,19].

The survival status (alive, dead, LT) of patients was assessed at the time of manuscript preparation. Also, for each patient, we evaluated, when available, the evolution over time of the dyspnea grade, chest CT findings, oxygen requirement, PFT findings (including DLCO, blood gas measurement, and 6-min walk test), right-heart catheterization parameters, and the use of pulmonary arterial hypertension (PAH)-targeted therapy, which was prescribed in accordance with the recommendations currently used at the time of the visit [18,19]. In case of LT, the histology of the lung explant was analyzed.

Continuous variables are reported with median (interquartile range [IQR]) and categorical variables with number (percentage). Overall survival was analyzed by the Kaplan-Meier method. The patients were censored at the date of death or LT. Spearman correlation analysis was used to explore the correlation of 1) mPAP with PaO₂, FEV₁ or DLCO at inclusion; 2) PaO₂ and FEV₁ or DLCO; and 3) the percentage of emphysema and PaO₂, DLCO, or FEV₁. $P \leq 0.05$ was considered statistically significant. Statistical analyses were performed with R v4.2.0.

Results

From our database, we identified 16 patients who fulfilled the inclusion criteria. Patients had their first diagnostic evaluation from 2010 to 2022.

Discussion

In this retrospective series of patients extracted from our database, we identified a subset with pulmonary emphysema presenting a particular phenotype: 1) a quite homogeneous clinical/functional profile characterized by marked exertional dyspnea, impaired pulmonary gas exchange with low DLCO, and marked oxygen desaturation at rest and/or upon exercise requiring at least long-term oxygen therapy upon exercise but with mild or moderate airway obstruction, in the absence at the time of diagnosis

Ethical approval

The study was approved by the ethics committee of the institutional review board of the French language society for respiratory medicine (*Société de Pneumologie de Langue Française. Comité d'évaluation des protocoles de recherche observationnelle 2021-044*).

Competing interests

GW reports non-financial support from CSL Behring, outside the submitted work; VB reports non-financial support from CSL Behring, outside the submitted work; CG, TG, DM, MS, RB, AM, MPD, CTM have nothing to disclose; HM reports personal fees from Boehringer, personal fees from Novartis, non-financial support from Pulmonx, non-financial support from LFB, personal fees from Grifols, and personal fees from CSL Behring, outside the submitted work.

Introduction and significance

Emphysema is an uncommon but important condition that often appears in the neonatal period. Diagnosis is based on CT, which identifies the affected lung lobe, which is treated with complete surgical resection.

Case presentation

We present a case of a child who had been suffering for about a year from recurrent respiratory infections without arriving at a clear and correct diagnosis. He was evaluated by us and diagnosed correctly despite the difficulty of distinguishing it from pneumothorax. The final treatment was surgical removal.

Clinical discussion

Emphysema is considered one of the important conditions that should be considered as a differential diagnosis if there is clear hyperinflated in the pulmonary lobe. The evaluation is mainly done through CT to reach the correct diagnosis and treatment.

Conclusion

Congenital lobar emphysema is a rare condition that primarily affects children. The majority of children with CLE experience symptoms and necessitate surgery.

1. Introduction

Congenital lobar emphysema (CLE) is a rare lung malformation affecting approximately 1 in 20,000 to 30,000 newborns [1]. It occurs when one or more lung lobes become abnormally due to a partial obstruction within the airways. This obstruction can compress adjacent organs and shift the mediastinum towards the opposite lung. Most CLE cases are diagnosed shortly after birth or in infancy. Adults with CLE are very uncommon, as symptoms typically appear early in life. Common symptoms include tachypnea and a cough [2], [3]. A high index of suspicion is essential for diagnosing CLE, especially given its rarity and potential misdiagnosed as pneumothorax. This misdiagnosis can lead to unnecessary procedures, such as chest tube insertion. Chest X-ray is the initial step in diagnosis, typically revealing an enlarged affected lobe and a displacement of the mediastinal structures [4]. In our case, we will present a case of CLE that was managed wonderfully and successfully without pneumothorax misdiagnosis and inappropriate pneumonia treatment, as this case developed an excellent plan for managing suspected cases of CLE.

This case is described in accordance with the criteria of SCARE [5].

2. Presentation of case

2.1. Patient information

We describe the case of a 2-year-old male with a history of recurrent respiratory infections since birth and multiple hospital admissions at an external hospital. The parents reported that the child presented with sudden shortness of breath without fever or cough, prompting a visit to the emergency department of our hospital.

3. Clinical findings

When he arrived at the emergency department, his general condition was good. No central or peripheral cyanosis was observed, and nor were there no signs of use of the accessory pectoral muscles.

4. Diagnostic assessment

Heart rate of 128 beats per minute, blood pressure of 103/62, temperature of 98.2 Fahrenheit, respiratory rate of 43 breaths per minute, and oxygen saturation of 88 % on room air.

A chest X-ray revealed a large air cyst in the upper lobe of the right lung, causing compression of the mediastinum ([Fig. 1A-B](#)).

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Fig. 1. A: Chest X-ray (anterior-posterior view) demonstrates hyperinflation of the right upper lobe with a shift of the mediastinal structures to the left.

B: Chest X-ray (lateral view) reveals a large air cyst in the right upper lobe.

C: Chest CT scan (cross-sectional view) shows emphysematous changes with cystic formations involving the right upper lobe. The left lung appears normal, without evidence of inflammatory infiltrates.

Computed tomography (CT) of the chest showed hyperinflation of the upper lobe of the right lung, with cystic cavities within it. The left lung appeared normal ([Fig. 1C](#)).

The echocardiogram showed a hyperkinetic heart with mild mitral and tricuspid valve insufficiency.

5. Therapeutic intervention

Based on the diagnostic findings, surgery was performed under general anesthesia. After isolating the right lung, a posterior lateral incision was made on the fifth right intercostal space, accessing the chest cavity. The right upper lobe remained enlarged even after lung isolation, confirming the presence of congenital lobar emphysema ([Fig. 2A](#)). To ensure adequate ventilation of the remaining lower and middle lobes, the entire upper lobe was resected ([Fig. 2B](#)). A chest tube was placed for drainage, and the chest wall was closed.

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Fig. 2. A: Intraoperative photograph clearly shows congenital lobar emphysema (CLE) in the right upper lobe.

B: Gross specimen of the resected right upper lobe.

Histopathological examination of the resected tissue demonstrated chronic nonspecific inflammation with moderate infiltrates and emphysematous changes within the lung parenchyma ([Fig. 3A-B](#)).

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Fig. 3. A: Hematoxylin and eosin (H&E) stained section shows inflammatory infiltrates and hemosiderin macrophages.

B: H&E stained section demonstrates moderate emphysematous changes.

The child was monitored for approximately five days. The chest tube was then removed, and they were discharged in good overall condition.

6. Discussion

CLE is a rare lung malformation that develops during fetal lung development, typically in the third trimester of pregnancy [6]. The exact cause remains unknown, but several factors are suspected to play a role, including:

- Abnormal cartilage formation: Defects in bronchial cartilage, crucial for airway support, are seen in about 25 % of diagnosed cases. This can lead to airway obstruction.
- Blocked airways: Other factors that can obstruct airways and contribute to CLE development include malformations of the blood vessels supplying the lungs and cytomegalovirus infection [7].

Males are slightly more likely to be affected by CLE than females. The malformation most commonly involves the left upper lobe, involvement of the lower lobes is rare.

Most CLE cases present in newborns or early childhood. Adult cases are uncommon. Symptoms of CLE can vary and may not appear immediately. They can be triggered by recurrent respiratory infections that trap air in the lungs.

Children with CLE may experience: respiratory distress, tachypnea, use of additional chest muscles to assist breathing, decreased breath sounds over the affected lung and high-pitched whistling sound (wheezing) in the affected area.

Chest X-ray is the initial imaging tool used to suspect CLE. It can reveal an overinflated lobe and a mediastinal shift towards the affected side.

In our case, a Chest X-ray is considered a typical and ideal image to describe CLE. It is observed that hyperinflated in the right upper lobe, in addition to the right upper lobe crossing the median line, which is not observed in the case of pneumothorax.

However, chest CT scan is considered the gold standard for diagnosis [8]. Providing a detailed view of surrounding lung tissue, the unaffected lung, and potential abnormalities such as vascular malformations or masses in the chest cavity.

Bronchoscopy has limited use in diagnosing CLE but may be considered if there's a history of foreign body aspiration or to assess airway variations [9].

Echocardiography, an ultrasound of the heart, is crucial due to the association between CLE and heart abnormalities in 14–20 % of cases [6]. It helps identify any coexisting heart problems.

Conservative management may be considered for patients with mild or no symptoms. These patients require close monitoring, as symptoms may develop, and surgery may become necessary in the future [10].

Surgery is the primary treatment for CLE, particularly for cases with severe symptoms. Early intervention is recommended to prevent complications. Most patients who undergo surgery experience symptom resolution and achieve normal growth and development.

7. Conclusion

Differentiating congenital lobar emphysema from pneumothorax is crucial. Our case serves as a textbook example of managing this rare condition effectively. By carefully evaluating clinical findings and radiographs, we avoided premature surgical intervention and ensured an accurate diagnosis and appropriate treatment plan.

Abbreviations

CLE

Congenital Lobar emphysema

CT

Computerized tomographic

Introduction

The impact of [obstructive lung disease](#) (OLD) and [emphysema](#) on lung cancer (LC) mortality in patients undergoing LC screening is controversial.

Methods

Patients with [spirometry](#) and LC diagnosed within the first three rounds of screening were selected from the [National Lung Screening Trial](#) (NLST) and from the Pamplona International Early Lung Cancer Detection Program (P-IELCAP). Medical and demographic data, tumor characteristics, comorbidities and presence of [emphysema](#) were collected. The effect of [OLD](#) and [emphysema](#) on the risk of [overall survival](#) was assessed using unadjusted and adjusted [Cox models](#), competing risk regression analysis, and [propensity score matching](#).

Results

Data from 353 patients with LC, including 291 with [OLD](#) and/or [emphysema](#) and 62 with neither, were analyzed. The median age was 67.3 years-old and 56.1% met OLD criteria, predominantly mild (1: 28.3%, 2: 65.2%). Emphysema was present in 69.4% of the patients. Patients with OLD and/or emphysema had worse survival on [univariate analysis](#) (HR: 1.40; 95% CI: 0.86–2.31; $p = 0.179$). However, after adjusting for LC stage, age, and sex, the HR was 1.02 (95% CI: 0.61–1.70; $p = 0.952$). Specific [LC survival](#) between both groups showed an adjusted HR of 0.90 (95% CI: 0.47–1.72; $p = 0.76$). Propensity score matching found no statistically significant difference in [overall survival](#) (HR: 1.03; 95% CI: 0.59–1.9; $p = 0.929$).

Conclusion

The survival of LC patients diagnosed in the context of screening is not negatively impacted by the coexistence of mild OLD and/or emphysema.

Graphical abstract

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Introduction

Lung cancer (LC) is the leading cause of cancer-related mortality worldwide.¹ Smoking is not only the primary risk factor for LC, but increases the risk of other respiratory diseases such as chronic obstructive pulmonary disease (COPD) and emphysema,² which in turn are strongly associated with the development of LC.^{3, 4, 5, 6, 7} Lung cancer screening is effective in reducing LC mortality,^{8, 9, 10} but there is a growing concern about the risks of screening patients with coexisting chronic lung disease.¹¹

The impact of COPD and emphysema on mortality in patients with LC remains controversial. Some studies have reported a worse prognosis attributed to post-treatment complications.^{12, 13, 14} However, improvements in bronchodilator treatment,^{13, 15} physical therapy,¹⁶ and even advances in thoracic surgery and radiation therapy¹⁷ have challenged these findings in other studies. Moreover, treatment of early-stage LC in patients with coexisting COPD or emphysema may even result in spirometric improvements due to a reduction of lung volume.^{18, 19, 20, 21, 22}

Evidence regarding the impact of these pulmonary diseases on LC mortality in lung cancer screening programs is limited, and generally based on secondary analyses of cohorts with limited sample size and/or underpowered statistical analysis.²³ Due to the large proportion of patients with COPD and/or emphysema participating in these programs,²⁴ there is a need for larger studies focused on the impact of these respiratory comorbidities on survival of patients who are diagnosed with LC in the context of screening. Our study focuses on patients who were screened for LC but also underwent lung function testing from the ACRIN sub-cohort of the National Lung Screening Trial (NLST) and the Pamplona (Spain) sub-cohort of the International Early Lung Action Program (P-IELCAP). To avoid confounding factors and to increase precision, competing risks and propensity score matching analysis were used.

Trials Oversight

The National Lung Screening Trial (NLST) was a randomized trial comparing screening for lung cancer using LDCT with chest radiography. Participants were invited to undergo three screening rounds (T0, T1, T2) at 1-year intervals. The NLST enrolled participants between August 2002 and September 2007, and followed through December 2009. The NLST was approved by the institutional review board at each of the 33 participating medical institutions. Details about the study protocol, participant

Baseline Clinical Characteristics, Lung Function and LC Stage

The characteristics of the study population are shown in Table 1. A total of 353 patients with LC from both cohorts were included in the analysis, of whom 291 had OLD and/or emphysema and 62 were classified as “smokers without lung disease”. The median ($p_{25};p_{75}$) age was 67.3 years (62.8;72.5). The cohort was predominantly male (66%) and heart disease (13.9%), chronic bronchitis (12.5%) and diabetes (10.5%) were the most common comorbidities. More than a half of the population (56.1%) met OLD

Discussion

Our results show that LC mortality in the context of screening is not influenced by the presence of mild OLD and/or emphysema as demonstrated by competing risk and propensity score matching of two lung cancer screening cohorts (NLST and P-IELCAP).

These results assuage concerns regarding the impact of OLD and emphysema on the risks and benefits of lung cancer screening.^{11, 30} Whether more severe forms of OLD or emphysema can affect screening outcomes has yet to be determined, since most

Abstract

Emphysema is a respiratory disease that causes the progressive loss of lung extracellular matrix (ECM) organisation, subsequently undermining lung integrity and reducing lung function. Fibroblasts must constantly repair damage to the lungs to preserve lung health, however, fibroblast ECM repair is reduced during [emphysema](#), causing ECM damage to outweigh fibroblast ECM maintenance. Current treatments for emphysema fail to address the root causes of emphysematous progression, highlighting the need for novel methods of treating emphysema. [Nitrofurantoin](#) is a broad-spectrum antibiotic indicated for the treatment of urinary tract infections that also displays potential as a novel avenue of emphysema treatment. Nitrofurantoin is known to potentially cause fibrotic effects that could be repurposed to increase fibroblast repair and outweigh the progressive ECM damage of the emphysematous lung. Therefore, this study examined the effects of nitrofurantoin treatment on primary human lung fibroblasts derived from emphysema patients to determine if the drug holds potential as a novel treatment for emphysema. Nitrofurantoin was shown to stimulate migration and alter fibroblast morphology by increasing cell area and reducing roundness, suggesting that it could induce an ECM-repair primed phenotype in fibroblasts. Interestingly, nitrofurantoin treatment did not alter collagen-IV, perlecan, periostin or tenascin-C deposition, though fibronectin deposition was significantly upregulated at a higher dosage (20 µg/mL). This study highlighted the nitrofurantoin induced changes to fibroblast motility and morphology that facilitate ECM repair. Thus, nitrofurantoin induced [pulmonary fibrosis](#) could be caused by a change in cell phenotype that subsequently upregulates ECM repair, indicating its potential as a treatment for emphysema.

1. Introduction

Fibroblasts are cells that reside within the [lung parenchyma](#) and work to maintain the integrity of the [extracellular matrix](#) (ECM), a dynamic network of [fibrous proteins](#) and [proteoglycans](#) that structurally supports cells and governs the mechanical properties of the lung, including stiffness and [lung elasticity](#) [1], [2], [3], [4], [5]. Fibroblasts work to repair damage to the lung caused by the introduction of [irritants](#), by lung injuries or by the gradual wear caused during the cyclic stretch of breathing [6], [7]. Fibroblast ECM maintenance is of critical importance to lung health, as a shift in the lung mechanical environment can have wide-reaching effects on the behaviour of mechanosensitive cells populations, cells that detect mechanical forces and alter cellular behaviour in response. Fibroblasts, among many other lung cell populations, are known to be mechanosensitive and drastically alter their behaviour due to a change in substrate stiffness or other forces [8], [9], [10], [11], [12].

Emphysema is characterised by severe alterations to the lung environment reflected by the progressive destruction of the lung ECM and loss of alveolar walls [3], [13], [14], [15]. The emphysematous lung is known to have decreased parenchymal stiffness and experience diminished stretch forces when breathing due to [lung hyperinflation](#), the inability to fully retract the lung during exhalation. This pathological hyperinflation causes reduced gas exchange and disrupts the stretch

forces, therefore distorting crucial regulatory mechanical cues experienced by fibroblasts in the emphysematous lung [16]. Furthermore, fibroblasts rely upon the regulatory cues provided by fibroblast-ECM protein interactions to guide ECM repair, yet the altered composition of the emphysematous lung likewise disturbs this form of regulation. The insufficient ECM maintenance performed by emphysematous fibroblasts is likely a result of cellular dysregulation due to altered biomechanical cues, such as reduced stretch forces, decreased lung stiffness, and altered ECM composition [17], [18]. Our recent study has suggested that the emphysematous lung environment may induce a fibroblast phenotype that is unsuited to ECM repair through pathological adaptation in [lung fibroblasts](#), disrupting the ability of emphysematous fibroblasts to correctly respond to lung regulatory cues. However, an increase in fibroblast ECM repair could reverse these pathological changes and may sufficiently increase fibroblast ECM deposition to halt emphysematous progression, restoring the regulatory cues of the healthy lung.

