Key words

breast cancer

Carcinoma en cuirasse

cutaneous metastases

Abbreviations used

AFND

acute febrile neutrophilic dermatosis

BMT

bone marrow transplantation

CeC

Carcinoma en cuirasse

HAART

highly active anti-retroviral drug therapy

History

A 76-year-old woman with limited past medical history presented to the hospital after a fall. She was found to have an extensive infiltrative and sclerotic red plaque comprised of papules and necrotic ulcerations of the right chest and abdomen (Fig 1) with associated right upper extremity edema. The skin changes had been initially noticed by the patient 5 months prior and self-diagnosed as shingles. A computerized tomography scan of the chest revealed bilateral pleural effusions with total collapse of the right lung, multiple pulmonary nodules, and enlarged mediastinal, hilar, and axillary lymph nodes. A biopsy from the right chest wall showed diffuse infiltration of the dermis with sparing of the overlying epidermis by cords and nests of atypical cells characterized by nuclear hyperchromasia, variably prominent nucleoli, and mitotic figures. Ductal differentiation and lymphovascular invasion were identified (Fig 2).

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Fig 1.

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Fig 2.

Question 1: What is the most likely clinical diagnosis?

- A.

Morpheaform Basal Cell Carcinoma

- B.

Carcinoma en Cuirasse

- C.

Cutaneous Tuberculosis

- D.

Vegetative Pyoderma Gangrenosum

- E.

Bullous Morphea

Answers:

- A.

Morpheaform Basal Cell Carcinoma – Incorrect. Though morpheaform basal cell carcinoma can similarly cause local tissue invasion and destroy the cutaneous architecture if left untreated, the presentation and progression of this lesion over 5 months is highly atypical for basal cell carcinoma. Histopathology demonstrates cords and nests of basaloid cells without ducts infiltrating dense sclerotic stroma.

- B.

Carcinoma en Cuirasse – Correct. The clinical history of metastatic lesions on imaging, rapid and destructive growth over 5 months, and visual appearance of an infiltrative plaque on the chest wall is most consistent with Carcinoma en cuirasse (CeC). The associated pathology demonstrated atypical tumor cells infiltrating the dermis and showing ductal differentiation, consistent with invasive breast carcinoma.

- C.

Cutaneous Tuberculosis – Incorrect. Cutaneous tuberculosis is the result of an often-chronic infection with mycobacterium tuberculosis. Histopathology demonstrates granulomas, which classically are surrounded by a collarette of lymphocytes and associated with pseudoepitheliomatous hyperplasia. Bacilli may be identified on special histochemical stains.

- D.

Vegetative Pyoderma Gangrenosum – Incorrect. Vegetative pyoderma gangrenosum is a superficial and often less-aggressive form of pyoderma gangrenosum, a type of neutrophilic dermatosis. Vegetative pyoderma gangrenosum is often seen in otherwise healthy individuals and typically presents with a well-demarcated violaceous border. Histopathology shows ulcer with a dense neutrophilic infiltrate that undermines the adjacent epidermis.

- E.

Bullous Morphea – Incorrect. Bullous morphea is an uncommon subtype of localized morphea that is characterized by intermittent blistering and results in atrophic, sclerotic skin. It is most commonly found on the lower extremities and is not expected to result in pulmonary nodules,

lymphadenopathy, or histopathologic findings of infiltrative tumor cells.[1] Histopathology demonstrates subepidermal bullae formation overlying sclerosis.

Question 2: What underlying primary malignancy is most commonly associated with this condition?

- A.

Breast

- B.

Lung

- C.

Liver

- D.

Acute Myelogenous Leukemia

- E.

Kidney

Answers:

- A.

Breast – Correct. CeC is most commonly associated with an underlying primary breast cancer. CeC can be the presenting sign or a sign of disease recurrence.[2]

- B.

Lung – Incorrect. Though lung cancer is another common malignancy associated with CeC, it is not the most commonly associated primary cancer.

- C.

Liver – Incorrect. Although liver cancer does cause cutaneous changes, it is much more associated with findings such as jaundice, spider angiomas, and rarely, pityriasis rotunda.

- D.

Acute Myelogenous Leukemia – Incorrect. Although acute myelogenous leukemia is often associated with cutaneous findings such as leukemia cutis and acute febrile neutrophilic dermatosis (Sweet Syndrome) among others, it has not been associated with CeC.[3,4] Leukemia cutis has a varied presentation but should be considered in patients with pink-red-violaceous indurated plaques or nodules. Acute febrile neutrophilic dermatosis most often manifests as well-circumscribed tender red plaques or nodules, and though it does have rare necrotizing subtypes, the histopathology does not demonstrate the expected diffuse neutrophilic infiltrate.

- E.

Kidney – Incorrect. Skin metastases of renal cell carcinoma are typically skin-color to red, vascular, nodular, rapidly growing lesions often seen on the scalp (but can be appreciated elsewhere).[5]

Question 3: Which of the following would be the most appropriate treatment for this condition?

- A.

Highly active anti-retroviral drug therapy

- B.

Multiagent anti-tuberculosis drug therapy

- C.

Bone marrow transplantation

- D.

Palliative chemotherapy/immunotherapy

- E.

High dose systemic corticosteroids

Answers:

- A.

Highly active anti-retroviral drug therapy – Incorrect. Highly active anti-retroviral drug therapy is the standard of care for HIV.[6] Although HIV is associated with the development of numerous secondary cutaneous infectious, such as tuberculosis, this patient's presentation is most consistent with CeC (not cutaneous tuberculosis), and the administration of the highly active anti-retroviral drug therapy would not be expected to successfully treat the underlying neoplastic pathophysiology.[7] Moreover, although HIV is associated with the development of certain cancers—such as Kaposi sarcoma, cervical cancer, and non-Hodgkin's lymphoma—it is not associated with the development of neither breast cancer nor CeC.[8]

- B.

Multiagent anti-tuberculosis drug therapy – Incorrect. Though multiagent anti-tuberculosis drug therapy would be an appropriate treatment approach for cutaneous tuberculosis, the absence of acid-fast bacilli on dermatopathology and presence of lymphovascular invasion with ductal differentiation should point the diagnostician toward a malignant process such as CeC.

- C.

Bone marrow transplantation – Incorrect. Bone marrow transplantation is the treatment of choice for hematologic malignancies such as leukemia, lymphoma, and myeloma. However, bone marrow transplantation would not be used to target the underlying pathophysiology of CeC secondary to a solid organ malignancy such as breast cancer.

- D.

Palliative chemotherapy/immunotherapy – Correct. This patient is presenting with extensive disease of the chest wall with evidence of diffuse metastatic breast cancer on histopathology as well as imaging (with multiple pulmonary nodules, enlarged lymph nodes, and pleural effusions). Given the underlying pathophysiology and extent of this patient's disease, palliative chemotherapy/immunotherapy is the best treatment option provided.

- E.

High dose systemic corticosteroids – Incorrect. Though systemic steroids are an appropriate initial treatment for vegetative pyoderma gangrenosum or bullous morphea, they are not appropriate as a monotherapy in the setting of diffuse infiltrative disease consistent with metastatic breast cancer.

Abstract

The chest radiographic evaluation of interstitial lung diseases is among the most difficult tasks in radiology. The following article reviews one system of pattern recognition used in the differential diagnosis of interstitial lung disease. The patterns reviewed include three reticular patterns: The Peripheral Reticular pattern, the Cystic pattern and the Linear pattern and two other patterns: the Nodular pattern and the ground-glass pattern. The majority of interstitial lung diseases may be classified into one of these patterns, which allows for improved differential diagnosis.

Introduction

The plain film interpretation of diffuse interstitial lung disease is a challenging topic, which often seems difficult initially; however, when armed with a systematic approach to analyze the radiograph, in many instances, a short differential diagnosis may be generated; and if sufficient clinical history is available, a specific diagnosis may be made. There are a variety of radiographic approaches to the interpretation of interstitial lung diseases, each with their own proponents. The following is a modification of one schema used at the University of Pennsylvania.

The vast majority of interstitial lung diseases may be classified as 'reticulonodular.' Any pattern of crisscrossing lines will create small polygonal networks: a reticular group. Focal interstitial thickening will appear as nodular opacities. Unfortunately, the category of 'reticulonodular' interstitial lung disease encompasses too broad a differential list; too many entities can be nonspecifically identified as 'reticulonodular.' Therefore, one of the first hurdles to overcome in separating the various interstitial diseases is to recognize the more specific and dominant underlying pattern. These underlying patterns may be classified as: Peripheral Reticular, Cystic, Linear and Nodular patterns. In addition, there is one mixed interstitial-alveolar pattern, which resembles interstitial diseases: the ground-glass pattern. These patterns will be reviewed in detail with particular attention to the means of distinguishing one from the other. These terms are designated with capital letters in order to emphasize that they represent a specific appearance and to distinguish them from the descriptive terms, 'reticular' and 'nodular' which generally describe the shape of opacities. The Peripheral Reticular, Cystic and Linear patterns all produce networks of lines and therefore are all reticular (with a small 'r').