The [antibiotic](#) nitrofurantoin is recommended as a first line therapy for [urinary tract infections](#) [19]; however, it has been known to potentially cause [adverse effects](#) such as [pulmonary fibrosis](#) [20], [21]. Despite the adverse effects, nitrofurantoin is an approved therapy by the Food and Drug Administration FDA-(FDA Reference ID: 3368447). Raised lung ECM stiffness, due to nitrofurantoin-induced pulmonary [fibrosis](#), could cause a further upregulation of fibroblast-mediated ECM maintenance due to the mechanosensitivity of fibroblasts. Furthermore, this increased fibroblast ECM repair is more likely to create a recursive loop of rising ECM stiffness and rising fibroblast activity that may overcome the progressive ECM damage of emphysema. Stimulation of ECM deposition through fibrosis represents a novel avenue of treatment for emphysema that could potentially address a major underlying cause of emphysema, rather than merely addressing symptoms, as done by current steroid and anti-inflammatory treatments [22]. Therefore, the present study aims to explore the effect of nitrofurantoin on fibroblasts and determine if the treatment can improve the dysfunctional ECM repair of emphysematous fibroblasts. The viability and potential of nitrofurantoin as a treatment for emphysema was investigated by assessing nitrofurantoin cellular toxicity and the influence of the drug on emphysematous fibroblast dynamic cellular behaviour. A range of emphysematous fibroblast processes relating to ECM maintenance, including cytokine expression (IL-6 and IL-8), [cellular motility](#), morphology, and [ECM protein](#) deposition were analysed to determine changes caused by nitrofurantoin treatment.

2. Materials and methods

2.1. Primary cell isolation

Primary human lung fibroblasts (PHLF) were extracted from the explanted or [resected lung](#) parenchyma of emphysema patients following resection or [lung transplantation](#). Written [informed consent](#) was obtained from each patient pre-operatively and the study was approved by a human research ethics committee (approval code #X14-0045). A segment of parenchymal tissue (1 cm^3) was taken from the discarded lung of each patient and divided into small pieces (1 mm^3). These smaller pieces of parenchymal tissue were washed with Hank's buffered salt solution (HBSS) and a minimum of ten washed pieces of parenchymal tissue were seeded in 75 cm^2 cell culture flasks (Corning Costar, Massachusetts, USA). The tissue segments were maintained in Dulbecco's Modified Eagle Medium (DMEM; Sigma-Aldrich, Sydney, Australia) supplemented with 5 % FBS (v/v) (FBS; Invitrogen, Melbourne, Australia) and 1 % [antibiotic antimycotic](#) solution (Sigma-Aldrich) at $37\text{ }^\circ\text{C}$ and 5 % CO_2 . After 2–4 weeks, the cells reached [confluence](#) and were identified as fibroblasts by observing the growth patterns and cellular morphology before being passaged. PHLF were then cultured in DMEM – low glucose (Sigma-Aldrich) supplemented with 10 % FBS (v/v; Invitrogen), 2 % (v/v) [HEPES](#) solution, 1 % (v/v) L-glutamine

solution (Gibco, Invitrogen, Melbourne, Australia) and 1 % antibiotic [antimycotic](#) solution (v/v; Sigma-Aldrich). All experiments on PHLF were performed between passages 2 and 6.

Additionally, PHLF were treated with the drug nitrofurantoin (Merck-Millipore, Massachusetts, USA) at 2, 10 or 20 µg/mL 24 h after seeding (standard treatment group) or 48 h after seeding (delayed treatment group). Pulmonary fibrosis is an uncommon [adverse effect](#) of nitrofurantoin treatment; therefore, a higher dose could be necessary to induce the desired fibroblast ECM deposition. Thus, 20 µg/mL was chosen as the maximum possible nitrofurantoin concentration (dose) that could induce ECM deposition without greatly reducing fibroblast viability ([Fig. 2](#)). Additionally, the concentrations 2 and 10 µg/mL were examined to provide an understanding of the effect of nitrofurantoin concentrations that did not negatively impact [cell viability](#) on fibroblasts and to determine if the impact of nitrofurantoin was dose dependent. Nitrofurantoin treatments were prepared by dissolving raw nitrofurantoin in [DMSO](#) then diluting to the desired concentration using cell culture media while ensuring that the final concentration of [DMSO](#) in solution was below 1 % (v/v).

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Fig. 1. An image of WI-38 fibroblasts (A) before and (B) after the application of the fibroblast detection model.

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Fig. 2. Dose-response curves of (A) healthy (WI-38 cells) and (B) emphysematous primary fibroblasts after being treated with varying concentrations of [nitrofurantoin](#) for 72 h and examined by [MTS assay](#). Absorbance values of untreated control cells were normalised to a 100 % value of fibroblast [cell viability](#) when treated with only a 1 % [DMSO](#) vehicle control. Dose-response curves were analysed by non-linear regression to fit a curve to the data and calculate the IC₅₀ and IC₈₀ of [nitrofurantoin](#) for healthy and emphysematous fibroblasts. (A) Nitrofurantoin was found to have an IC₅₀ of 78.1 ± 1.7 µg/mL and an IC₈₀ of 22.5 ± 3.0 µg/mL when applied to WI-38 fibroblasts. (B) Nitrofurantoin was found to have an IC₅₀ of 64.8 ± 0.4 µg/mL and an IC₈₀ of 19.6 ± 1.6 µg/mL when applied to primary emphysematous fibroblasts. Data is displayed as mean \pm SEM, n = 3.

2.2. Cell culture

The healthy human lung [fibroblast cell line](#), WI-38 (Code: 90020107) were purchased from CellBank Australia (CBA, Westmead, NSW, Australia) and maintained in minimum essential medium eagle (MEME; Sigma-Aldrich) supplemented with 10 % (v/v) FBS (Invitrogen), and 1 % (v/v) [sodium pyruvate](#) (Gibco). All experiments on WI-38 were performed between 22 and 40 population doublings.

All cell culture was performed in 6, 48 or 96 well plates (Corning Costar) coated with collagen type-I from rat tail (10 µg/cm²; Sigma-Aldrich) and all cells were treated with 2 ng/mL of TNF-α (InVitro Technologies, Melbourne, Australia) or 2 ng/mL of TGF-β (BioLegend, California, USA).

2.3. MTS assay

Cell viability was assessed using an [MTS assay](#) (CellTitre 96[®] Aqueous One Solution [Cell Proliferation Assay](#), Promega, Sydney, NSW, Australia), a [colorimetric method](#) that detects the reduction of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium inner salt (MTS) by mitochondrial [dehydrogenase](#) in living cells. All MTS assays were conducted according to the manufacturer's protocols. Fibroblasts were seeded at a density of $4 \times 10^3/\text{cm}^2$ in 96-well plates and subsequently treated with nitrofurantoin for 24 h post seeding. Nitrofurantoin treatments were prepared by first dissolving the drug in 100 % (v/v) DMSO and then serially diluting to the desired nitrofurantoin concentration (3.5 to 600 $\mu\text{g}/\text{mL}$) using [whole cell](#) culture media. The cells were treated with nitrofurantoin for 72 h then washed using [PBS](#) before MTS reagent was applied to each well for 2 h. The absorbance of each well was measured at 490 nm using a SpectraMax M5 spectrophotometer (Molecular Devices LLC, San Jose, California) and compared against the absorbance value of cells treated with a vehicle control to determine the percentage [cell viability](#) of each treatment group. Nitrofurantoin treatments contained a maximum of 1 % DMSO to avoid DMSO cytotoxicity negatively impacting cell viability. A vehicle control was prepared alongside a control group to validate that DMSO did not cause cytotoxicity. A lower cell density was necessary for the MTS assay compared to the [ELISA](#) and ECM ELISA assays to ensure that the absorbance of each well remained within the range of detection.

2.4. Live-cell microscopy

A Nikon Eclipse Ti inverted microscope (Nikon, Tokyo, Japan) was used to acquire time-lapses of PHLF after treatment with nitrofurantoin. The microscope was equipped with an S Plan Fluor ELWD 10X Ph2 ADM objective (Nikon, Tokyo, Japan), CoolSnap ES2 camera, CO₂ controller and TCH 882-G-COM Controller (Clear State Solutions, Melbourne, Victoria, Australia). Cells were seeded in 48 well plates (Corning Costar) at a density of $3.5 \times 10^3 \text{ cells/cm}^2$ and treated with 2, 10 or 20 $\mu\text{g}/\text{mL}$ of nitrofurantoin 24 h after seeding (standard treatment group) or 48 h after seeding (delayed treatment group). A time-lapse was captured through [phase contrast microscopy](#) by acquiring images in 15-minute intervals across a 24-hour time-period. Fibroblasts were incubated at 37 °C and 5 % CO₂ throughout the time-lapse acquisition. A deep learning model was trained using data gathered in these time-lapses to identify fibroblasts ([Fig. 1](#)) through the 'human-in-the-loop' training method using Cellpose [\[23\]](#), [\[24\]](#), an open-source machine learning program. The resulting fibroblast detection model was then used with the ImageJ plugin, LIMTracker [\[25\]](#), to detect and track fibroblasts throughout the time-lapse then to generate data on cell morphology and migration. The fibroblast detection model was developed from 40 images each containing approximately 20 regions of interest (ROIs; 800 total). These ROIs were used to generate masks and then analysed using a batch size of 1 with 500 epochs to train the fibroblast detection model. A minimum of $n > 50$ cells were tracked for each treatment group with cells that were detected for less than 10 consecutive frames or cells that were less than 1500 um^2 being excluded from analysis to eliminate erroneous fibroblast detection.

Fibroblast cell shape was analysed to determine roundness using the formula: $R = 4 \times \text{Area} \pi \times \text{MajorAxis}^2$. This formula produces a number between 0 and 1, where 0 represents a highly non-circular shape and 1 represents a perfect circle.

2.5. Assessment of cytokine concentrations using ELISA

PHLF and WI-38 cells were seeded in 6-well plates (Corning Costar) at a density of $2.7 \times 10^4 \text{ cells/cm}^2$ and subsequently treated with 2, 10 or 20 $\mu\text{g}/\text{mL}$ of nitrofurantoin 24 h post seeding (termed as standard treatment group) or 48 h post seeding (delayed treatment group). Supernatant was collected from all treatment groups 96 h after seeding (72 h or 48 h post treatment)

and the concentrations of interleukin-6 (IL-6) and interleukin (IL-8) were determined using enzyme-linked immunosorbent assay (ELISA) kits (BD OptEIA, BD Biosciences, Franklin Lakes, New Jersey, USA) according to the manufacturer instructions.

2.6. ECM ELISA

PHLF and WI-38 cells were seeded in 96-well plates (Corning Costar) at a density of 2.7×10^4 cells/cm² and then treated with 2, 10 or 20 µg/mL of nitrofurantoin 24 h after seeding (standard treatment group) or 48 h after seeding (delayed treatment group). The fibroblasts were washed with phosphate buffered saline (PBS) 96 h after seeding and further treated with 16 mM NH₄OH for 30 mins to remove all fibroblasts while preserving the adherent ECM produced by the fibroblasts. The decellularised ECM underwent further [PBS](#) washing and was blocked with 0.1 % [bovine serum albumin](#) (BSA; w/v; Sigma-Aldrich) to prevent non-specific binding. The blocked ECM was then treated with the following antibodies for 2 h: monoclonal mouse anti-collagen-IV primary antibody (diluted 1:1000; Sigma-Aldrich), monoclonal mouse anti-fibronectin primary antibody (diluted 1:4000; Invitrogen), monoclonal mouse anti-periostin primary antibody (diluted 1:2000, BD Biosciences), monoclonal mouse anti-perlecan primary antibody (diluted 1:2000; Invitrogen), monoclonal mouse anti-tenascin-C (diluted 1:10000; Sigma-Aldrich) and mouse IgG isotype (diluted 1:1000, BD Biosciences). The ECM was then treated with an HRP-linked anti-mouse secondary antibody (diluted 1:2000; [Cell Signaling](#) Technology, Danvers, Massachusetts, USA) followed by TMB substrate (Thermofisher, Sydney, NSW, Australia). Additionally, ECM was treated with mouse IgG isotype to test for false positive results. Finally, 1 M H₃PO₄ was applied to each well to halt the reaction and the absorbance of each well was measured using a SpectraMax M2 plate reader (Molecular Devices) at 450 nm, with plate correction at 570 nm. Expression of each ECM protein (collagen-IV, [fibronectin](#), [periostin](#), [perlecan](#) and Tenascin-C) was determined by comparing absorbance of each treatment group against the untreated control group for each cell line.

2.7. Statistical analysis

All results are presented as the mean ± the standard error of the mean (SEM) of three biological replicates for WI-38 results and five biological replicates ($n = 5$) for PHLF results. Statistical significance was determined using the software GraphPad Prism (version 8.2.1, San Diego, California, USA) via one-way ANOVA with Tukey's multiple comparison post-test or student's unpaired *t*-test with a threshold of significance of $p < 0.05$.

3. Results & discussion

The drug nitrofurantoin could have potential as a novel avenue of treatment for emphysema by [repurposing](#) the drug's pulmonary fibrosis adverse effect to counteract the loss of lung ECM during emphysematous progression. Nitrofurantoin was primarily chosen for this study due to its fibrotic adverse effect, however, the antibiotic nature of the drug could also assist in treating [respiratory infections](#), a frequent yet serious complication of emphysema [13], [26]. This study examined the effects of nitrofurantoin treatment on primary human lung fibroblasts derived from emphysema patients to determine the potential of this drug as a novel form of treatment for emphysema. Nitrofurantoin cellular toxicity and the influence of the drug on emphysematous fibroblast [cellular processes](#) relating to ECM maintenance, including cytokine expression (IL-6 and IL-8), [cellular motility](#), morphology, and ECM protein deposition were investigated to determine any changes induced by nitrofurantoin.

All fibroblasts in this study were seeded on cell culture plastic that had been coated with collagen-1 (10 µg/cm²; Sigma-Aldrich) to improve [cell adherence](#) and better replicate the ECM of the lung

parenchyma [27]. Furthermore, fibroblasts were treated with both 2 ng/mL of TNF- α (InVitro Technologies) and 2 ng/mL of TGF- β (BioLegend) in all studies to stimulate ECM protein deposition and to simulate aspects of the emphysematous lung inflammatory environment [28], [29], [30], [31]. A high seeding density was used in the [ELISA](#) and ECM ELISA assays to present sufficient ECM deposition to be within the range of analysis of ECM ELISA.

3.1. Nitrofurantoin toxicity

3.1.1. MTS assay

The toxicity of nitrofurantoin on healthy and emphysematous fibroblasts was first assessed using an MTS assay ([Fig. 2](#)). Both healthy and emphysematous fibroblasts were treated with nitrofurantoin across a broad range of concentrations 24 h after seeding, and MTS reagent was added for a further 72 h to assess changes in cell viability 24 h post [drug treatment](#). The vehicle control used in this experiment was not found to lower cell viability in comparison to untreated fibroblasts (data not shown). The range of concentrations examined by MTS was limited by the poor aqueous solubility of the drug (0.079 mg/mL at room temperature [32]). As a result, WI-38 fibroblasts were treated with nitrofurantoin concentrations ranging from 3.4 μ g/mL to 300 μ g/mL ([Fig. 2A](#)). Emphysematous fibroblasts were treated with nitrofurantoin concentrations ranging from 6.9 μ g/mL to 600 μ g/mL ([Fig. 2B](#)). The IC₅₀ and IC₈₀ were calculated by fitting a sigmoidal curve through non-linear regression. Nitrofurantoin was found to have an IC₅₀ of $78.1 \pm 2.9 \mu\text{g/mL}$ and an IC₈₀ of $22.5 \pm 5.2 \mu\text{g/mL}$ when applied to WI-38 fibroblasts ([Fig. 2A](#)), and an IC₅₀ of $64.8 \pm 0.6 \mu\text{g/mL}$ and an IC₈₀ of $19.6 \pm 2.3 \mu\text{g/mL}$ in case of primary emphysematous fibroblasts ([Fig. 2B](#)). These results are similar to previous research by Michiels et al, that determined the IC₅₀ of WI-38 cells treated with nitrofurantoin to be $12.1 \mu\text{g/mL}$ [33]. The difference in IC₅₀ results is most likely due to differences in nitrofurantoin [incubation time](#) or WI-38 [confluence](#) as the Michiels et al. study measured cell viability after 5 days of nitrofurantoin exposure, whereas this study measured cell viability after only 3 days post treatment.

Notably, the emphysematous fibroblast nitrofurantoin dose-response curve ([Fig. 2B](#)) had a lower Hill slope value (-1.16 ± 0.1) than that of the healthy fibroblasts in ([Fig. 2A](#); -1.12 ± 0.1), yet cell viability of the diseased primary fibroblasts was reduced at lower nitrofurantoin concentrations than healthy fibroblasts. A significant difference in IC₅₀ values was observed between WI-38 and emphysematous fibroblast cells ($78.1 \pm 1.7 \mu\text{g/mL}$ and $64.8 \pm 0.4 \mu\text{g/mL}$ respectively, [Table 1](#)), suggesting that the PHLF were more sensitive to nitrofurantoin toxicity than the healthy fibroblasts, however, the absolute difference in IC₅₀ of the healthy and PHLF cells remains similar. Furthermore, no significant differences in IC₈₀ values were found between WI-38 and emphysematous fibroblast cells ([Table 1](#)). The 3 concentrations/doses chosen for this study, 2, 10 and 20 $\mu\text{g/mL}$, were selected based on the IC₈₀ values of the two cell groups. Notably, nitrofurantoin cytotoxicity has previously been reduced through [pharmaceutical formulation](#) techniques such as spray drying [34] and would most likely also be reduced by nanoparticle-based formulations or polymeric [drug delivery systems](#).

Table 1. Comparison of IC₅₀, IC₈₀ and Hill slope values for the nitrofurantoin dose-response curves of healthy and emphysematous fibroblasts. (Unpaired Student *t*-test, Mean \pm SEM, *n* = 3, * *p* < 0.05).

Empty Cell	Healthy (WI-38)	Emphysematous Fibroblasts (PHLF)	<i>p</i> value
IC ₅₀ ($\mu\text{g/mL}$)	78.1 ± 1.7	64.8 ± 0.4	0.012*

Empty Cell	Healthy (WI-38)	Emphysematous Fibroblasts (PHLF)	p value
IC80 (µg/mL)	22.5 ± 3.0	19.6 ± 1.6	0.730
Hill slope	-1.12 ± 0.1	-1.16 ± 0.1	0.458

3.1.2. ELISA

The pro-inflammatory cytokine IL-6 and pleiotropic cytokine IL-8 were quantified using ELISA to examine the [cytokine release](#) profile of nitrofurantoin treated fibroblasts, as both emphysema and drug induced pulmonary fibrosis are closely linked to inflammation and irritation within the lung [35]. These specific cytokines were chosen for analysis due to the central roles they play in the inflammatory response. IL-6 is primarily pro-inflammatory and highly important to [biological processes](#) related to emphysematous progression, such as [chronic inflammation](#), [wound healing](#), the initiation of inflammatory responses and immune recruitment [36], [37], [38], [39]. Similarly, IL-8 is another major mediator of inflammatory responses and displays both pro and anti-inflammatory activity. Additionally, IL-8 is involved in the immune response by acting as a [neutrophil](#) chemotactic agent and encourages [neutrophil infiltration](#) [26], [40], [41]. IL-6 and IL-8 expression provides an understanding of the lung fibroblasts inflammatory response induced by nitrofurantoin treatment. Fibroblasts were treated with 2, 10 or 20 µg/mL of nitrofurantoin 24 h post seeding (described as immediate treatment), to simulate a preventative nitrofurantoin treatment. Alternatively, drug treatment occurred 48 h post seeding (described as delayed treatment) to simulate treating established emphysematous fibroblasts, such as what might be found in a more advanced case of emphysema. The supernatant of each treatment group was collected for analysis 3 days after nitrofurantoin application for the immediate treatment group (96 h after seeding) and 2 days after nitrofurantoin application for the delayed treatment group (96 h after seeding). This end point was chosen to allow cytokine expression ([Fig. 3](#)) and protein quantification by ECM ELISA ([Fig. 7](#)) to be measured at identical time points, as ECM ELISA requires a three-day incubation after treatment to detect a quantifiable difference in ECM protein abundance [34].

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Fig. 3. Changes in (A) IL-6 & (B) IL-8 production by primary emphysematous fibroblasts when seeded on a coating of collagen-1 and treated with TNF-α, TGF-β and left untreated (CTRL) or treated with varying concentrations of [nitrofurantoin](#). Data is represented as % change in IL-6/IL-8 expression with 100 % being the average IL-6/IL-8 expression of untreated (control) primary emphysematous fibroblasts. Data is displayed as mean ± SEM, n = 5 for all treatment groups with each cell line represented as a point. Statistical analysis was determined by one-way ANOVA with Tukey's multiple comparison post-test.

WI-38 (healthy) fibroblast IL-6 expression was not found to be significantly altered by the application of any nitrofurantoin concentration in the immediate or the delayed treatment groups. Mean IL-6 and IL-8 expression was not changed by nitrofurantoin treatment, and cytokine expression was found to be not statistically significant due to the wide variability in PHLF cytokine expression ([Fig. 3](#)).

The changes in cytokine expression after nitrofurantoin treatment were found to be neither significant, nor dose dependent, showing that nitrofurantoin treatment does not provoke an inflammatory response in lung fibroblasts.

3.2. Low concentration nitrofurantoin treatment increases fibroblast motility

Emphysematous fibroblast chemokinesis was examined over a 24 h time-lapse to investigate if nitrofurantoin treatment influenced the cellular migration speed of healthy and diseased fibroblasts. Migration is crucial to fibroblast maintenance of the lung ECM as the cells must frequently travel to sites of parenchymal damage through chemotaxis to commence ECM repair and maintenance [27], [30]. Notably, increased motility is a strong indication of fibroblast activation and therefore, upregulated ECM repair [42], [43]. The migration speed ($\mu\text{m}/\text{min}$) and mean squared displacement (MSD; μm^2) of both healthy and emphysematous fibroblasts were calculated from the tracked paths of individual fibroblasts over a 24 h time-lapse (Fig. 4).

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Fig. 4. Nitrofurantoin treatment raises the motility of primary emphysematous fibroblasts in a non-dose dependent manner. WI-38 and primary emphysematous cell lines were tracked over a 24 h period using phase-contrast microscopy after varying concentrations of nitrofurantoin treatment to examine changes in fibroblast motility. (A) Collated fibroblast migration speed ($\mu\text{m}/\text{min}$) is displayed as mean \pm SEM (displayed as red bars), $n > 50$ for WI-38 cells and $n > 250$ for all primary cells ($n > 50$ from each cell line), (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). Statistical analysis was performed using one-way ANOVA with Tukey's multiple comparison post-test. The mean squared displacement (μm^2) of WI-38 and primary emphysematous cell lines was calculated from cell trajectories after a 24 h time-lapse (1440 min). Data is displayed as mean \pm SEM, $n > 250$ for all treatment groups excluding CTRL (WI-38 cells) which had $n > 50$. (B) MSD of WI-38 and primary emphysematous cell lines when treated with nitrofurantoin 24 h after seeding (immediate treatment). (C) MSD of WI-38 and primary emphysematous cell lines when treated with nitrofurantoin 48 h after seeding (delayed treatment). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The mean migration speed of the WI-38 (healthy) fibroblasts was not found to be significantly different to that of the untreated primary emphysematous fibroblasts (Fig. 4A). However, emphysematous fibroblasts treated with 2 $\mu\text{g}/\text{mL}$ of nitrofurantoin (immediate treatment) displayed significantly increased migration speed compared to both WI-38 (2 $\mu\text{g}/\text{mL}$ immediate treatment: $0.686 \pm 0.02 \mu\text{m}/\text{min}$ vs. WI-38: $0.617 \pm 0.02 \mu\text{m}/\text{min}$; $p = 0.041$) and control emphysematous fibroblasts ($0.613 \pm 0.01 \mu\text{m}/\text{min}$; $p = 0.0014$). This nitrofurantoin induced migration speed upregulation could suggest that the fibroblasts have entered an activated phenotype and are therefore primed to repair ECM damage. Although the (likely multifactorial [44]) molecular mechanisms used by nitrofurantoin to alter fibroblast activity remain poorly understood, nitrofurantoin treatment may activate [signalling pathways](#) that induce fibroblast activation, such as the TGF- β /SMAD or NF- κ B pathways, thus stimulating ECM repair [45], [46]. Notably, nitrofurantoin interacts with [estrogen receptor \$\alpha\$](#) (ER α) [47], potentially stimulating oestrogen signalling pathway activity and thereby influencing the NF- κ B pathway [48].

The mean migration speed of fibroblasts immediately treated with 10 µg/mL of nitrofurantoin was not significantly different from the control group ($0.661 \pm 0.02 \mu\text{m}/\text{min}$ vs.

$0.613 \pm 0.01 \mu\text{m}/\text{min}$; $p = 0.242$) nor from the speed of fibroblasts immediately treated with 2 µg/mL ($0.661 \pm 0.02 \mu\text{m}/\text{min}$ vs. $0.686 \pm 0.02 \mu\text{m}/\text{min}$; $p = 0.91$). However, the mean migration speed of fibroblasts immediately treated with 20 µg/mL was significantly lower than that of the immediate 2 µg/mL treatment group ($0.602 \pm 0.02 \mu\text{g}/\text{mL}$ vs. $0.686 \pm 0.02 \mu\text{g}/\text{mL}$; $p = 0.0009$), indicating that the stimulatory effects of nitrofurantoin on fibroblast motility were not dose dependent.

Furthermore, the mean squared displacement values calculated from the cell tracking ([Fig. 4B](#)) supports the findings of [Fig. 4A](#), as the 2 µg/mL immediate nitrofurantoin treatment group was also found to have higher MSD compared to both the WI-38 fibroblasts and untreated emphysematous fibroblasts. Likewise, the MSD of the delayed nitrofurantoin treatment groups ([Fig. 4C](#)) supports the migration speed findings in [Fig. 4A](#), as all delayed treatment groups displayed lower MSD than the healthy fibroblasts and untreated fibroblasts control group.

The reduction in migration speed after the immediate 20 µg/mL nitrofurantoin treatment could be related to the toxicity of the drug, as the concentration is approaching the upper limit of the toxicity curve ([Fig. 2B](#)). The proximity of the PHLF nitrofurantoin IC₈₀ ($19.6 \pm 1.6 \mu\text{g}/\text{mL}$) and the 20 µg/mL treatment groups concentration could indicate that the 20 µg/mL nitrofurantoin treatment caused reduced motility due to cellular stress. The toxicity of nitrofurantoin potentially reversed the upregulated migration seen in the 2 µg/mL treatment group and thus, may have caused the effect to not be dose dependent. This [drug toxicity](#) suppressing fibroblast migration speed effect is also present in the 10 µg/mL treatment group, resulting in fibroblast migration speed that is neither significantly lower than the 2 µg/mL immediate treatment group, nor significantly higher than the untreated control group.

The influence of nitrofurantoin treatment on fibroblast motility was far less pronounced in the delayed treatment groups, as none were found to be significantly different from either the healthy control or the untreated emphysematous cells ([Fig. 4A](#)). The ineffectiveness of the delayed nitrofurantoin treatment could suggest that the influence of nitrofurantoin on cell motility had not yet taken effect. Notably, the delayed 20 µg/mL treatment group migration speed was significantly reduced compared to the 2 µg/mL immediate treatment group ($0.590 \pm 0.01 \mu\text{m}/\text{min}$ vs. $0.686 \pm 0.02 \mu\text{m}/\text{min}$; $p < 0.0001$). This significant decrease most likely suggests that although the migration speed of the delayed 2 µg/mL treatment group had not yet taken effect, the cytotoxicity of the delayed 20 µg/mL treatment group had reduced fibroblast migration speed. The altered migration speed of nitrofurantoin treated fibroblasts indicates that nitrofurantoin was most effective after immediate treatment, promoting migration when applied to fibroblasts at relatively low doses such as 2 µg/mL and. The increased migration speed of nitrofurantoin treated fibroblasts allows for more efficient ECM repair and could be caused by triggering an activated phenotype in fibroblasts [\[30\]](#).

The tracked paths taken by emphysematous cells were normalised to (0,0 µm) to visually demonstrate the impact of nitrofurantoin treatment on fibroblast motility via Wind-Rose plots ([Fig. 5](#)). [Fig. 5A](#) demonstrates that the paths taken by untreated emphysematous [fibroblast cell lines](#) appear to cluster around the point of origin (0,0 µm), whereas [Fig. 5B-D](#) show that fibroblasts appear to migrate farther from the site of origin after nitrofurantoin treatment. The Wind-Rose plots displaying the migration of fibroblasts after delayed treatment display similar migration to that of the untreated control cells ([Fig. 5E, F](#)).

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Fig. 5. Fibroblast displacement was raised after nitrofurantoin treatment. Windrose plots depicting the paths taken by emphysematous fibroblasts over a 24 h time-lapse normalised such that each path begins at the point (0,0 μm), $n > 50$. Fibroblasts were (A) left untreated, subjected to (B) 2 $\mu\text{g}/\text{mL}$, (C) 10 $\mu\text{g}/\text{mL}$, or (D) 20 $\mu\text{g}/\text{mL}$ of nitrofurantoin. Additionally, the fibroblasts were also examined after delayed treatment using (E) 2 $\mu\text{g}/\text{mL}$, (F) 20 $\mu\text{g}/\text{mL}$ or (G) 20 $\mu\text{g}/\text{mL}$ of nitrofurantoin.

The increase in fibroblast migration after nitrofurantoin treatment could suggest that fibroblasts are activated to begin ECM repair after 2 $\mu\text{g}/\text{mL}$ nitrofurantoin treatment. Notably, fibroblasts that have entered a pro-ECM repair phenotype typically display alterations to their behaviour beyond increased motility such as increased proliferation and shifts in cellular morphology, as fibroblasts' actin [cytoskeletons](#) undergo conformational changes [43], [49], [50], [51]. Cellular motility and morphology are closely linked as both are primarily governed by the actin cytoskeleton through conformational shifts that facilitate fibroblast functions such as migration or ECM deposition [52].

3.3. Nitrofurantoin treatment alters fibroblast morphology by increasing cell area and lowering roundness

The mean cell area (μm^2) and roundness of WI-38 and PHLF over a 24 h period was calculated to quantify changes in fibroblast morphology (Fig. 6A). The mean two-dimensional cell area of healthy fibroblasts was found to be significantly upregulated compared against untreated emphysematous cells ($3590 \pm 167.5 \mu\text{m}^2$ vs. $2926 \pm 75.7 \mu\text{m}^2$; $p = 0.0045$), however, nitrofurantoin treatment was shown to significantly upregulate cell area in all treatment groups, excluding delayed 2 $\mu\text{g}/\text{mL}$ treated fibroblasts (Fig. 6A). Fibroblast cell area did not respond to nitrofurantoin treatment in a dose dependent manner, as observed in the cell motility results (Fig. 5). However, unlike the non-dose dependent changes in migration speed, all fibroblasts displayed an approximately uniform increase in [cell size](#) (between 3518 ± 115.6 and $3863 \pm 127.8 \mu\text{m}^2$), except the delayed 2 $\mu\text{g}/\text{mL}$ treated fibroblasts ($3046 \pm 89.7 \mu\text{m}^2$). Interestingly, the elevated cell size of nitrofurantoin treated emphysematous fibroblasts was comparable to the size of healthy cells, suggesting that the nitrofurantoin treatment could be correcting aspects of diseased fibroblast behaviour. The increase in cell size is potentially priming the diseased fibroblasts to behave similarly to healthy fibroblasts, or alternatively, the drug may induce a proto-myofibroblastic or myofibroblastic phenotype that upregulates ECM protein deposition [49]. A study by Uhal et al. [53] that divided fibroblasts into a small, medium, and large populations based on cell size found that medium size fibroblasts had the greatest percentage of cells in the synthesis (S) and gap 2 (G2) phases of the cell cycle, where the cell prepares for mitosis, and therefore the greatest proliferation. Furthermore, only the large fibroblast population displayed α -smooth muscle actin (α -SMA), a marker of [myofibroblast](#) differentiation/fibroblast activation [53]. This study indicates that the nitrofurantoin induced upregulation in cell size likely encourages proliferative and activated fibroblast phenotypes by increasing the proportion of fibroblasts that possess 'large' or 'medium' phenotypes. Additionally, the results of Fig. 4A did not align with the changes in cell migration seen in Fig. 6A, as the 20 $\mu\text{g}/\text{mL}$ treatments failed to increase migration speed yet displayed the same increase in cell size as immediate 2 $\mu\text{g}/\text{mL}$ or 10 $\mu\text{g}/\text{mL}$ nitrofurantoin treatment. Although the cause of this discrepancy remains unclear, the differential response is potentially linked to cell size. For example, increased migration speed due to nitrofurantoin may only occur after cell size has increased, therefore the higher toxicity of the 20 $\mu\text{g}/\text{mL}$ immediate and delayed treatments may have caused the increase in

migration speed to be slowed due to cell stress. Furthermore, the increased migration speed of the 20 µg/mL treatment groups may become more apparent if given a longer [incubation time](#).

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Fig. 6. Nitrofurantoin treatment alters fibroblast morphology by increasing cell area and decreasing roundness. Fibroblasts were tracked to determine the average 2-dimensional cell area and roundness of primary emphysematous fibroblasts over a 24 h time-lapse after nitrofurantoin treatment. (A) Mean cell area of fibroblasts was presented as mean ± SEM (displayed as red bars). (B) Mean roundness of fibroblasts was presented as mean ± SEM (displayed as red bars). $n > 50$ for WI-38 cells and $n > 250$ for all primary cells ($n > 50$ from each cell line), (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). Statistical analysis was performed using one-way ANOVA with Tukey's multiple comparison post-test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Fig. 7. Nitrofurantoin treatment reduced the expression of tenascin-C, [periostin](#) and [perlecan](#) while raising [fibronectin](#) expression. The percentage change in deposition of the [ECM proteins](#) (A) collagen-4, (B) [perlecan](#), (C) [fibronectin](#), (D) tenascin-C, (E) [periostin](#) by primary emphysematous fibroblasts after nitrofurantoin treatment. Data is represented as % change in [ECM protein](#) expression where 100 % represents the average [protein expression](#) of untreated primary emphysematous fibroblasts. Data is displayed as mean ± SEM, $n = 5$ for all treatments with each cell line represented as a point, * Indicates a treatment group is significantly different from control (100 % [ECM protein](#) expression; * $p < 0.05$). Statistical analysis was determined by one-way ANOVA with Tukey's multiple comparison post-test.