Distribution of opacities such as upper, mid and/or lower lung zone predominance, or diffuse distribution may play an important supplementary role in distinguishing interstitial diseases as some entities have been noted to favor typical distributions. Occasionally, an individual case will fail to be easily categorized into one of these patterns. In that situation, clinical history is the most reliable means to determine the etiology of a given lung disorder.

Peripheral reticular pattern

As the name implies, the dominant feature of this group of diseases is the appearance of a fine network of lines producing a series of polygonal spaces. The most important characteristics of this pattern are the small size of the cystic spaces, usually less than 5 mm in diameter, and their peripheral distribution (Fig. 1, Fig. 2). At times, the pattern will appear as a faint increased opacity, white region, with many small black holes within it, an appearance which has been likened to lace.

Cystic pattern (central reticular pattern)

In this pattern the network of lines tends to take a rounded contour, producing moderately sized spaces about 5–15 mm, which are seen in the more central portions of the lung and spare the outermost one or two centimeter of the lung on the PA chest radiograph (Fig. 3, Fig. 4). This is in contrast to the Peripheral Reticular pattern, which has smaller spaces, usually less than 5 mm, and is seen in the peripheral regions of the lung. In some instances, the Cystic Pattern is seen as prominent ring

Linear pattern

The Linear pattern appears as long white lines, radiating from the hilum and also lines running perpendicular to the chest wall (Fig. 5, Fig. 6). These are Kerley A and Kerley B lines, respectively. These lines were first described by Peter Kerley in 1933. The increased Kerley A lines form a star burst centered on the hilum. Most radiologists can recognize Kerley B lines, which appear as fine, approximately one centimeter long horizontal lines perpendicular to the lateral chest wall on PA chest

Nodular pattern

The nodular pattern, as the name implies, appears as numerous tiny nodular densities. A cluster of small nodular opacities will appear as small white nodules with intervening black spaces between the nodules. The observer must learn to recognize that the white nodules are round and the intervening black spaces are irregular. This is the opposite of the reticular pattern where the black holes are round and the surrounding interstitial fibrosis is irregular.

Unfortunately, the Nodular pattern has

Ground-glass pattern

This is a term borrowed from the CT literature and is used to describe a faint opacity which slightly increase the overall opacity of the lungs. This appears as a faint haze throughout the lung fields on chest radiographs. It is often difficult to distinguish ground glass opacities from an underexposed radiograph. However, ground-glass opacities on chest radiographs will cause the vascular and interstitial lines to be indistinct and unlike ground-glass opacities on CT, the ground-glass pattern

Conclusion

Table 1 summarizes the causes of the five patterns of diffuse infiltrative lung disease. The plain film evaluation of diffuse interstitial lung disease is often a daunting task. However, armed with a systematic approach to evaluate the radiograph, a pertinent differential diagnosis may be generated and, with appropriate clinical history, a specific diagnoses may often be made.

Background

Pulmonary complications are associated with mortality in immunocompromised patients. The usefulness of bronchoscopy has been reported. However, clinical factors and procedures that influence diagnostic yield are still not established.

Materials and methods

We retrospectively analyzed 115 bronchoscopies performed on 108 immunocompromised patients, defined as those who take corticosteroids and/or immunosuppressants. We evaluated clinical factors, sampling procedures, final diagnosis, and severe complications of bronchoscopy.

Results

The clinical diagnosis was obtained in 51 patients (44%). Of those, 33 cases were diagnosed as infectious diseases and 18 as non-infectious diseases. Nine out of 115 cases (7.8%) initiated new immunosuppressive treatment for an underlying disorder based on the negative microbiological results obtained with bronchoscopy. Collagen vascular disease was the most common underlying disorders (62 patients, 54%). Bronchoscopy was useful regardless of whether the patient was immunosuppressed to treat collagen vascular disease (P = 0.47). Performing transbronchial biopsy correlated with better diagnostic yield of bronchoscopy (54.7% vs 35.5%, P = 0.049). Other clinical factors, such as radiological findings, respiratory failure or antibiotic use at the time of bronchoscopy did not significantly influence diagnostic yield. Respiratory failure requiring intubation after bronchoscopy occurred only in one case (0.9%).

Conclusions

Our study implied the transbronchial biopsy may be a useful procedure for reaching a diagnosis in immunocompromised patients with pulmonary infiltrates. In addition, our data suggest the usefulness of bronchoscopy for immunocompromised patients due to the treatment of collagen vascular disease as well as other underlying disorders.

Introduction

The number of immunocompromised patients has been increasing as the population of transplant recipients has grown [1,2]. In addition, the survival of immunocompromised patients had become longer due to emerging new immunosuppressive medications [3,4]. However, pulmonary complications are reported to be correlate with mortality in immunocompromised patients [[5], [6], [7], [8]]. Lung is one of the most common sites of infection, and immunocompromised patients can be infected with low virulence organisms. Pulmonary complications also include non-infectious disease associated with the underlying disorder. Treatment of infection is anti-pathogenic therapy and treatment of non-infectious lung complications is often immunosuppressive; thus, proper diagnosis of pulmonary complications is essential for immunocompromised patients.

Diagnosis of pulmonary complications in immunocompromised patients is often difficult. First, lung infection in immunocompromised patients can be caused by opportunistic pathogens, including low virulence bacteria, mycobacteria, fungus, and virus [9]. Second, immunocompromised patients can develop various non-infectious lung complications associated with underlying disorders. For example, patients with collagen vascular disease (CVD), who often need immunosuppressive medications, frequently develop interstitial pneumonia, drug-induced pneumonitis, or pulmonary hemorrhage [8,10]. Additionally, lung is sometimes involved with chronic graft versus host disease in patients who receive hematopoietic stem cell transplantation [6]. For microbiological or pathological examinations with lower respiratory tract samples, bronchoscopy would be useful to establish diagnosis of lung complications.

Several studies have investigated the diagnostic usefulness of bronchoscopy for immunocompromised patients. Recent reviews have demonstrated the diagnostic yield of bronchoscopy as 28–77% [11,12]. While diagnostic yield tends to be higher when underlying etiology is infectious disease [[13], [14], [15]], influence on diagnostic yield by other clinical factors or procedures that influence the diagnostic yield, such as transbronchial biopsy (TBB), are inconsistent among the previous studies. For example, Jain et al. reported that the diagnostic yields of TBB or bronchoalveolar lavage (BAL) for non-HIV-infected immunocompromised patients were similar (38%), and the diagnostic yield increased to 70% when TBB and BAL were combined [13]. On the other

hand, three retrospective studies, assessing the usefulness of bronchoscopy after hematopoietic stem cell transplantation, demonstrated that TBB provided limited additional diagnostic information compared to BAL [[16], [17], [18]].

Here, we report a retrospective observational study conducted at the University of Tokyo Hospital. The patients were immunocompromised and had lung complications. The aim of our study was to identify the contributing factors for diagnosis by bronchoscopy.

Study design and subjects

Bronchoscopy was performed at the Department of Respiratory Medicine in the University of Tokyo Hospital during November 2016 to October 2021. We evaluated each bronchoscopy independently for patients who underwent multiple bronchoscopies. Inclusion criteria were as follows; (1) immunocompromised patients with an abnormal lung shadow on computed tomography (CT) scan, (2) patients who underwent diagnostic flexible bronchoscopy for the suspicion of non-neoplastic lung disorder, and (3) patients

Patient characteristics

A total of 1259 bronchoscopic examinations were performed on 1063 patients during the study period, and 115 bronchoscopies performed on 108 immunocompromised patients were included in this study. One hundred and four patients underwent bronchoscopy once, 3 patients twice and 1 patient five times. The clinical characteristics are provided in Table 1. CVD was the most common underlying disorder for immunosuppression, followed by hematopoietic stem cell and solid organ transplantation. Respiratory

Discussion

The diagnostic usefulness of bronchoscopy for immunocompromised patients with lung complications has been controversial. In the present study, we found that the diagnostic yield with the patients who received TBB tended to be higher than those who did not. The diagnostic yield in patients with CVD was 47%, which was comparable to patients with other diseases. Additionally, we demonstrated that bronchoscopy can be safely performed in immunocompromised patients.

The overall diagnostic yield of

Conclusions

Overall, our study demonstrated the usefulness of bronchoscopy for diagnosing pulmonary complications of immunocompromised patients who took corticosteroids or immunosuppressants, including patients with CVD. Furthermore, our results imply that TBB was safe and can contribute to additional diagnostic information.

One hundred individuals who had undergone both high resolution computed tomography (HRCT) and chest radiography were studied to determine the accuracy of each technique in establishing the diagnosis of diffuse lung disease. The group consisted of 86 patients with a diagnosis of a chronic diffuse infiltrative lung disease and 14 normal subjects. Two independent observers assessed the HRCT examinations and chest radiographs and recorded the three most likely diagnoses. Overall a confident diagnosis was reached more often with HRCT (49%) than with chest radiography (41%). The diagnoses were correct in 82% of HRCT examinations and 69% of chest radiographs. Diagnoses made on HRCT, irrespective of the degree of certainty, were accurate more often than diagnoses made on chest radiography (56% and 47% respectively). Of the patients thought to have a normal chest radiograph, 42% had diffuse infiltrative lung disease (DILD). Of the patients thought to be

normal on HRCT, 18% had DILD. Conversely, normal subjects were correctly identified as such in 82% of chest radiographs and in 96% of HRCT examinations. This study emphasizes the important role of CT in helping to confirm or refute the presence of abnormality when the chest radiograph is normal or questionably abnormal, and underlines the superior diagnostic accuracy of HRCT compared with conventional chest radiography in DILD. Introduction

Lung squamous cell carcinoma (LUSC) usually shows expansive growth with large tumor nests; few reports on invasive growth patterns (INF) in LUSC have been associated with poor prognosis in gastrointestinal and urothelial cancers. In this study, we examine the association between INF and the prognosis of LUSC.