Changes in fibroblast cell size due to nitrofurantoin treatment were mirrored in the fibroblast mean roundness (Fig. 6B) as WI-38 fibroblasts had significantly lower roundness when compared against untreated emphysematous fibroblasts (0.18 ± 0.004 vs. 0.22 ± 0.004 ; $p < 0.0001$). Furthermore, all fibroblasts treated with nitrofurantoin displayed significantly lower roundness compared to untreated diseased cells. As with cell size (Fig. 6A), the decreased mean roundness of nitrofurantoin treated cells resulted in diseased cells of comparable morphology to healthy fibroblasts, in terms of roundness. Interestingly, the immediate treatment 2 µg/mL fibroblasts and delayed treatment 2 µg/mL fibroblasts displayed a significant difference as the delayed treatment group displayed higher roundness than the standard treatment group (0.18 ± 0.003 vs. 0.20 ± 0.003 ; $p = 0.042$). This difference was likely due to the low dosage of the delayed 2 µg/mL treatment group requiring a longer incubation time to alter fibroblast roundness. As was observed in the motility and cell area results (Fig. 5A, 6A), no dose dependency was found in the mean roundness of nitrofurantoin treated fibroblasts. A high roundness value alongside a lower cell area can be associated with a quiescent, deactivated fibroblast phenotype [54], therefore, the decreased roundness of fibroblasts after nitrofurantoin treatment supports the cell motility (Fig. 4A) results by further indicating that nitrofurantoin stimulates fibroblasts to enter an activated, potentially myofibroblastic phenotype. Notably, nitrofurantoin could exert a corrective effect on emphysematous fibroblasts morphology by

restoring the cell size and roundness to that of a healthy fibroblast. Correcting fibroblast morphology indicates that the cellular cytoskeleton has been altered, potentially facilitating ECM repair by improving the ECM homeostatic capability of the diseased cells after treatment. Together, the changes to both cell two-dimensional area and roundness support the notion that the nitrofurantoin could be inducing an activated phenotype in emphysematous fibroblasts.

3.4. Emphysematous fibroblast ECM protein deposition largely unaffected by nitrofurantoin treatment

Relative ECM protein deposition was quantified using ECM ELISA 72 h after immediate nitrofurantoin application and 48 h after delayed nitrofurantoin application to examine the effect of treatment on emphysematous fibroblast ECM protein deposition. Cells were seeded on a collagen-1 coating to improve cell adherence and to promote fibroblast function [55], and additionally, all fibroblasts were stimulated with both TNF- α and TGF- β at 2 ng/mL to further induce ECM deposition. All data is displayed as the relative percentage change in ECM protein deposition compared against the ECM protein deposition of untreated diseased fibroblasts. The [ECM proteins](#) collagen-IV, perlecan, tenascin-C, periostin and fibronectin were selected for analysis as they perform a range of functions within the lung ECM. For example, collagen-IV and fibronectin act as structural proteins, perlecan and periostin act as signalling proteins, whereas tenascin-C, fibronectin and periostin all influence dynamic cellular activity, such as adhesion and proliferation [56], [57], [58], [59].

Collagen-IV deposition was found to be unaffected by nitrofurantoin treatment regardless of treatment group, despite the extreme variability in collagen-IV expression for the immediate and delayed 20 μ g/mL treatment groups ([Fig. 7A](#)). The wide range of collagen-IV expression from the two 20 μ g/mL treatment groups is most likely caused by the inherent variability of primary cells. Furthermore, nitrofurantoin treatment did not cause a significant difference in collagen-IV treatment when compared against the collagen-IV expression of untreated cells. Perlecan expression remained consistent regardless of nitrofurantoin dosage or treatment method ([Fig. 7B](#)), except for immediate 20 μ g/mL nitrofurantoin treatment inducing a reduction in perlecan expression compared to untreated fibroblasts, potentially due to the cytotoxicity of nitrofurantoin at a concentration of 20 μ g/mL. However, the 20 μ g/mL delayed treatment group did not significantly lower the emphysematous fibroblast perlecan expression. Likewise, nitrofurantoin produced a similar pattern of tenascin-C expression as the [ECM protein expression](#) was unchanged by all treatment groups, excluding immediate 20 μ g/mL treatment which was shown to significantly lower tenascin-C expression ([Fig. 7C](#)). Periostin expression was again similar to that of tenascin-C and perlecan, as all 2 and 10 μ g/mL nitrofurantoin treatment groups did not significantly alter periostin production. However, both immediate and delayed 20 μ g/mL nitrofurantoin treatment groups significantly reduced periostin expression ([Fig. 7D](#)).

Unlike other [ECM proteins](#) examined in this study, the expression of fibronectin was significantly increased by all delayed nitrofurantoin treatment groups and by the 20 μ g/mL immediate treatment group ([Fig. 7E](#)). Fibronectin plays an essential role in wound repair and is a relatively ubiquitous protein throughout the body's ECM [60], [61]. Notably, lung fibronectin volume is known to increase during emphysema, however, this only occurs in the small airways of the lung, whereas the parenchyma and large airways do not display altered ECM composition in terms of fibronectin [62]. The increase in fibronectin deposition is highly important to ECM repair as fibronectin is linked to the expression of pro-fibrotic genes that could further support ECM repair activity in fibroblasts [63]. The increased production of fibronectin may indicate that nitrofurantoin indirectly causes pulmonary fibrosis through fibroblast-fibronectin interactions that alter fibroblast dynamic cellular activity by

upregulating ECM repair. Nitrofurantoin treatment was only found to alter ECM protein expressions when treated with 20 µg/mL, except for fibronectin.

The consistent pattern of significantly reductions in ECM protein deposition only after immediate or delayed 20 µg/mL nitrofurantoin treatment is most likely indicative of a reduction in ECM protein deposition due to cell stress caused by nitrofurantoin cytotoxicity. A reduction in cell viability due to the low nitrofurantoin IC₈₀ of PHLF cells is more likely to have limited ECM protein production than a downregulation in ECM protein expression after nitrofurantoin treatment. Moreover, the unchanged collagen-IV, periostin, perlecan and tenascin-C expression raises further questions about the mechanism used by nitrofurantoin to cause lung fibrosis in patients, as fibroblasts in a fibrotic lung would be expected to have upregulated deposition of a range of ECM proteins. These ECM ELISA results differ from the migration and morphology findings ([Fig. 4](#), [Fig. 6](#)) that suggest nitrofurantoin treatment induces an activated fibroblast phenotype that consequently stimulates ECM repair; however, the ECM ELISA results did not show that nitrofurantoin treatment would alter ECM maintenance in the emphysematous lung. Nitrofurantoin induced lung fibrosis may require a longer nitrofurantoin incubation to be modelled, as lung fibrosis is typically found in patients that are taking the drug for extended periods of time as prophylactic [\[20\]](#), [\[21\]](#). Furthermore, nitrofurantoin induced pulmonary fibrosis may be the result of complex interactions between multiple lung cell populations, rather than just lung fibroblasts, and would therefore require more complex co-culture-based models of the lung to be replicated *in vitro*. Although the ECM ELISA performed for this study did not find an increase in collagen-IV, perlecan, periostin or tenascin-C production, nitrofurantoin may primarily upregulate the production of other ECM proteins such as fibronectin and others that were not examined in this study. Alternatively, nitrofurantoin could upregulate fibroblast organisation of the ECM by stimulating behaviours that raise ECM stiffness without altering ECM protein deposition, such as ECM [protein crosslinking](#) [\[64\]](#).

4. Conclusion

The results suggest that nitrofurantoin holds promise as an innovative treatment for emphysema; however, its potential is impeded by associated toxicity. Additionally, the mechanism through which nitrofurantoin promotes extracellular matrix (ECM) production in fibroblasts remains unclear, posing limitations to its therapeutic viability. While nitrofurantoin did not demonstrate an increase in overall ECM deposition (excluding fibronectin), it may enhance the organization of emphysematous ECM protein deposition by inducing an activated phenotype in lung fibroblasts. This activation could lead to improved ECM organization without necessarily upregulating ECM deposition, such as through ECM proteins cross-linking. The findings from this research indicate that low-dose nitrofurantoin could serve as a promising treatment for emphysema, provided that the [drug's cytotoxic](#) effects are mitigated, potentially through the exploration of innovative [pharmaceutical formulation](#) strategies. Additional investigations are necessary to explore the impact of a wider spectrum of nitrofurantoin concentrations, duration and dosing schedule on both *in vitro* and *in vivo* models to better understand the impact of nitrofurantoin on fibroblast viability and motility.

Abstract

The escalating adoption of laparoscopic surgical techniques has demonstrated their capacity to yield improved clinical outcomes. However, concomitant with the advantages of this minimally invasive approach, certain adverse complications have been reported. In this report, we present a noteworthy case involving a 72-year-old male patient who underwent laparoscopic inguinal hernia repair. The surgical procedure proceeded without noteworthy complications, and the patient maintained hemodynamic stability throughout. However, the post-anesthetic recovery was compromised by the

onset of subcutaneous emphysema and bilateral tension pneumothorax. Immediate intervention was imperative, prompting the performance of an emergent needle thoracostomy, subsequently followed by the implementation of a closed drainage system within the thoracic cavity. These interventions proved efficacious in mitigating the patient's distressing symptoms. Although pneumothorax complications in the context of laparoscopic surgery are infrequent, it is imperative for anesthetists to remain vigilant regarding the potential occurrence of subcutaneous emphysema and pneumothorax in the perioperative period. This case underscores the significance of meticulous perioperative monitoring and rapid intervention, particularly in laparoscopic procedures, where the insufflation of carbon dioxide into the abdominal cavity can predispose patients to these rare yet potentially life-threatening complications. Heightened awareness among healthcare providers regarding the possibility of such events is pivotal in ensuring the safety and well-being of surgical patients.

1. Introduction

Nowadays, laparoscopic surgical techniques have become widely adopted as a minimally invasive approach for the treatment of inguinal hernia. Compared to conventional open surgery, it offers numerous benefits, including reduced blood loss and faster recovery for patients [1,2]. However, despite its advantages, this surgical method can also lead to complications, such as abdominal visceral injuries, vascular injuries, air embolism, cardiac arrhythmia, and abdominal wall injuries, among others [3, 4, 5, 6]. Among these, pneumothorax is a relatively rare but severe and potentially fatal complication that the anesthesiologists might encounter. Given the potentially fatal nature, it is critical for surgeons and anesthesiologists to be vigilant about close monitoring, prompt recognition and emergency management. In this report, we illustrate a case of subcutaneous emphysema and bilateral pneumothorax following laparoscopic inguinal hernia repair in a patient without any pre-existing pulmonary conditions.

2. Case presentation

A 72-year-old male (weight: 65 kg, height: 172 cm) was scheduled for elective laparoscopic right transabdominal preperitoneal (TAPP) inguinal hernia repair. The patient had an unremarkable medical history with no history of lung pathology. His preoperative chest radiograph (X-ray taken) the day before the surgery revealed possible inflammation in the lower lobe of the right lung (Fig. 1A). Laboratory examinations showed a mildly reduced platelet count ($50 \times 10^9/L$).

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Fig. 1. Perioperative chest anteroposterior radiograph of the patient. Prior to the surgery, the patient's chest radiograph A indicated no evident abnormalities. On the day of the surgery, the postoperative chest radiograph B revealed bilateral pneumothorax accompanied by extensive subcutaneous emphysema in the chest and neck regions.

Upon arrival in the operating room, standard intravenous access was established, and vital signs were monitored. The anesthesiologist initiated general anesthesia by administering midazolam 2 mg, sufentanil 15 µg, propofol 150 mg, and succinylcholine 100 mg intravenously in succession. Following successful mask ventilation, a No. 7.5 endotracheal tube was selected and smoothly inserted for intubation. Mechanical ventilation was set with a tidal volume (VT) of 450 mL, respiratory rate of 14 breaths per minute, inspiratory/expiratory ratio of 1:2 and positive end-expiratory pressure (PEEP) of

5 cm H₂O. Anesthesia was maintained with 1 % sevoflurane, 1 L/min nitrous oxide (N₂O), and propofol at 4 mg/kg/h. During the operation, a trocar was inserted through a 1 cm incision at the upper edge of the umbilicus, and the abdominal cavity was insufflated with CO₂ at a pressure of 13 mmHg to establish pneumoperitoneum. Operating forceps were introduced through incisions at the lateral edges of the rectus abdominis muscles at the umbilical level. The preperitoneal space was accessed and dissected along a transverse incision approximately 6 cm in length above the internal hernia opening. The hernia sac was carefully dissected from the surface of the spermatic cord and retracted into the abdominal cavity. Dissection continued medially along the spermatic cord to expose the direct hernial triangle and the medial pubic pecten ligament. Once the preperitoneal space was adequately prepared, a universal 3D inguinal hernia repair patch was introduced into this space. The peritoneal incision was then sutured continuously with absorbable sutures. Throughout the procedure, the patient's hemodynamics remained stable, and SpO₂ was maintained at 99–100 %. Adjustments were made to maintain end-tidal carbon dioxide partial pressure (PetCO₂) below 40 mmHg and peak inspiratory airway pressure between 19 and 24 cm H₂O.

The entire surgical procedure lasted approximately 1 h. Postoperatively, a minor amount of gas accumulation was observed in the patient's scrotal area. After the patient regained spontaneous respiration, he was transported to the Post-Anesthesia Care Unit (PACU) for further monitoring and removal of the endotracheal tube. Approximately 5 minutes later, the patient developed massive subcutaneous emphysema around the neck and face areas with high bilateral chest wall tension, indicating a possible pneumothorax. Due to the rapid increase in intrathoracic pressure, the patient exhibited retrograde flow even in the peripheral venous infusion. Upon observing these abnormalities, the PACU personnel promptly reported to the senior anesthesiologist. Following the diagnosis of bilateral tension pneumothorax, the anesthesiologist immediately performed chest wall needle decompression and informed the thoracic surgeons to initiate emergency closed drainage of thoracic cavity. Concurrently, bilateral pneumothorax was confirmed via bedside X-ray assessment ([Fig. 1B](#)). After an uneventful and successful surgical procedure, the patient was transferred to the Intensive Care Unit (ICU) for continued management. On postoperative day 7, the patient was discharged from the hospital with an unremarkable recovery, free of any further complications.

3. Discussion

The exact cause of subcutaneous emphysema and pneumothorax following laparoscopic inguinal hernia repair is often uncertain, but several previous cases have proposed that gas may extravasate from the abdominal cavity into the thoracic cavity [[7,8](#)]. Laparoscopic hernia surgery typically includes the totally extraperitoneal (TEP) and TAPP procedures. Upon reviewing case reports related to complications such as subcutaneous emphysema and pneumothorax in laparoscopic hernia surgery ([Table 1](#)), we found that a greater number of complications were associated with the TEP procedure (16 out of 21 cases). This may be attributable to the narrow preperitoneal space and the tendency for CO₂ insufflation in this space, causing dissection along the subcutaneous fascial plane. Studies have demonstrated that the diffusion of extraperitoneal CO₂ into the body is greater than that of intraperitoneal CO₂ [[5,9,10](#)]. However, some studies have indicated that there is no significant difference in the incidence of complications between the TAPP and TEP procedures [[\[11\]](#), [\[12\]](#), [\[13\]](#)]. In our case, the surgical method used was TAPP.

Table 1. Summary of pneumothorax and subcutaneous emphysema cases in laparoscopic hernia repair.

Reference	Age/Sex	Surgery	Technique	N ₂ O	Complication	Time of discovery	Treatment
Omeroglu et al. [33]	31 M	Laparoscopic inguinal hernia repair	TEP	Not mentioned	Bilateral pneumothorax and pneumomediastinum	During extubation	Sporadic resuscitation
Wallace et al. [34]	29 M	Laparoscopic inguinal hernia repair	TEP	Not mentioned	Bilateral subcutaneous emphysema, pneumomediastinum and pneumothorax	Following extubation	Chest drain
Kim et al. [35]	56 M	Laparoscopic inguinal hernia repair	TEP	Not mentioned	Right pneumothorax with subcutaneous emphysema	During surgery (about 50 minutes after CO ₂ insufflation)	Chest drain
Hagopian et al. [10]	Not mentioned	Laparoscopic inguinal hernia repair	TEP	Not mentioned	Unilateral pneumothorax with pneumomediastinum and subcutaneous emphysema	After surgery	Sporadic resuscitation
Ramia et al. [9]	52 M	Laparoscopic inguinal hernia repair	TEP	Not mentioned	Subcutaneous emphysema and pneumomediastinum	Immediately after extubation	Prolonged mechanical ventilation
Ishikawa et al. [36]	65 M	Robotic-assisted inguinal hernia repair	TAPP	Not mentioned	Bilateral pneumothorax	After surgery	Sporadic resuscitation
Ghaffar et al. [37]	2 M	Laparoscopic inguinal hernia repair and orchidopexy	TAPP	Not mentioned	Right pneumothorax	After surgery	Chest drain
Sucandy et al. [38]	48 M	Laparoscopic inguinal hernia repair	TEP	Not mentioned	Right pneumothorax	In the postanesthesia care unit	Sporadic resuscitation

Reference	Age/Sex	Surgery	Technique	N ₂ O	Complication	Time of discovery	Treatment
Bartelmaos et al. [39]	53 M	Laparoscopic inguinal hernia repair	TEP	Yes	Pneumomediastinum and right pneumothorax	In the recovery room	Spon reso
Ferzli et al. [40]	38 M	Laparoscopic inguinal hernia repair	TEP	Not mentioned	Bilateral pneumothorax and pneumomediastinum	At completion of surgery	Spon reso
	40 M	Laparoscopic inguinal hernia repair	TEP	Not mentioned	Massive subcutaneous emphysema, pneumomediastinum and right pneumothorax	During surgery	Spon reso
Aldakhil et al. [41]	28 M	Laparoscopic inguinal hernia repair	Not mentioned	Not mentioned	Subcutaneous emphysema and hypercarbia	During surgery	Spon reso
Schmidt et al. [42]	71 M	Laparoscopic inguinal hernia repair	TAPP	Not mentioned	Subcutaneous emphysema	At the first postoperative day	Spon reso
Benjamin et al. [43]	9-month F	Laparoscopic inguinal hernia repair	Not mentioned	Not mentioned	Subcutaneous emphysema	During recovery from anesthesia	Spon reso
Singh et al. [44]	53 M	Laparoscopic inguinal hernia repair	TEP	Not mentioned	Subcutaneous emphysema and hypercarbia	During surgery	Spon reso
Klopfenstein et al. [45]	59 M	Laparoscopic inguinal hernia repair	TEP	Yes	Subcutaneous emphysema and hypercarbia	During surgery	Spon reso

Reference	Age/Sex	Surgery	Technique	N ₂ O	Complication	Time of discovery	Treatment
Cheng et al. [46]	23 M	Laparoscopic inguinal hernia repair	TEP	No	bilateral pneumothoraces and subcutaneous emphysema	During surgery	chest drain
Nikolaos et al. [47]	73 M	Laparoscopic inguinal hernia repair	TEP	No	Bilateral subcutaneous emphysema and pneumothorax	After surgery	Con
Christopher et al. [48]	44 M	Laparoscopic inguinal hernia repair	TEP	No	Right pneumothorax	During surgery	chest drain
John et al. [49]	25 M	Laparoscopic inguinal hernia repair	TEP	Yes	Left pneumothorax	After surgery	chest drain
Madan et al. [25]	64 M	Laparoscopic inguinal hernia repair	TEP	Not mentioned	Pneumomediastinum.	After surgery	Con

M: Male; F: Female; TEP: Laparoscopic totally extraperitoneal inguinal hernia repair; TAPP: Transabdominal pre-peritoneal; N₂O: Nitrous oxide; CO₂: Carbon dioxide.