Materials and methods

We analyzed INF as a potential prognostic factor in 254 consecutive patients with LUSC who underwent complete surgical resection at our hospital between 2008 and 2017. INF was classified into 3 categories based on the structure of the tumor other than the large round solid nest of tumor cells.

Results

INF was categorized as INFa in 59 patients (23 %) with only well-demarcated large solid tumor cell nests, INFb in 89 patients (35 %) with medium to small, alongside large solid nests, and INFc in 98 patients (39 %) with cord-like/small nests or isolated cells plus large or medium solid nests. No significant lymph node metastasis differences were observed between INFc and INFa/b tumors. However, in patients with p-stage I, INFc had a poorer prognosis with regard to recurrence-free survival (RFS), with a 5-year RFS rate of 53.3 %, compared to 74.9 % for INFa/b ($p = 0.010$).

Conclusion

Our study highlights a novel pathological concept of INF in LUSC, and contributed to the proposal that it is a factor indicating an unfavorable prognosis in patients with early-stage LUSC. A prospective multicenter study is warranted for INFc patients, as careful follow-up and adjuvant chemotherapy might lead to the early detection and prevention of recurrence.

Introduction

Lung cancer is the leading cause of cancer-associated death, and the number of deaths has been reported to be 1.8 million annually worldwide [1]. The most common histological type of lung cancer is adenocarcinoma, followed by squamous cell carcinoma. In lung adenocarcinoma, morphological classification of the predominant subtype has been established, and there are many reports on the association between morphological class and patient prognosis [2]. On the other hand, there are few reports to date on the pathological factors associated with patient prognosis in lung squamous cell lung carcinoma (LUSC).

The most frequently observed LUSC was previously the central type, but in recent years, the peripheral type has increased in proportion [3]. The growth pattern of LUSC is generally focal tumor nests; however, infiltrating lesions with vesicular or solitary cells have also been observed [4], which closely resemble infiltrative growth or tumor budding, and has been reported as a prognostic factor of colorectal cancer [5]. The infiltrative growth pattern (INF) indicates the pattern of tumor infiltration into the surrounding tissue, and was reported to be associated with patient prognosis, mainly in gastrointestinal cancers. INF has also been shown to be a prognostic factor in urothelial carcinoma [6], in which focal tumor nests similar to those of LUSC are often observed.

In the present study, we investigated the pathological grading of INF and its association with prognosis in patients with surgically resected LUSC. We propose a novel pathological classification of INF in LUSC, which provides insights for prognosis prediction as well as the development of potential treatment strategies.

Patient cohort

A total of 1927 consecutive lung cancer patients underwent lobectomy or other surgical resection at our hospital from January 2008 to December 2017. Of these, 254 patients were diagnosed with LUSC. These patients underwent lobectomy or extended pneumonectomy, underwent systematic lymph node dissection, and had no visible residual cancer (R0). Patients with probable metastatic LUSC from other primary lesions and those who had received preoperative chemotherapy or radiation therapy were excluded.

High frequency of pathologically invasive factors in patients with INFc

The patient characteristics is shown in Supplement Table 1-2. Of 254 patients, 210 (83 %) were men, 253 (99 %) were smokers. In 246 patients (96.8 %), lobectomy or bilobectomy was performed, sleeve resection in 5, and pneumonectomy in 8 patients (3.2 %). Peripheral type of lung cancer was found in 229 patients (90 %), central type in 25 patients (10 %). Median tumor size was 3.4 (0.5–16) cm. Pathological lymph node metastasis (pN1 or pN2) was present in 86 patients (34 %). VPI was found in 110

Discussion

In this study, we aimed to investigate whether INF, a pathological factor associated with tumors, is associated with an unfavorable prognosis in patients with surgically resected LUSC. Our main findings were as follows. (i) INFc was strongly associated with visceral pleural invasion, vascular invasion, and lymphatic permeation, though lymphovascular invasion was frequent in INFa/b, (ii) INFc was associated with an unfavorable prognosis in patients with p-stage I surgically resected LUSC

Abstract

Background

Heated tobacco products (HTPs) have been marketed as safer alternatives to conventional cigarettes, but emerging evidence suggests potential respiratory risks. We present a case of pulmonary complications associated with IQOS, a popular HTP, contributing to the growing understanding of these risks.

Case description

A 40-year-old chronic smoker switched to IQOS, consuming 1.5 packs per day. He presented with incidental chest radiographic abnormalities and peripheral eosinophilia. Computed tomography of chest revealed pulmonary nodules and ground glass opacities. Bronchoscopy indicated mild eosinophilia. After ruling out other causes, a lung biopsy was recommended but declined. Discontinuation of IQOS led to symptom resolution and radiographic improvement. This case adds to a limited literature on HTP-induced lung injury, with a unique presentation and favorable response to cessation.

Conclusions

The case highlights potential pulmonary complications and the first describing an organizing pattern of lung injury associated with IQOS use, emphasizing the importance of recognizing and

discontinuing HTPs in patients with respiratory symptoms or radiographic abnormalities. Further research is needed to elucidate the mechanisms underlying the harmful effects of HTPs and inform public health policies. This case underscores the importance of monitoring and educating individuals about the potential risks of HTPs to respiratory health, especially in the context of smokers switching to these products.

Abbreviations

HTPs

Heated tobacco products

CCs

Conventional cigarettes

AEP

Acute eosinophilic pneumonia

HPHCs

Harmful and potentially harmful compounds

1. Introduction

Heated tobacco products (HTPs) are a new class of tobacco products that heat tobacco to a high temperature, but do not burn it. This results in the production of an aerosol that contains nicotine, but not the harmful carcinogens that are produced when tobacco is burned. Hence, HTPs have been marketed as purportedly safer alternatives to conventional cigarettes (CC) [1]. However, emerging evidence suggests that HTPs may still carry potential risks, including several pulmonary complications.

Caputi et al. highlighted an association between HTPs use and respiratory symptoms, including cough and phlegm production [2]. Furthermore, a systematic review by Glantz and Bareham indicated that HTPs aerosols contain a wide range of harmful chemicals, some of which are known to cause lung inflammation and damage [3]. One of the most popular HTPs is IQOS, which is made by Philip Morris International. IQOS is marketed as a "heat-not-burn" tobacco product, and it is sold in over 40 countries [4]. However, there have been several reports of pulmonary problems in people who use IQOS. A recent review described 58 cases of e-cigarette-related respiratory disease, including acute lung injury, organizing pneumonia, eosinophilic pneumonia, and acute respiratory distress syndrome [5]. These studies suggest that HTPs can cause several pulmonary problems.

Despite the limited data on the long-term health effects of HTPs, it is essential to document and analyze specific cases to further our understanding of the potential risks they pose to respiratory health. This case report aims to present and discuss a unique case of pulmonary complications associated with the use of IQOS after switching from CCs, contributing to the growing body of evidence regarding the adverse effects of HTPs on the respiratory system. In the context of HTPs, case reports can contribute to our understanding of the pathophysiology, clinical presentation, diagnostic challenges, and treatment considerations related to pulmonary complications.

2. Case description

A 40-year-old Lebanese gentleman was referred from the pre-employment medical commission department for further evaluation of an abnormal chest radiograph. He did not present with any systemic complaints such as fever, cough, chest pain, difficulty breathing, hemoptysis, anorexia, or weight loss. He had no significant past medical, surgical, or family history, except for a sleeve gastrectomy. The patient denied taking any long-term medications, supplements, alcohol, or recreational drugs. However, he had been a chronic smoker for the past 20 years, initially using CCs, smoking a pack per day, but in the last 6 months, he had switched to IQOS and increased his consumption to 1.5 packs of IQOS or more per day over the last few months prior to presentation, due to job-related stress. He recently started working as an entrepreneur and prior to 6 months he was the manager at a printing press with no exposure to printing materials. He does not have any pets at home.

On examination, the patient appeared well built and nourished with vital signs within normal limits. He had no pallor, clubbing, cyanosis, or jaundice on general examination. Chest examination revealed normal bilateral vesicular breath sounds with no added sounds. Other system examinations were unremarkable.

Blood tests revealed leukocytosis, with a white blood cell count of 10.8 × 10^3/μL, and eosinophilia of 3.2 × 10^3/μL. A peripheral smear indicated mild toxic features in the leukocytes, along with some reactive lymphocytes and moderate eosinophilia, possibly reactive in nature. Liver function and renal function tests were within normal limits. Chest radiograph reported ill-defined nodular shadows in the right mid and lower zones. A computed tomography (CT) scan of the chest revealed multiple scattered variable-sized nodules and patchy, partially solid opacities with surrounding ground glass opacification (Fig. 1). These findings were predominantly distributed sub-pleurally and along the peri-bronchovascular region. Centrilobular emphysematous changes were observed bilaterally in the upper lobes, while minimal pleural and pericardial fluid was detected. No significant mediastinal or hilar lymphadenopathy was identified. Bronchoscopy demonstrated normal bronchial anatomy, and the cytology differential showed 89 % macrophages, 5 % neutrophils, 4 % lymphocytes, and 2 % eosinophils. Cultures for bacteria, viruses, fungi, and tuberculosis were negative. Cytopathology results were negative for malignancy.