Furthermore, upon reviewing our case reports collection, we found that the majority of complications occurred in male patients (19 out of 21 cases), likely due to a higher incidence of

inguinal hernia in men. The larger inguinal canal in men predisposes them to the formation of inguinal hernias. Additionally, male hormones may influence the structure and function of abdominal wall muscles and connective tissues, thereby making inguinal hernias more prevalent in men [14,15]. In a separate study on the incidence of spontaneous pneumothorax by gender, it was found that the incidence in men was significantly higher than in women [16]. This discrepancy may be attributed to the fact that men, on average, are taller than women. The expansion stress at the apex of the lung is greater than in other parts, and taller individuals are more likely to experience bulla rupture due to gravitational effects [17,18]. Moreover, men are more prone to underlying lung diseases, often due to smoking and other habits, which further increases the risk of pneumothorax. Therefore, men may be more susceptible to these complications.

Additionally, 42.8 % (9 out of 21 cases) of these cases involved patients older than 45 years old, suggesting that pneumothorax and subcutaneous emphysema may be related to age-related factors. Although studies have not identified age as a direct risk factor for pneumothorax or subcutaneous emphysema during laparoscopic inguinal hernia repair surgery, the incidence of chronic obstructive pulmonary disease (COPD) and other pulmonary diseases increases with age. These conditions can lead to an imbalance in the pressure inside and outside the alveoli, potentially causing pneumothorax [19,20]. Additionally, during laparoscopic surgery, elderly patients, due to their multiple underlying diseases, prolonged operation times, loose subcutaneous tissue, and fragile connective tissue, are more susceptible to gas accumulation during pneumoperitoneum. This can result in complications such as subcutaneous emphysema and pneumothorax.

The insufflated gas from a pneumoperitoneum can enter the thoracic cavity through different routes, including diaphragmatic hiatuses (aortic, esophageal), congenital defects of the diaphragm, the Bochdalek foramen, or the retroperitoneal space [8,21]. In this case, there were neither signs of accidental diaphragmatic injury nor evidence of congenital defects. Notably, the only abnormality during the perioperative period preceding the subcutaneous emphysema and bilateral pneumothorax was the pneumatosis in the scrotum, which may be due to a failure to evacuate the gas in the hernia capsule. Additionally, the internal spermatic fascia is the continuation of the fascia transversalis, and further continuous with the endothoracic fascia. This allows the free gas to track along fascial planes and into the pleural space and subcutaneous tissue through the anterior gaps of the diaphragm [7,22]. In some cases, accidental damage to blood vessels during surgery may also cause intra-abdominal gas to enter the blood vessels, leading to rare but fatal complications such as gas embolism or pneumothorax [23,24].

In addition to the factors mentioned above, anesthetics are suspected to expand pneumothorax and subcutaneous emphysema. Although no definitive studies have established a direct link between N₂O and these complications, it is considered a potential risk factor for pneumothorax or subcutaneous emphysema in some studies [21,25]. The relatively low solubility in the blood of N₂O makes it a known rapid-onset and clearance inhaled anesthetic [26]. However, the inhalation of N₂O will rapidly diffuse into air-filled cavities, leading to rapid gas expansion and increased pressure in the closed space. As shown in an animal model, inhalation of N₂O for up to 30 minutes can increase the volume of a pneumothorax [27]. In this case, it is likely that the rapid diffusion of N₂O into the pleural space bilaterally, led to further expansion of free air in this space. As a result, the free gas was noted to extend into the tissues of the patient's neck in the PACU. Therefore, the inhalation of N₂O in our case might have been a critical factor contributing to the development of pneumothorax progression and extensive subcutaneous emphysema.

In our case, the patient's complications could be interpreted by one or more factors occurring simultaneously. Prior literatures have identified several other risk factors associated with

pneumothorax, including PetCO₂ exceeding 50 mmHg, operative durations exceeding 200 minutes, and the utilization of six or more operative ports [28]. In this particular case, the surgical intervention successfully maintained the patient's PetCO₂ consistently below 40 mmHg throughout the entire procedure, while also ensuring a relatively brief operative duration of 60 minutes. Both of these metrics stayed within acceptable limits. Furthermore, our patient exhibited no known underlying pulmonary disorders or other predisposing factors.

In this particular case, the presence of pneumothorax in the patient was diagnosed based on the extensive subcutaneous emphysema observed. Nonetheless, during the process of general anesthesia, if meticulous observation is not exercised, the symptoms of subcutaneous emphysema, and even pneumothorax, are highly likely to be masked, thereby potentially leading to delays in diagnosis and treatment. Due to the inherent difficulty in making a diagnosis based solely on clinical symptoms, pneumothorax often necessitates complementary imaging modalities for accurate detection [29]. In the present case, we employed chest X-ray examination to aid in the diagnosis. However, with the increasing prevalence of ultrasonography, chest ultrasonography has been shown to offer superior sensitivity in the diagnosis of pneumothorax compared to traditional X-ray techniques [30,31]. Furthermore, bedside pulmonary ultrasonography can be expeditiously performed, enabling real-time diagnosis of pneumothorax through the identification of four distinct ultrasonographic signs: vanishing pleural sliding, presence of lung points, disappearance of B-lines, and absence of lung pulse [32]. Therefore, it is imperative for clinical physicians to acquire proficiency in this technique through rigorous training and practice.

In clinical practice, anesthesiologists should exercise heightened vigilance during laparoscopic surgery in elderly patients, as this demographic is prone to more comorbidities, extended surgical durations, looser subcutaneous tissue, and more fragile connective tissue, which predispose them to an increased incidence of gas accumulation during pneumoperitoneum. Factors such as the intraoperative surgical approach, damage to the preperitoneal space and adjacent blood vessels, abrupt changes in airway pressure, elevated partial pressure of CO₂, and significant subcutaneous emphysema and pneumatoxisis can all precipitate severe complications. It is imperative that anesthesiologists remain cognizant of these potential issues throughout the surgical process.

4. Conclusion

In summary, although tension pneumothorax is a rare complication of laparoscopic inguinal hernia repair, it has the potential that can lead to fatal outcomes. In the context of critical scenarios, the ability to swiftly identify manifestations suggestive of pneumothorax during perioperative phases and to apply judicious interventions is of considerable importance. This highlights the significance of anesthesiologists prioritizing their attention on upcoming professional responsibilities.

In this review, we discuss physiological principles that guided the management of a lung transplant for emphysema related to alpha-1-antitrypsin deficiency, where a lung allograft to thoracic cavity size mismatch occurred (donor-to-recipient predicted total lung capacity [pTLC] ratio was 0.89, donor pTLC-to-recipient actual-TLC ratio 0.62). In emphysema, the loss of lung elastic recoil and airway obstruction leads to air trapping and lung hyperinflation. Remodeling of the thoracic cavity ("barrel chest") develops, which has implications for donor-to-recipient sizing and postoperative management of lung transplantation. We discuss the physiology of a relatively undersized allograft and the impact on chest tube, mechanical ventilation, and respiratory system mechanics management. This case also illustrates how chronic adaptations of the ventilatory pattern to advanced lung diseases are reversible and the chest cavity size can remodel back to normal after lung transplantation. Background

In this review, we discuss 5 physiological principles (I-V) that guided the management of a lung transplant for severe emphysema related to alpha-1-antitrypsin deficiency. In emphysema, the loss of lung elastic recoil and airway obstruction leads to air trapping and lung hyperinflation. Remodeling of the thoracic cavity ("barrel chest") (I) develops, which has implications for donor-to-recipient sizing and postoperative management of lung transplantation. Collateral ventilation (II) pathways in emphysema can complicate the intraoperative course. During the postoperative period of lung transplantation, numerous changes and adaptations occur in lung and chest cavity physiology. We discuss the physiology of a relatively undersized allograft and the impact on chest tube (III), mechanical ventilation and respiratory system mechanics management. The increased thoracic cavity volume can return to more normal size following lung transplantation (IV). The time course for such a reduction in thoracic cavity size toward normal size takes months. Managing the post-transplant period based on physiological principles can allow for an excellent long-term outcome and our recipient experienced excellent long-term allograft function with supranormal expiratory airflows (V).

Case presentation

A 57-year-old male, 180 cm tall, with alpha-1-antitrypsin deficiency (ZZ-genotype) developed severe emphysema (forced expiratory volume in 1 second [FEV₁]: 17% predicted, diffusion capacity for carbon monoxide: 17% predicted) and lung hyperinflation (TLC: 10.03 liters [141% predicted], functional residual capacity [FRC]: 8.15 liters [197% predicted]) ([Table 1](#)). Computed tomography (CT) imaging of the chest demonstrated severe emphysema at the bases as well as a barrel chest ([Figure 1A](#) and B). He required 8 liters per minute of O₂ at rest and intermittently assisted mechanical ventilation via a mask interface for baseline hypercarbia (partial pressure of carbon dioxide in arterial blood: 63 mm Hg). The patient also exhibited pulmonary hypertension (pulmonary arterial pressure: 70/38 mm Hg).

Table 1. Spirometry and Lung Volumes Over Time

Lung function parameter	Pre-Tx recipient	Donor	D-to-R ratio	6 mo post-Tx	8 mo post-Tx	10 mo post-Tx	12 mo post-Tx
Height (cm)	180	170	0.95				180
FRC (liter)	8.15	3.43 ^a	0.42				3.48
FRC % pred.	197						96
RV (liter)	7.54	1.6 ^a	0.20				2.65
RV % pred.	309						106
RV/TLC	75						23
Actual TLC (liter)	10.03	6.3 ^a	0.62				6.52
pTLC (liter)	7.1	6.3	0.89				7.1
TLC % pred.	141						91

Abbreviations: D, donor; FEF, forced expiratory flow; FEV₁, forced expiratory volume in 1 second; FRC, functional residual capacity; FVC, forced vital capacity; Pred., predicted; pTLC, predicted total lung capacity; R, recipient; RV, residual volume; TLC, total lung capacity; Tx, transplant.

a

Based on recipient-predicted values calculated from regression equations based on sex, age, and height.

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Figure 1. (A, B) Pretransplant computed tomography of the chest. (A) Coronal view showing lower lobe predominant emphysematous changes. (B) Sagittal view showing intact major fissure and lower lobe predominant emphysematous changes. (C) Intraoperative situs following clamshell incision. (D) Left explanted native recipient lung. The explant remained hyperinflated following pneumonectomy and did not fit into a 3.5-liters specimen container. (E) The pleura of the left lower lobe is punctured and immediately following the pleural puncture (F) the explant fully deflated.

A suitable donor for a bilateral lung transplant became available. After clamshell incision, the native lungs protruded out of the chest ([Figure 1C](#)). Pulmonary arterial pressures decreased to 44/20 mm Hg, oxygenation and hemodynamics improved, allowing an off-pump bilateral sequential lung transplant. Following surgical pneumonectomy, the native lung explant was neither deflated nor fitted into the specimen container (volume 3.5 liters, [Figure 1D](#)). Puncturing the upper lobe had no significant effect. Only after puncturing the pleura of the lower lobe did the explant deflate ([Figure 1E](#) and F, Supplemental Video 1).

The following is the Supplementary material related to this article [Video 1](#).

Media player

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Video 1. Collateral ventilation.

The donor (18-year-old male, 170 cm tall) had a predicted total lung capacity (pTLC) of 6.3 liters. The donor-to-recipient pTLC ratio was 0.89 and the donor pTLC-to-recipient actual-TLC ratio was 0.62 ([Table 1](#)). Residual pleural space was noted after implantation ([Figure 2](#)).

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Figure 2. (A) Intraoperative situs following right allograft implantation. The hyperinflated left native lung is filling the entire left hemithorax, while the right allograft is not fully reaching the apex in the right hemithorax. (B) Intraoperative situs following bilateral allograft implantations. Bilateral allografts are not fully reaching the apex of the hemithoraces and a size mismatch is apparent. (C) Post-transplant computed tomography of the chest. Residual pleural space from a significant size mismatch between a smaller allograft and a larger recipient's chest cavity is shown in axial view (C), sagittal view (D), and coronal view (E).

There was excellent early allograft function with primary graft dysfunction grade 0 at all time points. The recipient was extubated 9 hours post-transplant. During the first 24 hours, 1.7 liters of serosanguineous drainage from the chest tubes (~20 cm H₂O suction) was recorded. There was no evidence of active bleeding. Over the next 24 hours, chest tube output remained high at 2.1 liters. The recipient had minimal oxygen requirements (2 liters per minute via nasal cannula). However, on bedside exam, he did exhibit evidence of high work of breathing, using accessory respiratory muscles. The chest tubes were placed to waterseal, which was associated with a substantial decrease in chest tube output to on average 300 ml per 24 hours. The work of breathing improved as the chest tubes were placed on waterseal, however remained high. Hypercarbic respiratory failure (pH 7.29 and partial pressure of carbon dioxide in arterial blood of 76 mm Hg) necessitated reintubation and subsequent tracheostomy on postoperative day 4. A CT scan of the chest on postoperative day 4 demonstrated a size mismatch between the recipient's chest cavity and smaller allografts ([Figure 2](#)), with significant residual pleural space.

The post-transplant period was complicated by a chest wall hematoma complicated by hemorrhagic shock requiring massive transfusions of blood products and surgical evacuation. He developed acute lung allograft dysfunction from antibody-mediated rejection, which resolved after treatment with plasma-exchange, rituximab and intravenous immunoglobulin. Likely as a complication of his blood product transfusion, he had a primary infection with cytomegalovirus (CMV, serostatus donor, and recipient negative) and CMV pneumonitis complicated by acute lung allograft dysfunction. Secondary to the above complications, he required a prolonged period of ventilatory support. By postoperative month 3, he was weaned from nocturnal mechanical ventilation. By postoperative month 4, the tracheostomy tube was removed. He underwent surgical closure of the tracheostomy stoma 5 months post-transplant. His first pulmonary function studies 6 months post-transplant showed supranormal expiratory airflow ([Figure 3](#)) with FEV₁/forced vital capacity (FVC) ratio of 97%, forced expiratory flow (FEF)_{25-75%} of 5.93 liters/s (190% of predicted), FVC of 2.45 liters (50% predicted), and FEV₁ of 2.37 liters (63% predicted) ([Table 1](#)). He had a gradual increase in his FVC and FEV₁ to 3.22 (63% predicted) and 3.16 (81% predicted) respectively, by 10 months after transplant. However, he maintained supranormal expiratory airflow (FEV₁/FVC ratio 98%, FEF_{25-75%} 6.26 liter/s [194% predicted]) ([Table 1](#)). A follow-up chest CT at 11 months post-transplant showed normal lung parenchyma and complete resolution of previous pneumothoraces ([Figure 3](#)).

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Figure 3. (A) Pretransplant FVL and (B) first post-transplant FVL. (C) Follow-up chest CT at 11 months post-transplant shows normal lung parenchyma and resolution of previous pneumothorax. (D) Axial and (E) sagittal views. CT, computed tomography; FVL, flow volume loop.

Discussion

The transplant of our recipient highlights a series of physiological phenomena and the importance of applying basic physiological principles to the clinical management at the bedside. Throughout the discussion, we aim to highlight the general applicability of these physiological principles in end-stage lung disease from COPD/emphysema (I and II) and lung transplantation (III-V).

Air trapping and hyperinflation in emphysema

Loss of lung elastic recoil and air trapping from severe emphysema can lead to significant lung hyperinflation, which can cause intrinsic (or “auto”) positive end-expiratory pressure in the lung, which may result in substantial elevation of intrathoracic pressure. This can have adverse hemodynamic consequences, leading to pulmonary arterial hypertension and diastolic dysfunction. Our patient likely had significant auto-positive end-expiratory pressure and elevations in intrathoracic pressure preoperatively. After clamshell incision and decompression of his chest cavity by the protrusion of his native lungs out of his chest ([Figure 1C](#)), his pulmonary arterial pressures significantly decreased, and his hemodynamics improved. This allowed an off-bypass bilateral sequential lung transplant ([Figure 1A-F](#)).

Collateral ventilation in emphysema

In severe emphysema, collateral ventilation can be a lower resistance pathway for airflow compared to the airway tree. Collateral ventilation can connect airspaces of an entire lung lobe and if there are incomplete lung fissures present it can connect airspaces of an entire lung. When the pleura is

punctured in the setting of a severely emphysematous and hyperinflated lung, transpleural airflow through the pleural tear can be a pathway of least resistance. In such circumstances, we have previously reported complete transpleural exhalation via pleural tears during lung transplantation for severe emphysema.[1](#), [2](#), [3](#), [4](#) Such a phenomenon can be characterized by complete loss of the end-tidal CO₂ waveform, as well as complete loss of measured expiratory volume in the breathing circuit.[1](#) The measured inspiratory volume, however, may exhibit no change. Intraoperative loss of expiratory airflow and detectable end-tidal CO₂ during mechanical ventilation would ordinarily be considered an anesthetic emergency. However, some patients may maintain stable oxygenation and ventilation in such circumstances.[1](#) Alveolar ventilation becomes more effective for CO₂ elimination via “transpleural” exhalation, often requiring reduced minute ventilation to maintain normal pH and eucapnia.[2](#)

This physiology of collateral ventilation and severe emphysema is highlighted in this case by the significant deflation that occurred after the explanted lung's lower lobe was punctured in only 1 defined location ([Figure 1D-F](#), Supplemental Video 1).

Physiology of an undersized allograft

Size matching based on pTLC indicated an almost ideal size match, with a donor-to-recipient pTLC ratio of 0.89. However long-standing hyperinflation in our patient resulted in a barrel chest, with a substantial increase in total thoracic volume. The FRC of our patient was elevated at 8.15 liters, which was higher than the pTLC of the allograft at 6.3 liters. The measurement of the recipient's FRC was confounded by severe emphysema. However, in the setting of a barrel chest, the recipient's FRC in the immediate postoperative period was likely similar in magnitude to the preoperative TLC of the allograft. The residual pleural space on a CT of the chest on postoperative day 4 supports the presence of a significant donor-to-recipient size mismatch ([Figure 2A-F](#)). An allograft expanded in the range of its TLC would be associated with a substantial increase in elastic recoil and would be limited by its physical boundaries to further inflation. This physiology of an undersized allograft has implications for post-transplant patient management.[5](#), [6](#), [7](#), [8](#), [9](#), [10](#)

Chest tube management

Chest tubes are frequently placed on continuous regulated suction in the immediate postoperative period of lung transplantation, to facilitate the removal of intrapleural air and fluid. However, high negative pleural pressure applied to a large residual pleural space, as may occur with an undersized allograft, can lead to severe hyperinflation of the graft.[5](#) Such hyperinflation may predispose the allograft to volutrauma, inflammation, and pulmonary edema.[5](#) A similar situation is often reported when suction is applied to a postpneumonectomy residual pleural space and is referred to as “postpneumonectomy” pulmonary edema. Thus, if chest tube suction is applied to a residual pleural space in the setting of a very undersized allograft, hydrostatic shifts of fluid into the allograft and pleural space may occur, resulting in pulmonary edema and high chest tube drainage. Instead of placing the chest tubes on continuous suction, it may be more appropriate to place them on waterseal if clinically feasible.[5](#)

Mechanical ventilation

In the setting of an undersized allograft, it is important to manage mechanical ventilation according to the characteristics of the donor lung.[6](#), [7](#), [8](#), [9](#), [10](#) Specifically setting the size of the tidal volume based on donor lung size, rather than recipient predicted body weight, is critical to assure lung-protective ventilation ([Figure S1](#)). We previously described the relationship between donor-recipient lung-size mismatch and postoperative mechanical ventilation tidal volumes according to recipient-

and donor-predicted body weights in a cohort of bilateral lung transplant patients and highlighted that undersized allografts received relatively higher tidal volumes compared with oversized allografts when the tidal volumes were related to donor-predicted body weights (as a measure of allograft size) ([Figure S1](#)).⁷ It is common practice to set tidal volumes based on recipient characteristics.⁸ However, in our opinion, the mechanical ventilation strategy should be based on donor characteristics (using donor-predicted body weight as a parameter of actual allograft size), rather than recipient characteristics.^{8, 9, 10} Donor-based lung-protective ventilation is associated with decreased risk of primary graft dysfunction grade 3 at 48 to 72 hours and decreased 1-year mortality.¹⁰

Work of breathing

An allograft inflated in the range of its TLC significantly increases the work of breathing for the recipient ([Figure S2](#)).¹¹ At TLC, the lung elastic recoil is in the range of 30 cmH₂O ([Figure S2](#)). In addition, the compliance of the lung at TLC is substantially reduced. Attempts to further inflate the lung will be associated with substantial increases in the work of breathing and may be limited by the physical boundaries of the allograft ([Figure S2](#)). Such factors may increase the required work of breathing in the early postoperative period, leading to hypercarbic respiratory failure as we observed in this patient ([Figure S2](#)). It has been shown that the changes in the thoracic cavity based on the underlying lung disease are not permanent.¹² On the contrary, irrespective of the underlying lung disease, it has been shown that the thoracic cavity remodels to near-normal size in the postoperative period.^{12, 13} Certainly, vigilance during the early postoperative period is mandated given the mechanical limitations of the undersized allograft and risk for acute respiratory failure. However, the long-term prognosis for our recipient, at least regarding the mechanical synergy between the lungs and chest wall, was predicted to be excellent. After the thoracic cavity of the recipient returns to a more normal size, the allograft is better matched, as indicated by the donor-to-recipient pTLC ratio of 0.89.

Time course of remodeling of the thoracic cavity size

After lung transplantation, the chronic adaptations of the ventilatory pattern to advanced lung diseases are reversible and indicate that the main contributing factor is the lung itself rather than the systemic effects of the disease.¹² The exact time course of chest cavity remodeling toward normal has not been described. Using CT chest and lung volume measurements before and 1 year after transplant, Yu et al have shown that disease-specific chest remodeling caused by lung fibrosis in restrictive lung disease and lung hyperinflation in obstructive lung disease is reversible after lung transplant.¹³ After lung transplant, the chest remodeling occurs in the opposite direction to the lung disease-specific remodeling caused by the underlying lung disease in recipients ([Figure S3](#)).¹³ In our patient, CT imaging and lung volume measurements returned to normal at around 1 year after transplant ([Table 1](#)). The period of prolonged ventilatory support in this patient is best explained by the postoperative course complicated by chest wall hematoma, antibody-mediated rejection with allograft dysfunction, and CMV pneumonitis.

Supranormal expiratory airflow following lung transplantation

All our recipient's post-transplant pulmonary function studies showed supranormal expiratory airflow ([Figure 3](#), [Table 1](#)). We have previously defined and described the supranormal expiratory airflow pattern following lung transplantation and found that it was associated with improved survival after lung transplantation and a lower risk of bronchiolitis obliterans syndrome.^{14, 15}

In our previous studies, we found that restriction of donor lungs in a relatively smaller recipient thorax was the likely cause of the supranormal expiratory airflow pattern, as a higher pTLC ratio

(suggestive of oversized allografts) was strongly associated with it.¹⁴ Increased elastic recoil from limitations on inspiration and lower airway resistance from larger transplanted airways may explain the supranormal expiratory flow associated with an oversized allograft. The physiology of an oversized allograft restricted in a smaller recipient thorax has similarities to the old physiological experiment of chest wall strapping, which also causes increased elastic recoil and increased expiratory airflow.^{16, 17} In our recipient, the donor allograft was undersized relative to the recipient's chest cavity size. Elastic recoil of the lung is the key determinant of expiratory airflow capacity. Lung recoil can be from tissue forces (elastic structures of the parenchyma) and surface forces (at the air-liquid interface of the alveoli) ([Figure S4](#)). In the case of the relatively undersized allograft in our recipient, it is likely that increased tissue forces (from stretch of collagen and elastin fibers) are the source of increased lung recoil and the supranormal expiratory airflow.

Conclusion

The transplant journey of our recipient highlights a series of lung physiological phenomena and the importance of applying basic physiological principles to the clinical management at the bedside. We have previously shown that undersized allografts are associated with an increased risk of complications, primary graft dysfunction, and increased resource utilization.^{18, 19, 20, 21} However, we believe that an understanding of the physiology of an undersized allograft may allow for adjustments to postoperative mechanical ventilation and chest tube management strategies to protect the allograft, as highlighted in our recipient. After lung transplantation the chronic adaptations of the ventilatory pattern to advanced lung diseases are reversible and the chest cavity size can remodel back to normal. This indicates that the main contributing factor to chest cavity changes before transplant is the diseased lung itself rather than systemic effects of the disease leading to permanent chest wall and chest cavity changes.

Background

Patients with [chronic obstructive pulmonary disease](#) (COPD) often suffer from [cachexia](#) and malnutrition. Less is known about body composition and nutritional behaviour in patients with advanced COPD and [pulmonary emphysema](#).

Methods

We performed a single-center prospective analysis of patients with COPD GOLD III/IV. Metabolic parameters, dietary and exercise behavior, lung function, exercise capacity and body composition by [bioelectrical impedance analysis](#) (BIA) were analyzed. Patients with severe [emphysema](#) (emphysema index [EI] >20%) were compared to patients with mild [emphysema](#) (EI ≤ 20%).

Results

A total of 121 patients (45.5% female, mean age 64.8 ± 8.1 years, mean FEV₁ $31.0 \pm 8.6\%$, mean [RV](#) $234.7 \pm 50.6\%$) were analyzed, of whom 14.1% were underweight. Only 5% of the patients substituted protein and only about 1/3 performed regular exercise training. BIA showed an unfavourable body composition: body fat ↑, ECM/BCM-index ↑, phase angle ↓ ($5.0 \pm 0.9^\circ$), cell percentage ↓, FFMI (fat-free mass index) ↓. The 94 patients with severe emphysema (mean EI $36.6 \pm 8.5\%$) had lower body-mass-index (22.8 ± 4.3 vs. $31.1 \pm 5.8 \text{ kg/m}^2$, $p < 0.001$), FFMI, [body weight](#) and body fat, but did not differ significantly in the quality of body composition (e.g. phase angle). Their lipid and [glucose metabolism](#) were even better than in mild emphysema patients.

Conclusion

The finding of significantly lower BMI but similar body composition and better metabolic status in severe emphysema patients needs further investigation. However, it should not distract from the necessity to implement dietary and exercise recommendations for advanced COPD patients.

1. Introduction

[Chronic obstructive pulmonary disease](#) (COPD) is a progressive [lung disease](#) and represents the third leading cause of death worldwide [1]. [Pulmonary emphysema](#) develops in COPD patients as a consequence of parenchymal destruction and loss of normal [elastic recoil](#) that cause severe [airflow obstruction](#) and gas exchange impairment. Furthermore, [emphysema](#) is associated with a rapid decline in [forced expiratory volume](#) in 1 second (FEV₁) and mortality [2]. Patients with COPD and pulmonary [emphysema](#) are severely impaired in their activity levels. Endoscopic lung volume reduction (ELVR) procedures have been established as therapy option for progressed emphysema patients to improve lung function, exercise capacity and [quality of life](#) [3]. From ELVR literature and practice, an emphysema index (EI) of the lung above 20%, measured by quantitative [computed tomography](#), is considered as severe emphysema [4].

30–60% of all COPD patients exhibit malnutrition, which can lead to pulmonary [cachexia](#) and [sarcopenia](#) by progressive weight loss. The prevalence of [cachexia](#) increases with COPD GOLD (Global Initiative for Chronic Obstructive Lung disease) disease stage. [Muscle wasting](#) and diaphragmatic [weakness](#) translate into worsened exercise tolerance, which in turn accelerates [disease progression](#) [5].

Different [nutritional assessments](#) have been established beyond [body mass index](#) (BMI), because malnutrition is often underestimated with BMI measurement alone. [Bioelectrical impedance analysis](#) (BIA) is a simple and non-invasive technique to assess body composition. It is based on the electrical resistance of the human body, which is measured by applying [electric current](#) [6] and provides body composition parameters derived from a three-compartment-model ([Fig. 1](#)). BIA measurement has been performed over different COPD disease stages and showed severe malnutrition in terms of reduced fat-free-mass (FFM) [7] and phase angle (PhA), which correlated with FEV₁, FFM and even mortality [8]. Also COPD exacerbations seem to be associated with BMI and FFM [9]. Less data are available for COPD patients with severe pulmonary emphysema [10,11], suggesting that the amount of emphysema may be associated with [skeletal muscle](#) loss and [fat mass](#) [12]. To our knowledge, no data are available delivering detailed BIA parameters for severe COPD and mild vs. severe emphysema patients.

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Fig. 1. Three-compartment model of bioelectrical impedance analysis.

This study was designed to gain insight into nutritional status, dietary behaviour and activity level of severe COPD GOLD III/IV patients and provide details of body composition in patients with and without a relevant amount of pulmonary emphysema.

2. Methods

This is a prospective, observational study. The study was approved by the ethics committee of the Medical Faculty Heidelberg (S-047/2021) and is in accordance with the Declaration of Helsinki in its current version. Patients that presented at Thoraxklinik Heidelberg from March 2021 until March 2022 for evaluation of ELVR therapy options, were asked for participation and included in the study after giving written [informed consent](#).

- *Inclusion criteria:* age ≥18 years, [COPD](#) GOLD III-IV, ability to perform bodyplethysmography and 6-min-walking test (6-MWT), willing and able to consent for study participation.
- *Exclusion criteria:* implanted electronical device (e.g. pacemaker, brain stimulator), participation in an interventional endoscopic study, active cancer, muscle disease (e.g. [muscular dystrophy](#), amyotrophic lateral sclerosis), tuberculosis or consumptive illness with progressive weight loss.

2.1. Data collection

Data collection was performed prospectively. Patients were asked if they perform housework and walking (yes/no), muscle exercise training (yes/no), [respiratory therapy](#) (yes/no) and physiotherapy (yes/no). Furthermore, dietary behavior (no specialties/vegan/vegetarian/protein supplementation), exacerbation history, medication history (especially oral [cortisone](#) long term therapy, [inhalation therapy](#), roflumilast), respiratory insufficiency and pre-existing diseases (e.g. diabetes mellitus, arterial hypertension, [hyperlipidemia](#), nicotine consumption in package years [py], [osteoporosis](#) [known diagnosis or highly suspected diagnosis in multi-detector-computed tomography (MDCT) with [vertebral fractures](#) vs. suspected osteopenia/osteoporosis on MDCT without fractures vs. no]) were assessed. The results of the following examinations were recorded: laboratory (HbA1c, Cholesterol, HDL-Cholesterol, LDL-Cholesterol, [triglycerides](#), [thyroid](#) parameters), questionnaires (mMRC [modified [medical research council](#)], CAT [COPD Assessment test]), vital data (body weight [BW] and body height measured by a standardized, calibrated [body weight](#) scale and length gauge of “seca” [seca Deutschland, medical measurement systems and scales, Hamburg, Germany], [body mass index](#) [BMI], calculated in kg/m²), 6-MWT, bodyplethysmography (FEV₁, RV [residual volume], VC [vital capacity], TLC [total lung capacity] in l and %). [Emphysema](#) index (EI) was obtained from the Heidelberg-based quantitative [computed tomography](#) software Yacta (yet another [CT scan](#) analyzer), calculating emphysema as percentage of low attenuation areas below –950 Hounsfield units (HU) [[13](#)] on the basis of a MDCT.

2.2. Bioelectrical impedance analysis

BIA measurement was performed with the multifrequency bioelectrical-impedance-analysis device “Nutriguard-MS” (Version 2.0, 2019, Data Input GmbH, Pöcking, Germany) at 5 kHz, 50 kHz and 100 kHz, according to instructions for use (supine position, measurement in the morning after fasting >6 h, no clothing except underwear, no sports/alcohol in the last 12 hours, on the dominant side of the patient) and data were obtained from the Nutriguard-MS-software (version 2.0, 2019, Data Input GmbH). The following parameters were obtained: [basal metabolic rate](#) in kilocalories (kcal), total body water (TBW) in liters, body fat (BF) in kg and %, PhA in °, intracellular water (ICW) and extracellular water (ECW) in liters, ECM (extra cellular mass, consisting of [interstitium](#), bone, connective tissue) in kg and %, BCM (body cell mass, sum of all metabolic active cells, consisting of muscle and organ cell mass) in kg and %, BCM/ECM-index in %, cell percentage in %. In addition, fat-free mass (FFM = BCM + ECM) in kg and fat-free mass index (FFMI = FFM/height x height) in kg/m² were calculated. A special focus in the evaluation was placed on the phase angle, which is a raw measurement parameter and therefore largely independent of measurement problems or other

sources of error. It is a general parameter for cell density and integrity of the cell membranes (cell health) and directly proportional to BCM. The amount of total body water (consisting of 43% extracellular and 57% intracellular water) is also mainly determined by BCM. Cell percentage is the amount of BCM cells in the [lean body mass](#) and stands for overall nutritional and training status. Elevated ECM/BCM index and low cell percentage are hints for water storage [14].