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Fig. 1. Computed Tomography Chest Images of Pulmonary window at the level of outset of segmental bronchus.

Panel A: Variable sized nodular and patchy part solid opacities with surrounding ground glass opacification, showing subpleural and peribronchovascular predominance distribution. Panel B: Resolution of the opacities.

Considering the differential diagnosis, further assessment with a lung biopsy was recommended, but the patient declined any further invasive interventions. IQOS cessation was strongly advised, leading him to stop using IQOS and switch back to conventional cigarettes. A repeat chest CT scan after 6 weeks showed resolution of the multiple bilateral lung lesions observed in the previous scan. No medications were administered during this period.

3. Discussion

In our patient, the presence of peripheral eosinophilia and the observed improvement in nodular pulmonary opacities following cessation of IQOS usage strongly suggests the occurrence of acute lung injury in an organizing pneumonia pattern secondary to IQOS. Through a thorough evaluation, we have systematically ruled out other potential causes, including acute and chronic infections, vasculitis, autoimmune etiologies, or concomitant medications that could lead to lung injury. Notably, our patient remained asymptomatic, and the radiographic findings were incidental. However, given more time, there is a potential for the condition to have evolved into eosinophilic pneumonia, which, to date, is the only pathology attributed to the use of HTPs. The radiological findings our case predominantly correspond to an organizing pneumonia pattern. To our current knowledge, this is the first case report describing and detailing such a pattern of pulmonary injury.

There are only four other documented case reports in the literature describing the development of acute eosinophilic pneumonia (AEP) following exposure to HTPs, as summarized in Table 1 [[6], [7], [8], [9]]. AEP is a rare but serious lung condition that is characterized by inflammation and the accumulation of eosinophils, in the lungs. Most patients with AEP with identifiable causes are current smokers, and changes in smoking habits, such as newly starting smoking, increasing the number of cigarettes smoked, or changing the cigarette brand, can cause AEP [10,11]. AEP potentially induced by switching from CCs smoking to HTPs has only been described in one of the cited cases. All four summarized cases exhibited a relatively short duration of presentation and demonstrated a favorable response to discontinuation of HTPs usage along with a course of corticosteroids. It is noteworthy that one of the reported cases presented as fulminant AEP, necessitating critical care and extracorporeal membrane oxygenation support. The accumulation of these case reports, including our current case, highlights the need for increased awareness regarding the potential pulmonary complications associated with the use of IQOS and similar HTPs. These findings underscore the importance of promptly recognizing and discontinuing HTP usage in patients presenting with respiratory symptoms or radiographic abnormalities. Furthermore, the successful response to corticosteroid therapy in the reported cases suggests a potential role for immunomodulatory treatment in managing such cases of HTP-induced acute lung injury.

Table 1. Summary of case reports of HTPs induced lung injury.

Study	Patient	Symptom	Chest Radiograph	CT chest	Bronchoalveolar Lavage	Treatment
Tajiri et al. [6]	47-year-old lady switched from CC to HTP, 4 months prior to referral	Nonproductive cough followed by fever and malaise. Duration: short (exact duration not available)	Bilateral infiltrates	Patchy GGO with interstitial thickening	Eosinophil: 72 %	Prednisolone
Kamada et al. [7]	20-year-old male on HTP for 6 months and doubled the	Fever and shortness of breath requiring 10L/min oxygen: Duration: 1 day	Bilateral infiltrates	Bilateral infiltration with smooth interlobular septal	Eosinophil: 60 % Lymphocyte: 20 %	Prednisolone for weeks with dram improvement on radiologically. No recurrence on

Study	Patient	Symptom	Chest Radiograph	CT chest	Bronchoalveolar Lavage	Treatment
	daily use 2 weeks prior to symptoms			thickening and pleural effusion		stopping predniso after stopping HT
Aokage et al. [8]	16-year-old man known to have childhood asthma on HTP	Severe cough, fatigue and dyspnea Duration: 2 weeks Progressed to ARDS requiring ECMO despite IV methylprednisolone	GGO on CXR	Consolidation with mosaic pattern	Eosinophil: 14.7 % Neutrophil: 51.7 % Lymphocyte:33.6 %	Methylprednisolo 1 gm IV x 3 days followed by prednisolone 60 for 5 days. On ECMO for 10 with CT chest on showing healthy
Kang BH et al. [9]	22-year-old lady smoking HTP's for 2 weeks and increased from 6/day to 15/day just before symptoms onset	Duration: 1 day	Bilateral infiltrates	Bilateral multifocal patchy consolidation with smaller nodular GGO and interlobular septal thickening	Eosinophil:62 % Lymphocyte:15 % Macrophage:14 %	Rapid improveme with IV methyl prednisolone wit normal CXR at 14

HTPs contain tobacco leaves that are heated and therefore have a unique aerosol with distinct toxicity different from e-cigarettes and conventional cigarettes (CC). The product that our patient was smoking, IQOS, was the first HTP launched in 2014. It comprises of disposable heat sticks, which are glycerin-soaked tobacco sticks heated by an electric blade when inserted in a holder [12]. HTPs were created to resemble the taste of CC and serve as an alternative for cases where e-cigarettes failed to create the desired effect of CC authenticity [13]. However, HTPs have been shown in peer-reviewed articles, mostly funded by HTP manufacturers, to reduce exposure to harmful and potentially harmful compounds (HPHCs) that are produced only at combustion temperatures [[14], [15], [16], [17]]. Nevertheless, HTPs contain more than 50 constituents that are cytotoxic and genotoxic, which are not included in the FDA's list of HPHCs, and they are present at significantly higher percentages than in CCs [18].

The portrayal of HTPs as a safe product has created a concerning trend of attracting non-smokers to start using HTPs, in addition to the profile of conventional smokers who want to reduce or stop smoking CC, similar to the profile of electronic cigarettes [[19], [20], [21]]. Because HTPs are less satisfying than CC, they are used more frequently, often exceeding specified doses, although nicotine levels are similar to CC [22,23]. The results of in vitro experiments on human primary keratinocytes and bronchial epithelial cells highlight the comparable cytotoxic effects of cigarette smoke extract from CCs and HTPs, indicating their potential carcinogenicity [24]. HTP

aerosol exposure was found to induce cytotoxicity, oxidative stress, and inflammatory responses, with a greater generation of reactive oxygen species observed in intensive HTP exposure scenarios compared to CCs [13]. Transcriptomic changes associated with oxidative stress were more prominent at 4 hours of exposure, suggesting early adaptive mechanisms and antioxidant responses that varied based on the intensity of exposure [13]. Moreover, in a mouse model exposed to an intensive puffing regime of HTPs, characterized by 5 hours of exposure per day over a 2-week period, significant increases in proinflammatory cytokines, chemokines, and CD4+RORγt + T cells were observed in bronchoalveolar lavage fluid [[25], [26], [27], [28], [29]]. These findings shed light on the underlying cellular mechanisms contributing to various inflammatory pathologies like organizing pneumonia in our case, respiratory infections, inflammation, autoimmune diseases, asthma, and chronic obstructive pulmonary disease (COPD).

4. Conclusions

The data presented in this study emphasize the potential health risks associated with HTPs, as they induce cytotoxicity, oxidative stress, and inflammation. Further research is warranted to elucidate the specific molecular pathways involved in these processes and to evaluate the long-term effects of HTPs use on human health. Understanding the mechanisms underlying the harmful effects of CCs and HTPs can inform public health policies and interventions aimed at reducing tobacco-related diseases and promoting healthier alternatives.

Severe COVID-19 can lead to extensive lung disease causing lung architectural distortion. In this study we employed machine learning and statistical atlas-based approaches to explore possible changes in lung shape among COVID-19 patients and evaluated whether the extent of these changes was associated with COVID-19 severity. On a large multi-institutional dataset (N = 3443), three different populations were defined; a) healthy (no COVID-19), b) mild COVID-19 (no ventilator required), c) severe COVID-19 (ventilator required), and the presence of lung shape differences between them were explored using baseline chest CT. Significant lung shape differences were observed along mediastinal surfaces of the lungs across all severity of COVID-19 disease. Additionally, differences were seen on basal surfaces of the lung when compared between healthy and severe COVID-19 patients. Finally, an AI model (a 3D residual convolutional network) characterizing these shape differences coupled with lung infiltrates (ground-glass opacities and consolidation regions) was found to be associated with COVID-19 severity. Abbreviations

COVID-19

Coronavirus disease of 2019

CT

Computed Tomography

AI

Artificial Intelligence

ARDS

Acute respiratory distress syndrome

RT-PCR

Reverse transcription polymerase chain reaction

NLST

National Lung Screening Trial

CNN

convolutional neural network

SDF

Signed distance function

AUC

Area under the receiver operating characteristic curve

DSC

Dice similarity coefficient

GLCM

Gray Level Cooccurence Matrix

GLRLM

Gray Level Run Length Matrix

GLSZM

Gray Level Size Zone Matrix

GLDM

Gray Level Dependence Matrix

NGTDM

Neighboring Gray Tone Difference Matrix

LASSO

Least Absolute Shrinkage and Selection Operator

ROC:

receiver operating characteristic curve

1. Introduction

The on-going Coronavirus disease of 2019 (COVID-19) pandemic has prevailed in the form of multiple mutant variants including the most recent Omicron variants BA.4 and BA.5 [1,2]. In spite of administration of multiple vaccine doses, COVID-19 has led to severe respiratory complications and numerous deaths [3]. Chest computed tomography (CT) examination demonstrates pathological changes in the lung, mostly as lung infiltrates (ground-glass opacities and consolidations), especially in the peripheral regions of the lung [4]. Recently, studies have illustrated the importance of chest CTs not only for diagnosis of COVID-19 but also for staging and monitoring disease progression [5].

With COVID-19 cases continuing to remain high, artificial intelligence (AI) based approaches have been developed using CT or chest radiographs for COVID-19 diagnosis [6,7] and distinguishing COVID-19 disease from other causes of pneumonia ([8], p. 1). Additionally, a few studies have also developed AI-based biomarkers for COVID-19 prognosis identifying severe COVID-19 patients with high risk of mortality [9] or those with need of mechanical ventilator [10].

Recent studies have shown that severe COVID-19 disease can lead to complications such as pneumonia, acute respiratory distress syndrome (ARDS), and sepsis [11]. These complications may further cause acute lung damage followed by pulmonary fibrosis and chronic impairment of lung function ([12], p. 19). While for some patients, damage caused to the lung may ameliorate over time, for others, lung damage may persistent and cause permanent loss of lung function [13]. While it is known that severe COVID-19 disease may potentially cause extensive lung damage and persistent architectural distortion of the lung, to the best of our knowledge, there has been no quantitative effort to study impact of disease severity on lung structure, shape and morphology during early onset of the disease.

While previous studies have used deep learning approaches on CT scans for COVID-19 prognosis [14,15], most of these approaches rely on biomarkers derived from lung infiltrates for their analysis. However, the objective of this study was to explore the impact of severity of the disease on lung shape and morphology using baseline CT scans acquired at the time of COVID-19 diagnosis. On a large multi-institutional dataset (N = 3443), at first, three different populations were defined; a) healthy (no COVID-19), b) mild COVID-19 (no ventilator required), c) severe COVID-19 (ventilator required) and separate statistical population-atlas models were built and compared to understand differences in shape and appearance of the lung. Subsequently, these statistical models were further integrated with deep learning to learn the extent of lung deformation associated with severity of the disease. Finally, deep learning derived features of lung deformation and shape differences were integrated with deep learning derived features of lung infiltrates to create a prognostic biomarker for COVID-19.

2. Methods and materials

2.1. COVID-19 study population

Patients with COVID-19 disease as confirmed with reverse transcription polymerase chain reaction (RT-PCR) who underwent CT evaluation for diagnosis were identified for this study. This study included a retrospective cohort of N = 3230 patients with data acquired from four different sources; N = 835 patients from D_1 (Renmin Hospital of Wuhan University), N = 113 from D_2 (University Hospitals Cleveland Medical Center), N = 2000 from D_3 **(**a publicly available challenge dataset: Study of Thoracic CT in COVID-19 (STOIC) ([16], p.)), N = 282 from D_4 (a publicly available dataset from The Cancer Imaging Archive (TCIA) release by the Stony Brook University). The dataset from source D_1 was stratified based on the severity, and randomly split into 50 % training (D_1^{train}: N = 416) and 50 % testing (D_1^{test}**:** N = 419), while data from the sources D_2, D_3 and D_4 were all used as an external independent test set to test our hypothesis. Summary characteristics with detailed information of cohorts D_1-D_4 including inclusion and exclusion criteria are provided in the supplementary section, S1. Additionally, details of CT acquisition parameters are presented in Table S1.

Additionally, a randomly selected set of N = 213 patients from *the National Lung Screening Trial (NLST) Spiral CT abnormalities dataset* ([17,18]; [19]; [20]) marked as "Other minor abnormality noted" was used as part of a control cohort (D_5).

All patients from datasets D_1-D_5 were grouped into three categories based on severity; a) healthy patients (control cohort) with no COVID-19 disease), b) mildly affected COVID-19 patients (patients who did not require a mechanical ventilator), c) severely affected COVID-19 patients (patients who required a mechanical ventilator).

2.2. Manual delineations of lung and lung infiltrates

A board-certified cardiothoracic radiologist with 14 years of experience delineated lung regions and lung infiltrates (ground-glass opacities and consolidations) on the training set D_1^{train} (N = 416). The annotations were made using an open source software 3D Slicer v4.10 [21]. For the rest of the patients in the test sets (D_1^{test}, D_2, D_3 and D_4), automated lung and lung infiltrate segmentations were obtained using a U-Net based convolutional neural network (CNN) trained from D_1^{train}.

2.3. Population based lung shape difference atlas (D_A) construction

2.3.1. Atlas construction with single split of training data

Difference atlases are often used to identify significant shape variations in anatomic regions between different populations using statistical approaches [22,23]. The characteristics of shape differences as identified by difference atlases are further used to predict disease diagnosis and prognosis. In our study, we constructed a difference atlas ($\mathbf{D_A}$) to identify significant shape differences between COVID-19 patients with mild and severe disease. Fig. 1 shows the flowchart explaining construction of a difference atlas ($\mathbf{D_A}$) through a population-atlas model. The population atlas model takes CT scans of two sets of population separately as inputs and outputs $\mathbf{D_A}$ depicting statistically significant shape differences between the input populations.

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Fig. 1. Population-based difference atlas construction identifying statistically significant shape differences between the two populations. At first, the lung regions in the chest CTs are manually annotated and a template is chosen based on the median lung volume of a particular population. As part of the second step, all the CT volumes are registered to the chosen template and corresponding transformations are applied to the lung masks. Subsequently, a signed distance function is calculated on all the lung masks in the template space to represent shape information as a volume. Finally, a difference atlas ($\mathbf{D_A}$) is generated using a generalized linear model-based T-test with 500 permutations.

Detailed steps of generating $\mathbf{D_A}$ are given below.

- Step 1: Population splits

COVID-19 CT scans were divided into two populations based on the severity of the disease; For example, patients with mild COVID-19 disease defined as one set of population and patients with severe COVID-19 disease requiring a mechanical ventilator as the other set of population.

- Step 2: Template selection

A representative template $\boldsymbol{T}$ was chosen based on median lung volume from patients with milder disease among the two populations. For example, when constructing $\mathbf{D_A}$ between mild and severe COVID-19 patients, a template is chosen from mild COVID-19 cohort. For, $\mathbf{D_A}$ between healthy

patients healthy and mild COVID-19 patients, template is chosen from the healthy cohort. Similarly, for constructing $\mathbf{D_A}$ between healthy and severe COVID-19 patients, template is chosen from the healthy cohort**.**

- Step 3: Registration of all CT scans to the template space, **T**

All CT scans were registered to a common canonical frame of reference, ***T***. A rigid followed by a b-spline deformable co-registration was performed. For rigid registration, mattes mutual information was used as a similarity metric with number of histogram bins set to 32. The similarity metric was optimized using gradient descent, a maximum of 500 iterations per resolution. A linear interpolator was used for rigid registration. Subsequently, to initialize deformable registration manually segmented lung masks were given as inputs to registration framework to provide anatomical constraints and improve registration accuracy. The anatomical constraints would help in making sure that the lung CT was sufficiently registered at the same time not introducing unnatural deformations in the lung. For the deformable registration, standard gradient descent optimizer and normalized correlation coefficient to measure the similarity between fixed and transformed volumes [24] was used. The step size along the search direction for gradient descent was determined as a decaying function of the iteration loop with scalar constant set at 25,000 to attain a close fit. A multi-resolution approach with 5 different levels was used with the number of iterations for each resolution set at 2500. Elastix tool v4.9 was used to perform both rigid and deformable registrations in this study [25].

- Step 4: Volumetric representation of the shape of the lung using signed distance function

All the registered volumes were first isotopically scaled to 2 mm^3 resolution. A signed distance function (SDF) was applied to the corresponding binary lung masks in the template space. As opposed to a binary representation of a lung mask where each voxel within the lung is represented as 1 and voxels outside the lung are represented as 0, the SDF is defined as;ßßßßßSDF={−d(x,ß)(x∈ß+)0(x∈ß)d(x,ß)(x∈ß−)Where β represents the boundary of the lung, β+ represents the voxels inside the boundary and β-represents the voxels outside the boundary. −d(x1x2) represents the Euclidean distance between the voxels x1 and x2. Python 3.11 with modules numpy (v1.26.4) and SimpleITK (v2.3.1) was used to apply SDF to volumetric images.