2.3. Statistical analysis

All analyses have been performed using R Studio (version 2023.06.0, Posit PBC) and R programming language for statistical computing and graphics (version 4.2.2). Data are presented as mean \pm standard deviation (SD) or, for parameters with skewed distributions/outliers, as median (interquartile range [IQR]). Frequency data are presented as absolute numbers and percentages. BIA variables were analyzed in the whole patient group and according to sex. [Linear regression analyses](#) were performed to assess correlations between BW, BMI, BF (in kg and %), cell percentage, ECM/BCM, PhA, FFMI and clinically relevant parameters as EI, FEV₁ and RV (in l and %), and 6-MWD (6-min-walking distance). Correlations were considered high for R² 0.7–0.9, moderate for R² 0.4–0.69, weak for R² 0.3–0.39 and not relevant for R² < 0.3. Furthermore, subgroup analyses to identify differences in BMI, ECM/BCM, FFMI, BF and PhA with regard to binary variables (oral [cortisone](#) long term therapy, protein supplementation, housework/walking, physiotherapy, muscle exercise training yes/no) were performed, using t-test for independent samples. p-Values are reported as * for p \leq 0.05, ** for p \leq 0.01 and *** for p \leq 0.001. A comparative analysis between patients with EI >20% vs. EI \leq 20% was performed with regard to general [patient characteristics](#), functional parameters and BIA parameters. The hypothesis test functions used by default were chi quadrat test for categorical variables (with continuity correction) and oneway tests for continuous variables (with equal variance assumption, i.e., regular ANOVA). Two-group ANOVA is equivalent to t-test.

3. Results

3.1. Patient recruitment and characteristics

A total of 130 patients was screened for participation, of whom 121 fulfilled inclusion criteria and were analyzed. 5 patients were excluded due to FEV₁ >50%, 3 with active cancer disease and 1 patient was not able to perform bodyplethysmography. All baseline characteristics of the group can be viewed in the supplement ([Table S1](#)).

Lung function was significantly impaired due to hyperinflation with a mean FEV₁ of $31.0 \pm 8.6\%$ and [RV](#) of $234.7 \pm 50.6\%$. Oral [cortisone](#) long term therapy was established in 14.9% of all patients. The mean emphysema index of the lung was $31.3 \pm 12.6\%$ (min EI 1% - max EI 58%).

3.2. Activity level, dietary behavior and body weight

72.7% of all patients were still able to perform housework and walking activities regularly. The mean distance in 6-MWT was 252.3 ± 104.0 m (min 40 m, max 474 m). Muscle exercise training was only performed by 28.1% of all patients, [respiratory therapy](#) by 34.7% and physiotherapy by 24%. Most of the patients (92.6%) showed no special dietary behavior, with only 5% taking protein supplementation and 3.3% consuming vegan/vegetarian food. The mean BMI was 24.7 ± 5.8 kg/m². Body weight categories were (according to the world health organization classification = WHO [15]) 14.1% underweight, 43.8% normal weight (BMI 18.5–24.9 kg/m²) and 42.5% overweight.

3.3. Body composition

BIA measurement revealed reduced values for men and women regarding phase angle (mean $4.96 \pm 0.86^\circ$), cell percentage, total body water, lean body mass, body cell mass and basal metabolic rate compared to age- and sex-matched, normal weight healthy controls ([Table 1](#)). Body weight and body fat were increased in men, while women showed reduced body weight and only slightly elevated body fat. ECM and ECM/BCM-index were higher in both groups compared to the average values of a healthy population. FFMI was on a low level in both groups (mean FFMI 18.7 ± 2.6 for men, 15.8 ± 2.0 for women). The parameters in summary showed an “unhealthy” body composition.

Table 1. BIA measurement results of all patients in comparison to average values of [healthy persons](#).

Characteristics	All (n = 121)	Men (n = 66)	Average values for healthy men^a	Women (n = 55)	Average values for healthy women^a
BIA values	Measurement at right side of the body	112 (92.6)	–	–	–
Age	64.8 ± 8.1	65.5 ± 7.7	–	64.0 ± 8.7	–
FEV ₁ (%)	31.00 ± 8.55	30.39 ± 8.15	–	31.72 ± 9.02	–
RV (%)	234.71 ± 50.64	229.80 ± 50.54	–	240.50 ± 50.61	–
EI (%)	31.34 ± 12.60	30.58 ± 13.13	–	32.25 ± 11.98	–
Body weight (kg)	69.71 ± 18.17	76.84 ± 16.08	72.7 ± 6.8	61.16 ± 16.89	63.2 ± 5.9
BMI (kg/m ²)	24.66 ± 5.76	25.72 ± 4.95	–	23.38 ± 6.41	–
Body fat (kg)	20.37 ± 10.43	20.76 ± 8.68	14.1 ± 4.5	19.92 ± 12.28	18.6 ± 4.0
Body fat (%)	27.84 ± 9.13	26.08 ± 6.80	19.2 ± 5.3	29.95 ± 11.01	29.2 ± 4.8
TBW (l)	36.08 ± 7.78	41.00 ± 6.63	42.9 ± 4.0	30.18 ± 4.07	32.7 ± 2.9
LBM (kg)	49.29 ± 10.64	56.02 ± 9.06	58.6 ± 5.4	41.21 ± 5.56	44.6 ± 3.9
ECM (kg)	26.48 ± 5.52	29.50 ± 5.23	29.0 ± 4.0	22.86 ± 3.21	22.7 ± 2.6
BCM (kg)	22.81 ± 6.19	26.52 ± 5.39	29.6 ± 4.0	18.37 ± 3.63	22.0 ± 2.8
ECM/BCM [median]	1.16 [0.29]	1.13 [0–29]	1.00 ± 0.2	1.22 [0.36]	1.05 ± 0.17

Characteristics	All (n = 121)	Men (n = 66)	Average values for healthy men ^a	Women (n = 55)	Average values for healthy women ^a
Cell percentage (%)	45.93 ± 5.22	47.22 ± 5.13	50.5 ± 4.9	44.39 ± 4.93	49.2 ± 4.1
Basal metabolic rate (kcal)	1336.36 ± 195.64	1454.09 ± 169.76	1550 ± 125	1195.09 ± 114.47	1310 ± 90
Phase angle (°)	4.96 ± 0.86	5.18 ± 0.89	5.8 ± 1.1	4.70 ± 0.76	5.5 ± 0.8
ECW (l)	14.07 ± 4.52	16.55 ± 3.95	—	11.09 ± 3.17	—
ICW (l)	22.01 ± 3.42	24.45 ± 2.71	—	19.09 ± 1.04	—
FFM (kg)	49.29 ± 10.63	56.01 ± 9.05	—	41.22 ± 5.55	—
FFMI (kg/m ²)	17.39 ± 2.77	18.74 ± 2.59	16.7– 19.8 ^b	15.77 ± 2.03	14.6– 16.8 ^b

Abbreviations: BIA; bioelectrical impedance analysis; FEV₁, forced expiratory volume in 1 s; RV, residual volume; EI, emphysema index; BMI, body mass index; TBW, total body water; LBM, lean body mass; ECM, extracellular mass; BCM, body cell mass; ECW, extracellular water; ICW, intracellular water; FFM, fat-free mass; FFMI, fat-free mass index.

Values are reported in mean ± SD or N (percentage) or median [IQR]

a

Average values for healthy and normal weight (BMI 19–24.9 kg/m²) persons, age matched for 60–69 years (Source: Data Input, based on analysis of n = 29.409 women and n = 2224 men) [14].

b

There are no absolute [normal values](#) for FFMI, but these values are considered normal [16].

3.4. Factors influencing body composition

The [linear regression analyses](#) revealed no relevant correlations between lung function (RV, FEV₁) or exercise level (6-MWT) and BIA parameters. Moderate effects were observed for emphysema index and body weight, emphysema index and BMI, emphysema index and absolute body fat ([Fig. 2](#)). It is important to mention, that no significant correlations could be detected between emphysema index and other BIA parameters like phase angle, cell percentage or ECM/BCM. Patients with protein supplementation had significantly lower FFMI (14.6 ± 1.7 vs. 17.5 ± 2.7 kg/m², **), lower BMI (18.0 ± 2.9 vs. 25.0 ± 5.6 kg/m², **) and less body fat (18.5 ± 4.7% vs. 28.3 ± 9.1 %, **) than patients

without protein supplementation. Phase angle was better if they were still able to perform housework and walking activities and lower if they took oral [cortisone](#) long term therapy ([Fig. 3](#)). Patients performing muscle exercise training had more body fat (30.2 ± 7.5 vs. $26.9 \pm 9.6\%$, *). No differences were obtained for patients with and without physiotherapy.

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Fig. 2. Linear regression analyses for emphysema index and parameters of body composition

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Fig. 3. Phase angle in patients with and without oral cortisone long term therapy and housework/walking.

3.5. Comparison of mild vs. severe emphysema patients

22.3% (n = 27) of all patients had mild emphysema with an EI $\leq 20\%$ and were compared to 94 patients with EI $>20\%$. Medical treatment including oral [cortisone](#) long term therapy revealed no differences between both groups as well as most activity measurements including 6-MWT. The severe emphysema cohort suffered of significantly more impaired lung function with less FEV₁ and more hyperinflation. Patients with severe emphysema less often performed physiotherapy, less often suffered from diabetes mellitus, had lower [HbA1c](#), lower triglycerides and higher HDL-cholesterol. Body weight and BMI were significantly lower in the severe emphysema group (22.8 ± 4.3 vs. 31.0 ± 5.8 kg/m², p < 0.001), as well as various BIA parameters (basal metabolic rate, body fat, TBW, ECM, BCM, lean mass, ECW, ICW, FFM and FFMI). Phase angle was lower in the high emphysema group, but this was not statistically significant, neither were ECM/BCM-Index or cell percentage ([Table 2a](#) and [2b](#)).

Table 2a. Comparison of patients with mild vs. severe emphysema, baseline characteristics.

Empty Cell	EI $\leq 20\%$ (n = 27)	EI $> 20\%$ (n = 94)	p-value
Patient Characteristics			
Emphysema index (%)	13.07 ± 5.39	36.59 ± 8.48	<0.001
Age (years)	64.26 ± 8.80	64.99 ± 7.99	0.683
Sex, female	9 (33.3)	46 (48.9)	0.224
≥ 1 moderate/severe exacerbation in last year	23 (85.2)	64 (68.1)	0.357

Empty Cell	EI ≤ 20% (n = 27)	EI > 20% (n = 94)	p-value
Patient Characteristics			
CAT	24.08 ± 4.83	25.22 ± 7.39	0.460
mMRC: 0 points	0	1 (1.1)	0.648
1 point	0	6 (6.4)	
2 points	5 (18.5)	20 (21.3)	
3 points	9 (33.3)	25 (26.6)	
4 points	13 (48.1)	42 (44.7)	
Medication and comorbidities			
Oral cortisone long term therapy	5 (18.5)	13 (13.8)	0.767
Use of statins	11 (40.7)	33 (35.1)	0.757
Triple therapy (LABA + LAMA + ICS)	21 (77.8)	61 (64.9)	0.303
Prescription of diuretics	11 (40.7)	26 (27.7)	0.288
Osteoporosis specific medical treatment	1 (3.7)	2 (2.1)	1.000
Roflumilast	4 (14.8)	17 (18.1)	0.915
Prescription of l-thyroxine	4 (14.8)	14 (14.9)	1.000
Diabetes mellitus type 1	0 (0)	1 (1.1)	0.001
Diabetes mellitus type 2	7 (25.9)	3 (3.2)	
Respiratory Insufficiency Type I, LTOT	16 (59.3)	62 (66.0)	0.556
Respiratory Insufficiency Type II, LTOT and NIV	2 (7.4)	10 (10.6)	
Osteoporosis (proven diagnosis or radiological hints with fractures on MDCT)	7 (25.9)	17 (18.1)	0.581
Suspected osteopenia/osteoporosis on MDCT	18 (66.7)	72 (76.6)	

Empty Cell	EI ≤ 20% (n = 27)	EI > 20% (n = 94)	p-value
Patient Characteristics			
Physical activity			
Housework/walking	21 (77.8)	67 (71.3)	0.672
Respiratory therapy	11 (40.7)	31 (33.0)	0.605
Physiotherapy	12 (44.4)	17 (18.1)	0.010
Muscle exercise training	7 (25.9)	27 (28.7)	0.966
6-MWD (m)	242.65 ± 95.98	255.25 ± 106.72	0.592
Dietary behaviour			
Special dietary behaviour	1 (3.7)	8 (8.5)	0.672
Protein supplementation	1 (3.7)	5 (5.3)	1.000
Vegan/vegetarian	0 (0)	4 (4.3)	0.632
Lung function			
FEV ₁ (l)	1.07 ± 0.34	0.85 ± 0.27	0.001
FEV ₁ (%)	34.80 ± 8.79	29.91 ± 8.20	0.008
RV (l)	4.68 ± 1.01	5.41 ± 1.21	0.006
RV (%)	203.22 ± 40.56	243.41 ± 49.85	<0.001
VC (l)	2.54 ± 0.82	2.38 ± 0.80	0.378
VC (%)	65.84 ± 16.02	67.19 ± 16.50	0.707
GOLD stage III	17 (63.0)	43 (45.7)	0.174
GOLD stage IV	10 (37.0)	51 (54.3)	

* Vales are reported in mean ± SD or n (percentage). p-values <0.05 are considered statistically significant and in **bold**.

Abbreviations: CAT, COPD-assessment-test; mMRC, modified Medical Research Council; LABA, long-acting beta-agonist; LAMA, long-acting [muscarinic antagonist](#); ICS, inhaled corticosteroid; LTOT, long-term oxygen therapy; NIV, non-invasive ventilation therapy; MDCT, multi-detector computed tomography; EI, emphysema index; 6-MWD, 6-min-walking distance; FEV₁, forced expiratory volume in 1 s; RV, residual volume; VC, vital capacity; GOLD, Global Initiative for Chronic Obstructive Lung Disease.

Table 2b. Comparison of patients with mild vs. severe emphysema, laboratory parameters and body composition.

Empty Cell	EI ≤ 20% (n = 27)	EI > 20% (n = 94)	p-value
Laboratory parameters			
HbA1c (%)	6.03 ± 0.69	5.65 ± 0.44	0.004
HDL cholesterol (mg/dl)	56.57 ± 17.99	73.08 ± 22.48	0.003
LDL cholesterol (mg/dl)	113.71 ± 39.48	104.64 ± 36.95	0.346
Cholesterol (mg/dl)	202.33 ± 45.93	196.81 ± 38.12	0.591
Triglycerides [median] (mg/dl)	148 [284.00]	95.00 [273.00]	0.005
TSH [median] (U/ml)	1.23 [4.76]	1.06 [7.71]	0.378
fT3 (U/ml)	3.21 ± 0.45	3.30 ± 0.48	0.438
fT4 (U/ml)	12.82 ± 2.15	14.34 ± 2.10	0.006
BIA values			
BMI (kg/m ²)	31.03 ± 5.83	22.83 ± 4.26	<0.001
Body weight (kg)	89.02 ± 17.75	64.17 ± 14.09	<0.001
Body fat (kg)	31.49 ± 11.43	17.18 ± 7.60	<0.001
Body fat (%)	34.58 ± 9.00	25.90 ± 8.24	<0.001
TBW (l)	42.11 ± 8.22	34.35 ± 6.75	<0.001
LBM (kg)	57.53 ± 11.23	46.92 ± 9.24	<0.001
ECM (kg)	30.26 ± 6.38	25.39 ± 4.75	<0.001

Empty Cell	EI ≤ 20% (n = 27)	EI > 20% (n = 94)	p-value
BCM (kg)	27.27 ± 6.04	21.53 ± 5.64	<0.001
ECM/BCM [median]	1.14 [0.76]	1.19 [1.69]	0.187
Cell percentage (%)	47.33 ± 4.81	45.53 ± 5.29	0.115
Basal metabolic rate (kcal)	1477.41 ± 191.30	1295.85 ± 178.11	<0.001
Phase angle (°)	5.19 ± 0.86	4.89 ± 0.86	0.120
ECW (l)	18.03 ± 4.62	12.93 ± 3.81	<0.001
ICW (l)	24.09 ± 3.93	21.42 ± 3.02	<0.001
FFM (kg)	57.53 ± 11.22	46.92 ± 9.23	<0.001
FFMI (kg/m ²)	19.94 ± 2.80	16.65 ± 2.30	<0.001

* Values are reported in mean ± SD or n (percentage) or median [IQR]. p-values <0.05 are considered statistically significant and in **bold**.

Abbreviations: BIA, bioelectrical impedance analysis; BMI, body mass index; TBW, total body water; LBM, lean body mass; ECM, extracellular mass; BCM, body cell mass; ECW, extracellular water; ICW, intracellular water; FFM, fat-free mass; FFMI, fat-free mass index.

4. Discussion

We investigated dietary behaviour, activity level and body composition in a group of 121 patients with COPD GOLD III/IV. This is the first study providing detailed BIA parameters in patients with advanced COPD and emphysema. Although activity levels were significantly impaired and underweight was present, efforts to improve body composition through exercise training or special dietary behaviour were low. BIA showed an unfavourable body composition with poor overall fitness and low muscle mass. Severe emphysema patients were significantly thinner, but body composition was not equally worse and lipid and [glucose metabolism](#) were even better.