- Step 5: Difference atlas (D_A) using a non-parametric Generalized Linear Model (GLM) based *t*-test

As described in a previous study [24], at each voxel location in the template space (***T***), distributions of voxel values (based on signed distance function) between CT scans of two populations were tested for statistical significance using Generalized Linear Model (GLM) based *t*-test. The statistical testing was performed with 500 random permutation testing correcting for multiple comparisons [26]. Threshold free cluster enhancement (TFCE) method was used to correct for multiple comparisons [26]. The categorical variable, patient population (for example mild or severe COVID-19 disease) was considered as an exploratory variable and the signed distance function as a dependent variable for GLM based *t*-test. A “randomize” function from “fsl” library [27] was used to perform GLM based *t*-test. The method provides a test statistic image and sets of p-values**.** The p-values obtained from the statistical test were further represented as $\mathbf{D_A}$**.**

2.3.2. Ensemble difference atlas (D^E_A) construction using multiple splits of training data

To make the process of $\mathbf{D_A}$ construction more robust, populations were divided into three randomly selected overlapping subsets (~67 % of total samples in each subset) and three different $\mathbf{D_A}$s were

constructed. All the three $\mathbf{D_A}$s were further thresholded at $p > 0.05$. Subsequently, an ensemble method with majority voting technique on the thresholded binary masks of $\mathbf{D_A}$ to create a final ensemble difference atlas ($\mathbf{D^E_A}$). The supplementary figure, Fig. S1, illustrates the process of generating $\mathbf{D^E_A}$.

Considering pairs of populations, $\mathbf{D^E_A}$s were constructed identifying regions of statistically lung shape differences between; a) healthy patients from D_5 and mild COVID-19 patients from D_1^{train}, b) healthy patients from D_5 and severe COVID-19 patients from D_1^{train} and c) mild and severe patients from D_1^{train}. Additionally, differences in lung volumes and mean intensities within the lung between the populations were also reported using violin plots.

2.4. AI based quantification of lung shape differences

At first, the ensemble difference atlas ($\mathbf{D^E_A}$) constructed between mild and severe COVID-19 patients was transformed back from template space to the corresponding original image space using the registration approach as used for atlas creation. In this case, the moving image for registration was considered to be the ensemble difference atlas ($\mathbf{D^E_A}$) while the corresponding input CT scans were considered as fixed images. Previous studies Eppel, [28] have shown that using binary mask of regions of interest (ROIs) as an additional channel (priors) along with an image input can help the network focus their attention on the ROIs while still extracting contextual information from the whole image. Similarly, in this study, a 3D CNN (M_1) was used to characterize population lung shape differences for COVID-19 prognosis. At first, $\mathbf{D^E_A}$ constructed between mild and severe COVID-19 patients was used as a shape prior $\mathbf{S_P}$ (Fig. 2). $\mathbf{S_P}$ was used as an auxiliary channel along with chest CT scans to train M_1 (Fig. 2). A 3-fold cross validation of M_1 was performed on D_1^{train} and ensemble predictions (average predictions) of M_1 from 3-fold cross validation was used to evaluate the model's performance of the test sets D_1^{test}, D_2, D_3 and D_4. Three different base CNN architectures 3D-SEResNet-50 [29,30], 3D-DenseNet-121 [30], 3D-ResNeXt-50 [30] were tested for evaluating architecture sensitivity. The architecture yielding the best cross-validation area under the receiver operating characteristic curve (AUC) on D_1^{train} was chosen as the final base architecture for M_1. Data preprocessing and further implementation details of M_1 are provided in the supplementary section, S2, S3. PyTorch v2.1.2 python module was used to train all CNNs for AI based quantification of lung shape differences [31].

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Fig. 2. Flowchart of CovSafeNet. CovSafeNet consists of two parallel 3D CNNs (M_1, M_2). While M_1 encodes shape differences of the lung between mild and severe COVID-19 patients via a shape prior $\mathbf{S_P}$ (top row), M_2 encodes spatial information of automatically segmented lung infiltrates, $\mathbf{I_P}$ (bottom row). The decisions from M_1 and M_2 are fused at the decision fusion node ($\mathbf{N_F}$) to obtain the final predictions predicting severity of COVID-19.

2.5. Automated segmentations of lung and lung infiltrates

A 3D U-Net [32] model was used to segment lung and lung infiltrate regions on CT scans. All CT scans were first pre-processed by converting them from Hounsfield units to image intensities by assuming the air in the lungs corresponds to a zero-intensity value. To narrow the field of view to the lung within the chest CTs, intensities greater than −300HU were thresholded and subsequently a bounding box containing the largest connected component was extracted. The cropped region was

further resized to 128 × 192 × 96 (x, y, z) and was provided as input to a 3D U-Net. PyTorch v2.1.2 python module was used to train all CNNs for 3D segmentations of lung and lung infiltrates.

2.6. CovSafeNet: integrating lung shape difference biomarker with lung infiltrates

At first, separate 3D U-Net [32] models were used for segmenting lung regions and lung infiltrates on CT. D_1^{train} was further divided into sub-cohorts D_1^{train1} (N = 367) and D_1^{train2} (N = 49) and the 3D U-Net models were trained on D_1^{train1} and evaluated on D_1^{train2} and were subsequently used to automatically segment the lung and lung infiltrate regions of D_1^{test}, D_2, D_3 and D_4.

The segmentation maps outputted by the networks were used as infiltrate prior ($\mathbf{I_P}$) to evaluate detection performance of the network in detecting the infiltrate regions. A region was considered as being detected if ≥ 0.2 Dice similarity coefficient (DSC) overlap existed between the network segmentation map and the ground-truth delineation of that corresponding region. The segmentation accuracy of the detected regions was reported in terms of DSC on D_1^{train2}.

Subsequently, $\mathbf{I_P}$ was then used as an auxiliary channel along with chest CT scans to train M_2 (Fig. 2) to identify COVID-19 patients with severe disease. A 3-fold cross validation of M_2 was performed on D_1^{train} and ensemble predictions (average predictions) of M_2 from 3-fold cross validation was used to evaluate the model's performance of the test sets D_1^{test}, D_2, D_3 and D_4. Three different base CNN architectures 3D-SEResNet-50 [29,30], 3D-DenseNet-121 [30], 3D-ResNeXt-50 [30] were tested for architecture sensitivity. The architecture yielding the best cross-validation AUC on D_1^{train} was chosen as the final base architecture for M_2. Data preprocessing and further implementation details of M_2 are provided in the supplementary section, S2, S3.

Subsequently, CovSafeNet (Fig. 2) framework was built based on decision fusion of two different residual classifiers M_1 and M_2 (average predictions from M_1 and M_2). While M_1 encoded lung structure differences of the lung between mild and severe COVID-19 patients via a shape prior $\mathbf{S_P}$ (Fig. 2), M_2 encoded spatial information of automatically segmented lung infiltrates, $\mathbf{I_P}$ (Fig. 2). A similar 3-fold cross validation strategy on D_1^{train} was used on CovSafeNet and the ensemble predictions (average predictions) of CovSafeNet from 3-fold cross validation was used to evaluate the model's performance of the test sets D_1^{test}, D_2, D_3 and D_4. PyTorch v2.1.2 python module was used to train CovSafeNet [31].

2.7. Comparison of CovSafeNet with clinical and radiomics based machine learning model

2.7.1. Radiomics model, M_R

PyRadiomics [33] package was used to extract the radiomic features from regions of lung shape differences and lung infiltrates. Extracted features included first order statistics, 2D and 3D shape base features, Gray Level Cooccurence Matrix (GLCM), Gray Level Run Length Matrix (GLRLM), Gray Level Size Zone Matrix (GLSZM), Gray Level Dependence Matrix (GLDM), Neighboring Gray Tone Difference Matrix (NGTDM). All CT volumes were resampled to 2 mm^3 isotropic resolution prior to the feature extraction. Two different models M_{R1} and M_{R2} were trained on D_1^{train} with features extracted from regions of population lung shape differences and lung infiltrates, and subsequently evaluated on the test sets D_1^{test}, D_2, D_3 and D_4. The final radiomic classifier M_R was based on decision fusion of M_{R1} and M_{R2}. (addition of predictions from M_{R1} and M_{R2}). The following steps were involved in training the models M_{R1} and M_{R2} at each iteration on every cross-validation fold.

- *Step 1:* Correlated features with a Pearson correlation >0.9 were removed.

- *Step 2*: Constant features which were repeated for more that 25 % of the samples were removed.
- *Step 3:* On the remaining features an outlier removal step was performed, allowing for clipping of the feature values between 1st and 99th percentile and resultant features were normalized in the range [0,1].
- *Step 4*: A Least Absolute Shrinkage and Selection Operator (LASSO) [34] feature selection strategy was applied to select the most optimal radiomic features.
- *Step 5*: XGBoost classifier was trained based on the features selected based on LASSO feature selection.

Python module scikit-learn v1.3.2 was used to train the radiomics model $\mathbf{M_R}$.

2.7.2. Clinical model, M_C

Clinical data was not available for the dataset source D_3. Therefore, clinical features common to D_1^{train}, D_2 and D_4 were selected for the analysis. Creatinine (Cr), aspartate aminotransferase (AST), alanine aminotransferase (ALT), neutrophils count (Neu), lymphocytes count (LYM) and procalcitonin (PCT) information was available for D_1^{train}, D_2 and D_4 cohorts. Subsequently, a similar preprocessing and model training strategy used for training the radiomics based classifiers was used to develop the clinical model $\mathbf{M_C}$. Python module scikit-learn v1.3.2 was used to train the clinical model $\mathbf{M_C}$.

2.8. Statistical analysis

Registration accuracies for atlas construction was evaluated using DSC between binary masks of lung regions of the fixed image (template mask) and moving image (image registered to the template) respectively. Additionally, DSC was also used to evaluate the performance of the automated segmentation of the lung and lung infiltrate regions using a 3D CNN. Detection performance of the lung infiltrate regions was evaluated by sensitivity and positive predictive value.

The outcome of interest in this study for disease severity was invasive mechanical ventilation/death vs. no invasive ventilation support (no respiratory distress, oxygen supplementation, non-invasive ventilation). Mean and standard deviation of AUC was used to indicate the cross-validation performance of all AI models on training set D_1^{train}. Further, AUC with 95 % confidence intervals (CI) was used to capture performance of all AI models on test sets (D_1^{test}, D_2, D_3 and D_4). The DeLong test [35] was used to compare the statistical significance with respect to difference in AUCs between the two models. Optimal cut-off point on receiver operating characteristic curve (ROC) was chosen by maximizing F1 score on the D_1^{train}. Furthermore, sensitivity, specificity and F1 score were reported on test sets (D_1^{test}, D_2, D_3 and D_4) based off the optimal cut-off point. pROC and cutpointr packages of R statistical software was used to perform the DeLong's test and calculate optimizal cut-off point respectively.

3. Results

3.1. COVID-19 study population

A total of N = 3230 patients acquired from four different sources (D_1: N = 835, D_2: N = 113, D_3: N = 2000, D_4: N = 282) were used in this study. An additional N = 213 patients from *the National Lung Screening Trial (NLST) Spiral CT abnormalities dataset* ([17,18]; [19]; [20]) marked as “Other minor abnormality noted” were used as a control cohort (D_5) for positive COVID-19 patients. Table 1 shows

patient demographics. Median age with interquartile ranges among the cohorts D_1 and D_2 were found to be 59 (47–67) and 62 (53–72) respectively. Median ages for D_3 and D_4 could not be evaluated as accurate age information was not available for subjects within the cohorts D_3 and D_4. All patients from datasets D_1-D_5 were grouped into three categories based on severity; a) healthy patients (control cohort) with no COVID-19 disease, b) mildly affected COVID-19 patients (patients who did not require a mechanical ventilator), c) severely affected COVID-19 patients (patients who required a mechanical ventilator).

Table 1. Patient demographics of datasets **D_1-D_3.** All the demographic information was available for cohorts **D_1**, **D_2**. Although cohort C had age information, they were specified as ranges. Hence median and interquartile range related statistics could not be calculated. No demographic information was available for dataset **D_4.** μmol: micromoles; U: units; L: liter; ng: nanograms; mL: milliliter.

Variables	**D_1**	**D_2**	**D_3**
Age Median (IQR)	59 (47–67)	62 (53–72)	–
Gender Male (%)	46.30	45.31	–
Laboratory findings Median (IQR)			
Creatinine (Cr) (μmol/L)	60.0 (50.0–72.0)	99.0 (75.4–142.3)	84.4 (69.0–110.9)
Aspartate aminotransferase (AST) (U/L)	24.0 (19.0–37.0)	30.0 (21.0–47.0)	38.0 (26.0–61.0)
Alanine aminotransferase (ALT) (U/L)	23.0 (16.0–39.0)	21.0 (15.0–33.0)	30.0 (19.0–54.0)
Neutrophils count (Neu) (10^9 L^{-1})	3.63 (2.25–5.11)	4.82 (2.75–6.17)	5.3 (3.8–7.0)
Lymphocytes count (LYM) (10^9 L^{-1})	1.21 (0.88–1.68)	1.05 (0.77–1.30)	0.89 (0.65–1.36)
Procalcitonin (PCT) (ng/mL)	0.16 (0.055–0.24)	0.17 (0.08–0.54)	0.17 (0.09–0.34)

The dataset from source D_1 was stratified based on the severity, and randomly split into 50 % training (D_1^{train}: N = 416) and 50 % testing (D_1^{test}**:** N = 419), while data from the sources D_2, D_3 and D_4 were all used as an external independent test set to test our hypothesis. Supplementary table, Table S2 shows the distribution of mild and severe COVID-19 patients on the training (D_1^{train}) and test sets (D_1^{test}, D_2, D_3 and D_4).

3.2. Chest CT reveals significant volume and lung shape differences between mild and severe COVID-19 patients

The registration accuracy for atlas creation, **D_A** was observed to be high with a DSC of 0.91 ± 0.08 between the template, T and the registered lung masks. Additionally, the supplementary figure, Fig. S2 depicts deformation fields of deformable registration performed by registering the CT scans to a template volume. One can notice that applying anatomic constraints helps in making sure that no

unnatural deformations are introduced during the registration processing. Furthermore, from supplementary figure, Fig. S2, one can notice that the deformation fields are oriented towards the ground lung infiltrates (glass opacities and consolidation regions). Hence, this shows that a COVID-19 patient with severe disease with the existence of more lung infiltrates presents higher deformations as compared to patients with milder COVID-19 disease with little or no lung infiltrates. Statistically significant volume differences were found between healthy, mild and severe patients in D_1^{train} (Fig. 3). One can observe that lung volumes decreased with increasing severity of COVID-19 disease (Fig. 3). Similarly, it may also be observed that mean intensity values within the lung on CTs increased with increasing severity (Fig. 3). Additionally, we also conducted the same analysis separately for male patients and female patients and found the similar trends in lung volumes and mean CT intensities (supplementary figure, Fig. S3).

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Fig. 3. Differences in lung volumes and mean intensities within in lung as seen on CTs between three different populations a) severe COVID-19 patients (patients who required a mechanical ventilator) from the training set $\mathbf{D_1^{train}}$ (green), b) mild COVID-19 patients (patients who did not require a mechanical ventilator) from the training set $\mathbf{D_1^{train}}$ (orange), c) healthy patients (no COVID-19) from $\mathbf{D_5}$ (blue). The plot shows that as the severity of COVID-19 disease increases, the lung volume decreases. Similarly, it can be observed that mean lung intensities increases as the severity of COVID-19 disease increases.

Population difference atlases ($\mathbf{D_A}$) and subsequently ensemble of population shape difference atlases ($\mathbf{D^E_A}$) (Fig. 1 shows construction of $\mathbf{D_A}$ and supplementary figure, Fig. S1 depicts construction of $\mathbf{D^E_A}$) were constructed between pairs of populations (healthy, mild and severe COVID-19 patients). The $\mathbf{D^E_A}$ between mild and severe COVID-19 patients (Fig. 4) shows that the majority of the lung shape differences were observed at the mediastinal surface of both lungs. Furthermore, it may be observed that lung shape differences between healthy and severe COVID-19 patients (supplementary figure, Fig. S4) was extended to the basal surfaces of both the lungs along with mediastinal surfaces. However, lung shape differences were found only on the mediastinal surfaces for $\mathbf{D^E_A}$ between healthy and mild COVID-19 patients (supplementary figure, Fig. S5). Additionally, when $\mathbf{D^E_A}$ between healthy and mild COVID-19 patients, was compared with $\mathbf{D^E_A}$ between mild and severe COVID-19 patients, one can notice that differences between healthy and mild COVID-19 patients was more significant as compared to further worsening of the disease between mild and severe COVID-19 patients.

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Fig. 4. Ensemble difference atlas ($\mathbf{D^E_A}$) between mild (patients who did not require a mechanical ventilator) and severe (patients who required a mechanical ventilator) COVID-19 patients. Majority of the lung shape differences between $\boldsymbol{V}^-$ and $\boldsymbol{V}^+$ COVID-19 patients were found at the mediastinal surface of both lungs (Blue: No statistically significant shape difference between the populations. Red: Regions with statistically significant shape difference between the populations).

3.3. AI based quantification of lung shape differences associated with severity of COVID-19

A 3D CNN (M_1: Fig. 2) was used to quantify the lung shape differences for predicting COVID-19 severity (distinguishing between mild and severe COVID-19 patients). Two channels were given as input to M_1 (Fig. 2) with first being the lung CT volume and the second being a binary mask of regions with lung shape differences. The base architecture for M_1 was chosen based on 3-fold cross-validation performance (AUC = 0.810 ± 0.045) on D_1^{train} (supplementary table, Table S3). M_1 resulted in AUC of 0.850; 95 % CI [0.814, 0.888], 0.714; 95 % CI [0.612, 0.816], 0.709; 95 % CI [0.681, 0.738] and 0.610; 95 % CI [0.542, 0.678] on D_1^{test}, D_2, D_3 and D_4 respectively. Additionally, M_1 yielded AUC = 0.655; 95 % CI [0.633, 0.678] when evaluated on the combined dataset (D_1^{test}, D_2, D_3, D_4).

Fig. 5 (top 2 rows) shows Gradient-weighted Class Activation Maps[36] (Grad-CAM) interpretability results for M_1. These activation maps depict that CNN is focus its attention on regions with significant lung shape differences during the classification phase.