Our cohort of 121 patients represents a typical severe COPD cohort with impaired lung function and reduced exercise capacity. 27.3% were no longer able to perform regular housework and walking activities. Despite the fact, that 14.1% of all patients were underweight, only 5% received protein supplementation. Physiotherapy and respiratory therapy, which are an essential part of conservative COPD treatment, were only used by about one third of the patients. Muscle exercise training was also rarely performed. Malnourished COPD patients have reduced lung function and lower [quality of life](#) [17]. [Nutritional supplementation](#) is therefore recommended by GOLD guidelines [18] to promote weight gain, improvements in [respiratory muscle](#) strength, quality of life and exercise capacity. From our data, we can conclude that there is still a lot to do for COPD patients, both in terms of education and the implementation of dietary recommendations and exercise programs. It should also be noted that [osteoporosis](#) was often a known diagnosis in these patients or [vertebral fractures](#) were already

visible in the [MDCT](#) without specific treatment having been initiated. There is also a need to raise awareness of osteoporosis in this vulnerable patient group.

BIA analysis revealed low FFMI levels, reduced phase angle, cell percentage, body cell mass and increased values for extracellular mass, ECM/BCM index and body fat in our cohort. This shows an overall unhealthy body composition, especially because phase angle [8,19] and FFMI [20] have been shown to predict mortality in COPD patients. The phase angle is the best studied BIA marker in COPD patients, being associated with physical function, disease severity and prognosis [21]. FFMI correlates with exercise capacity, dyspnea, respiratory muscle function and [pulmonary function](#) [22]. In comparison, De Blasio and colleagues measured body composition in 212 COPD patients (mean FEV₁ 45.3%, GOLD stage I-IV) vs. 115 age- and BMI-matched controls, but underweight patients were not included (BMI 20–35 kg/m²). The mean FFMI was 19.3 ± 1.4 in male and 17.4 ± 1.3 in female COPD patients (both lower in our cohort). FFM and the 5/250 impedance ratio were decreased and negatively influenced by disease severity [7]. Benedetto and colleagues have shown reduced cell mass in patients with severe COPD, [skeletal muscle](#) depletion and worsening gas exchange [8]. In the COSYCONET (Systemic Consequences-Comorbidities Network) study [23], the largest German cohort of COPD patients (n = 2137), mean FFMI (18.0 ± 2.8 kg/m² COSYCONET vs. 17.39 ± 2.77 kg/m² our cohort) and BMI (25.8 ± 4.1 kg/m² vs. 24.66 ± 5.76 kg/m²) were slightly higher than in our group. This is due to the higher proportion of underweight patients in our cohort (14.1% vs. 12.3% in COSYCONET) and the earlier COPD cohort GOLD stage I-IV in COSYCONET (mean FEV₁ 52.5 ± 18.8%). They could show that higher FFMI was associated with better exercise capacity, but only in underweight patients. Detailed BIA parameters other than phase angle and FFMI are rarely reported in the literature and patient cohorts differ in disease severity and weight classification. This highlights the need to publish detailed BIA results to establish comparative parameters.

Our analysis revealed moderate correlations between emphysema index and BMI, absolute body weight and absolute body fat. This was not due to impaired lung function but must be due to emphysema, as FEV₁ and RV did not show relevant correlations despite a known association between poor lung function and poor nutritional status in the literature [17]. On the other side, emphysema index showed no influence on other BIA parameters like phase angle, ECM/BCM-index, and FFMI. The subgroup analyses must be evaluated critically in view of widely varying group sizes. Patients should be encouraged to stop oral cortisone long term therapy and to keep moving, as this was correlated with better phase angle in our analysis. The finding that protein supplementation was negatively associated with BMI, body fat and FFMI, seems to be more of a coincidence, that malnourished patients are encouraged to supplement proteins. The correlation between muscle exercise training and increased [body fat mass](#) does not seem logical either.

As expected from the previous results, high emphysema index was associated with significantly lower body weight, BMI, body fat and FFMI. Although phase angle, ECM/BCM-index and cell percentage were lower, these differences were not statistically significant. Maybe it would require larger patient cohorts to reveal significant differences. The metabolic status in terms of glucose and [lipid metabolism](#) was better in severe emphysema patients (less diabetes mellitus, lower triglycerides, higher HDL-cholesterol). This is in line with data from the COPDGene [Study Cohort](#) [24] that showed a higher prevalence of diabetes and [metabolic syndrome](#) in non-emphysematous COPD and lower weight in the emphysema-group, although definitions for non-emphysema (EI <5%) vs. emphysema (EI >10%) were different. These findings need further evaluation as they challenge the reported protective effect of obesity (“obesity paradox”) in COPD patients [25]. Bioelectrical impedance analysis has rarely been performed in emphysema patients. Mainly low FFM/FFMI have been reported to be associated with severe emphysema [10,12,22] and to correlate with some measures

of lung function as well as exacerbation frequency, 6-MWD, respiratory muscle strength [22] and handgrip strength [12]. The extent of emphysema seems also associated with skeletal muscle loss and [fat mass](#) [12]. There is still a debate about cutoff levels for emphysema, which depend on HU that are used for quantification (-950 HU vs. -910 HU). A different grading of emphysema severity would mean different results for body composition. As an aside, it should be mentioned that there is a selection bias for COPD patients with emphysema in this study and therefore it is not surprising that men and women in our analysis have similar severe emphysema indices (32.3% EI women vs. 30.6% EI men), despite the differences in emphysema prevalence reported in the literature [26]. We were able to provide a first detailed insight into the body composition of severe pulmonary emphysema patients and found evidence that, despite a BMI difference $> 8 \text{ kg/m}^2$ in comparison to mild emphysema patients, body composition of emphysema patients was not necessarily equally worse and the metabolic status was even better.

The presented study has several limitations. Firstly, the studied cohort is a highly selected cohort of severe COPD patients and does not represent all GOLD stages. The results can therefore only be extrapolated to similar COPD cohorts. Secondly, the subgroups and emphysema cohorts were unbalanced in size and not matched. This should be considered for future studies. Thirdly, and most important, the distinction between mild and severe emphysema is based on the treatment benefit of ELVR studies. Future studies with larger patient cohorts may chose differentiated classifications of emphysema and also distinguish other phenotypes of COPD (e.g. [chronic bronchitis](#), frequent exacerbator) according to their body composition.

5. Conclusion

Patients with progressed COPD and poor lung function have an unfavourable body composition. While severe emphysema patients have significantly lower weight, the body composition must not necessarily be worse and the metabolic status in terms of lipid and [glucose metabolism](#) may even be better. Further studies to establish comparative parameters for body composition in advanced COPD cohorts are needed. Awareness must be created about dietary behaviour and exercise training, as protein supplementation and physical training are essential therapy tools that are not used by a large proportion of advanced COPD patients.

Implantable cardioverter-defibrillators (ICD) are highly effective in treating life-threatening [ventricular arrhythmias](#) and are commonly implanted in patients at risk of [sudden cardiac death](#). Successful [defibrillation](#) by an ICD depends on its ability to deliver [shocks](#) that exceed defibrillation thresholds. In a properly implanted and normal-functioning ICD system, extracardiac conditions (eg, [pneumothorax](#), [pleural effusion](#), excessive soft tissue) can increase shock impedances and divert the shock pathway, resulting in defibrillation failure. Among patients with transvenous ICD (TV-ICD), persistent elevation in high-voltage impedance, defibrillation threshold testing (DFT) failure, and ICD shock failures have all been reported in patients with pneumothorax, air pockets around the [pulse generator](#), and subcutaneous [emphysema](#).^{1, 2, 3} Undersensing of [ventricular fibrillation](#) and oversensing leading to inappropriate shocks secondary to air entrapment has been reported with subcutaneous ICDs.^{4,5} We present a unique case of late S-ICD malfunction owing to air entrapment from [disease progression](#) in a patient with advanced emphysema. This case elucidates the importance of proper screening and work-up of patients with a history of emphysema prior to considering them for S-ICD implantation.

Case report

A 57-year-old male with history of witnessed [sudden cardiac arrest](#) owing to [ventricular fibrillation](#) underwent a successful subcutaneous [ICD](#) (S-ICD) (Boston Scientific, Marlborough, MA) implantation for secondary prevention of sudden cardiac death at an outside medical institution 6 years ago. During [DFT](#) at implantation, the first 80 J [shock](#) was successful and high-voltage shock impedance was 86 ohms in primary sensing vector. Postimplant [chest radiographs](#) showed appropriate locations of the [pulse generator](#) and defibrillation electrode. Radiolucency within the [lung parenchyma](#) adjacent to the S-ICD pulse generator and defibrillation coil was notable as well ([Figure 1A](#)). Evaluations at the time of the patient's cardiac arrest, including routine laboratory tests, electrocardiogram, and [echocardiogram](#), were unremarkable. [Coronary angiography](#) showed patent [coronary arteries](#). The patient's [past medical history](#) was significant for 35 pack-years of smoking and smoking-related advanced [emphysema](#), for which he had undergone [lung volume reduction surgery](#) 3 years prior. He had tested negative for α 1-antitrypsin deficiency PiZZ phenotype. He was on continuous home oxygen and inhaled long-acting beta-2 agonist and steroid combination. Family history was negative for malignant arrhythmia, cardiomyopathy, or sudden cardiac death. The patient received 2 appropriate shocks from his device for fast [ventricular tachycardia](#) during the first year of device implantation and was arrhythmia free on [sotalol](#) since then. A year ago, during a routine device check, he was noted to have intermittent loss of sensing and failure to register [R waves](#) ([Supplemental Figure 1](#)). The chest radiograph ([Figure 1B](#)) and a computed tomography (CT) scan ([Figure 2](#)) showed advanced emphysema, traction [bronchiectasis](#), and a large emphysematous bulla compressing the lung and shifting the mediastinum to the right. The S-ICD pulse generator and [defibrillator](#) electrode were in good locations. A DFT and pulse generator change was recommended, as his device was near elective replacement indicator. In the [electrophysiology](#) laboratory, [general anesthesia](#) was administered. The patient was prepped and draped in a standard sterile manner using 4-piece [surgical drape](#). Low-voltage shock impedance with 10 J energy in [sinus rhythm](#) was 120 ohms. Ventricular fibrillation was induced by 40 Hz pacing. After initial sensing dropouts, the sensing and charging were appropriate, but 65 J shock from the device was unsuccessful ([Figure 3](#)). High-voltage shock impedance was 130 ohms. Following the delivery of shock, complete loss of sensing was noted ([Figure 3](#)). A total of three attempts to defibrillate with 360 J external shocks through anterior and lateral chest wall defibrillator pads were unsuccessful. The patient's chest was exposed and another external shock at 360 J with paddles applied anteriorly and posteriorly was successful in restoring sinus rhythm. The S-ICD system was explanted, followed by implantation of a conventional transvenous dual-chamber dual-coil ICD ([Supplemental Figure 2](#)). DFT was successful with the first 36 J shock and high-voltage shock impedance was 60 ohms. At 24 months follow-up, the patient was arrhythmia-free on sotalol. The sensed R wave was 11 mV, capture threshold 1 V @ 0.4 ms, pace impedance 660 ohms, and high-voltage shock impedance 64 ohms.

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Figure 1. [Chest radiographs](#), posteroanterior views. **A:** Obtained at subcutaneous implantable cardioverter-defibrillator implantation, showing hyperinflated lungs with radiolucent areas bilaterally, more pronounced on left side. **B:** At device malfunction, showing progressive disease, traction [bronchiectasis](#), and expansion of emphysematous bullae.

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Figure 2. Computed tomography scan of the chest showing advanced emphysema, traction bronchiectasis, and a large emphysematous bulla occupying entire left lower hemithorax in shock path. **A:** Coronal view. **B:** Sagittal view. EB = emphysematous bulla; PG = pulse generator.

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Figure 3. Subcutaneous implantable cardioverter-defibrillator tracing at defibrillation threshold testing showing sensing dropouts (*yellow stars*), failed shock (*blue star*), and complete loss of sensing (*blue arrows*).

Discussion

We report a case of late S-ICD malfunction owing to [disease progression](#) and air entrapment in a patient with advanced emphysema in whom DFT at implantation was successful and high-voltage shock impedance acceptable despite evidence of significant [lung disease](#).

The S-ICD system is noninferior to conventional TV-ICD, with a probability of successful termination of malignant [ventricular arrhythmia](#) approaching >95%.^{6, 7, 8} The utilization of the S-ICD has eliminated acute and chronic endovascular complications associated with conventional TV-ICD systems (eg, chronic [venous occlusions](#), endovascular infections, and potentially fatal complications during indicated lead extractions).⁹ S-ICD is therefore an attractive alternative to TV-ICD for prevention of sudden cardiac death from malignant ventricular arrhythmia in the absence of indications for pacing for [bradyarrhythmia](#), cardiac resynchronization, or treatment of antitachycardia pacing-responsive [ventricular tachycardia](#).¹⁰ Following U.S. Food & Drug Administration approval in 2012, the use of S-ICD was initially limited to younger patients with mild or no structural [heart diseases](#) and fewer comorbidities. The efficacy and safety of S-ICD systems in older patients with structural heart disease and more comorbidities has been reported to be noninferior to TV-ICD in a large representative cohort.^{11,12} Hence, it is now being implanted frequently in older patients with structural heart diseases and more comorbidities, especially in those with increased risk of infection (eg, immune compromised) or in whom preservation of venous patency is desired (eg, chronic kidney disease).

In comparison to the TV-ICD, the S-ICD system is exclusively extravascular and extracardiac, thus making its functionality potentially more vulnerable. Any amount of extra insulation in the form of air, fluid, or tissue in the shock pathway may have a significant impact on the proper functioning of the device. Hence, appropriate positioning and approximation of the pulse generator and defibrillation electrode to the left lateral rib cage and to the [sternum](#), respectively, are crucial for successful defibrillation in the S-ICD system. For the same reasons, in appropriately selected patients who pass preimplant screening, a DFT at the time of S-ICD implantation is routinely performed as a class I recommendation to ensure proper sensing of ventricular fibrillation and confirm successful defibrillation.^{9,13} A shock impedance of <90 ohms has been shown to correlate with a defibrillation threshold success rate of >95%.¹⁴ Alternatively, R-wave synchronous shock with high (65 J) or low (10 J) energy in [sinus rhythm](#) has been shown to assess the overall system integrity in patients in whom DFT is not performed for safety reasons.¹³ If any shock administration is deemed to be inappropriate

owing to safety concerns, a noninvasive postimplant Praetorian score can be used as an alternative to DFT and shock impedance testing, using 3 independent determinants: (1) position of the pulse generator with respect to left mid [axillary line](#), (2) thickness of the [subcutaneous fat](#) between the pulse generator and the thoracic wall, and (3) the number of coil widths of fat tissue between the S-ICD coil and the sternum. A Praetorian score of <90 predicts a low risk and a score ≥150 predicts a high risk of shock failure.¹⁴ Hence, many S-ICD implanters are omitting DFT and are relying on sinus rhythm low-voltage shock impedances or sometimes on postimplant Praetorian scoring.

Emphysema is a distal airspace disease, characterized by destruction of [lung parenchyma](#) with loss of [elastic tissue](#) without fibrosis, resulting in airway obstruction and air trapping. Emphysema is a progressive disease, even after cessation of smoking. The presence of emphysema, defined by [CT scan](#) imaging of lungs, among smokers is associated with progression of emphysema in all GOLD stages, regardless of presence or absence of symptoms and [spirometry](#) abnormalities.¹⁵

Our patient had evidence of air trapping in the shock pathway, which was overlooked as a potential cause of shock failure. A preimplant [quantitative CT](#) scan of the chest might have allowed better assessment of disease severity and air entrapment and prompted against S-ICD implantation. A successful DFT with a high-voltage shock impedance of <90 ohms and successful treatment of 2 episodes of fast ventricular tachycardia postimplant were reassuring but did not reliably predict long-term efficacy of S-ICD owing to his progression of emphysema. Progressive air trapping and expansion of emphysematous bullae resulted in sensing abnormalities, increased high-voltage shock impedance, and DFT failure, necessitating exchanging of the S-ICD system with a conventional TV-ICD.

Emphysema is not an uncommon comorbidity among older patients undergoing ICD implantations. Such patients should undergo careful evaluation before they are deemed candidates for S-ICD, including a quantitative CT scan of the chest to assess for the presence and severity of air trapping, especially in the lower lobe of the left lung, which could be subtle and easily overlooked on plain radiograph of the chest. A normal DFT and high- or low-voltage shock impedance <90 ohms at the time of implant in patients with mild or even moderate emphysema may not necessarily predict long-term success from progression of emphysema, especially in patients with [bullosic disease](#). A [pneumothorax](#) resulting from rupture of small bulla, which may be even remote to the shocking vector, can have similar detrimental effects in S-ICD functions. Patients with severe or advanced emphysema and those with bullous disease, especially involving the lower lobe of the left lung, should be considered for conventional TV-ICD systems. Issues related to progressive emphysema may occur in TV-ICD, but options of adding a left subclavian or [coronary sinus](#) defibrillation coil in such systems may allow feasible troubleshooting. Extravascular ICDs such as the Aurora EV-ICD System (Medtronic, Dublin, Ireland), a new addition to extravascular and extracardiac ICD systems, potentially can have similar challenges and are also better avoided in such patients. Patients with severe and advanced emphysema and S-ICD should be carefully monitored for device malfunction and emphysema progression. A CT scan of the chest should be performed after [lung volume reduction surgery](#) for assessing worsening air trapping, especially in the path of the shocking vector.

Conclusion

Emphysema is a progressive disease that may result in significant air trapping from bullae expansion and pneumothorax and causes S-ICD failure. Proper preimplant screening including a [quantitative CT](#) scan of the chest should be incorporated in the assessment process for the candidacy for S-ICD systems in patients with emphysema. Should such patients undergo S-ICD implantations, close

monitoring for S-ICD malfunction and disease progression will allow timely and appropriate interventions.