3.4. CovSafeNet: a novel framework integrating biomarkers of lung shape differences and lung infiltrates for COVID-19 prognosis

A 3D CNN architecture (M_2: Fig. 2) was used for characterization of lung infiltrates. Details of lung infiltrates segmentation and segmentation results are presented in the supplementary section, S4. The supplementary figure, Fig. S6 shows some exemplar segmentations of lung and lung infiltrate regions. M_2 resulted in a 3-fold cross validation AUC = 0.815 ± 0.005 on D_1^{train} (supplementary table, Table S3). Furthermore, M_2 resulted in AUC of 0.867; 95 % CI [0.833, 0.901], 0.770; 95 % CI [0.667, 0.873], 0.697; 95 % CI [0.666, 0.728] and 0.650; 95 % CI [0.579, 0.723] on D_1^{test}, D_2, D_3 and D_4 respectively. Additionally, M_2 yielded AUC = 0.680; 95 % CI [0.658, 0.702] when evaluated on the combined test set (D_1^{test}, D_2, D_3, D_4).

Gradient-weighted Class Activation Maps [36] (Grad-CAM) (Fig. 5) results for M_2 are presented in Fig. 5 (bottom two rows). Similar to M_1, one can notice that the auxiliary channel can aid the network in focusing on regions of interest (here lung infiltrates) to make its final decisions. Additionally, from Fig. 5 one may observe that M_2 successfully classified a patient as having mild or severe COVID-19 even though lung infiltrates had similar appearance. For instance, from Fig. 5, mild COVID-19 patients (2a, 2b) and severe COVID-19 patients (2e, 2f) had similar appearance and size (smaller lung infiltrates), and M_2 correctly identified the severity of the patients' disease. Similarly, although mild COVID-19 patients (2c, 2d) and severe COVID-19 patients (2g, 2f) have similar appearance and sizes (larger lung infiltrates), M_2 correctly identified them as mild versus severe COVID-19 patients.

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Fig. 5. Grad-CAM interpretability results with binary masks, S_P and I_P encoded into M_1 (1a-1h) and M_2 (2a-2h) respectively as auxiliary channels to the network. These maps show that these auxiliary channels can aid the network in setting an attention region helping the network to focus on these regions, while at the same time, providing the context of the whole lung region. Blue regions indicate areas contributing to predictions as mild COVID-19 disease while red regions indicate areas contributing to severe COVID-19 disease. The color bar gradient corresponds to the strength of the contribution.

Subsequently, CovSafeNet (Fig. 2) was constructed based on decision fusion of M_1 and M_2. CovSafeNet resulted in an improved performance over M_1 and M_2, yielding a 3-fold cross validation AUC of 0.838 ± 0.015 on D_1^{train}. Similarly, from Table 2, it may be observed that CovSafeNet significantly outperforms M_1 and M_2 resulting in an AUC of 0.693; 95 % confidence interval (CI) [0.671,0.716] on combined test set (D_1^{test}, D_2, D_3, D_4).

Table 2. Test set performance (AUC with 95 % confidence intervals (CI)) of M_1, M_2 and CovSafeNet on D_1^{test}, D_2, D_3 and D_4 datasets. The performance of CovSafeNet was compared with models M_1 and M_2 using DeLong's test. * indicates statistically significant improvement as indicated by DeLong's test.

Model AUC (95 % CI)	Empty Cell	**D_1^{test} N = 419**	**D_2 N = 113**	**D_3 N = 2000**	**D_4 N = 282**	**Combined N = 2814**
M_1		0.850 (0.814, 0.888)	0.714 (0.612, 0.816)	0.709 (0.681, 0.738)	0.610 (0.542, 0.678)	0.655 (0.633, 0.678)
M_2		0.867 (0.833, 0.901)	**0.770 (0.667, 0.873)**	0.697 (0.666, 0.728)	0.650 (0.579, 0.723)	0.680 (0.658, 0.702)
CovSafeNet		**0.890* (0.860, 0.921)**	0.769 (0.667, 0.870)	**0.732 (0.704, 0.761)**	**0.654 (0.583, 0.724)**	**0.693* (0.671, 0.716)**
	DeLong's Test with M_1	p = 0.0342	p = 0.9877	p < 0.0001	p = 0.812	p = 0.0123
	DeLong's Test with M_2	p = 0.0001	p = 0.0589	p = 0.0548	p = 0.0558	p < 0.0001

CovSafeNet's performance evaluated on individual cohorts D_1^{test}, D_2, D_3 and D_4 was found to be 0.890; 95 % CI [0.860,1.921], 0.769; 95 % CI [0.667, 0.870], 0.732; 95 % CI [0.704,0.761] and 0.654; 95 % CI [0.583, 0.724] respectively. CovSafeNet resulted in statistically significant improvement over M_1 and M_2 on D_1^{test} (AUC = 0.890; 95 % CI [0.860,0.921]). However, similar statistically significant improvement was not found between M_1 and M_2 on cohorts D_3 and D_4 (Table 2). Also, on D_2, CovSafeNet performed similar to M_2 yielding an AUC = 0.769; 95 % CI [0.667,0.870]. Furthermore, other performance metrics such as sensitivity, specificity and F1 score are presented in the supplementary table, Table S4. CovSafeNet resulted in a sensitivity, specificity and F1 score of 86 %, 36 % and 51 % respectively on the combined test set (D_1^{test}, D_2, D_3, D_4).

3.5. Comparison of CovSafeNet with clinical and radiomics based machine learning model

Radiomics and clinical machine learning classifiers M_R and M_C were trained with radiomic and clinical features respectively. Clinical features were not available for cohort D_3. Therefore, comparisons of M_R and M_C with CovSafeNet were only made with D_1^{test}, D_2, and D_4. Among the clinical features, aspartate aminotransferase (AST), neutrophils count (Neu), lymphocytes count (LYM) were

selected as the most prominent clinical features. From Table 3 one can notice that CovSafeNet with an AUC = 0.688 95 % CI [0.653,0.724] significantly outperforms both M_R (AUC = 0.651; 95 % CI [0.614, 0.689]) and M_C (AUC = 0.607; 95 % CI [0.568, 0.646]) on the combined cohorts D_1^{test}, D_2, and D_4. Additionally, the combination CovSafeNet with the clinical model (M_C) by pooling the decisions (average of decisions from CovSafeNet and M_C) yielded AUC = 0.685; 95 % CI [0.649, 0.721] with no statistically significant difference (p = 0.6694) found compared to the performance of the CovSafeNet model. Additionally, by performing a multivariable analysis (Supplementary table, Table S5) we also show that CovSafeNet was independently associated with other clinical variables (age, Cr, AST, ALT, LYM, Neu, PCM).

Table 3. Test set performance (AUC with 95 % confidence intervals (CI)) of the radiomics based machine model (M_R), and clinical based machine learning model (M_C) and CovSafeNet on D_1^{test}, D_2, D_3 and D_4 datasets. The performance of CovSafeNet was compared with models M_R and M_C using DeLong's test. * indicates statistically significant improvement as indicated by DeLong's test.

Model AUC (95 % CI)	Empty Cell	**D_1^{test} N = 419**	**D_2 N = 113**	**D_3 N = 2000**	**D_4 N = 282**	**Combined (D_1^{test}, D_2, D_3 D_4) N = 2814**	**Combined (D_1^{test}, D_2, D_4) N = 814**
Radiomics, M_R		**0.893 (0.856, 0.931)**	0.641 (0.523, 0.752)	0.723 (0.692, 0.742)	0.579 (0.514, 0.648)	0.662 (0.631, 0.684)	0.674 (0.631, 0.703)
Clinical, M_C		0.686 (0.638, 0.726)	0.668 (0.549, 0.781)	–	**0.664 (0.599, 0.732)**	–	0.602 (0.568, 0.651)
CovSafeNet		0.890 (0.860, 0.921)	**0.769 (0.667, 0.870)**	**0.732 (0.704, 0.761)**	0.654 (0.583, 0.724)	**0.693* (0.671, 0.716)**	**0.688* (0.653, 0.724)**
	DeLong's Test with M_R	(p = 0.6028)	(p = 0.0485)	(p = 0.4116)	(p = 0.0323)	(p < 0.0001)	(p < 0.0001)
	DeLong's Test with clinical model M_C	(p < 0.0001)	(p = 0.0925)	–	(p = 0.6321)	–	(p < 0.0001)

3.6. Ablation studies

3.6.1. Sensitivity of template selection to construct difference atlas

To understand the effect of template selection on quantification of lung shape differences, we constructed $\mathbf{D}^{E}_{A}$ using a randomly selected template ($\boldsymbol{T}^{r}$), instead of template $\boldsymbol{T}$ chosen based off median lung volume. AI based quantification of lung shape differences using $\boldsymbol{T}^{r}$ as template was then

compared to the quantification using $\boldsymbol{T}$ as the template. The resulting M_1 trained using $\boldsymbol{T}'$ as the template yielded an AUC of 0.847, 0.728, 0.689 and 0.569 on D_1^{test}, D_2, D_3 and D_4 respectively. DeLong's test indicated no significant differences ($p > 0.12$) on D_1^{test}, D_2, D_3, except for D_4 ($p = 0.0350$).

3.6.2. 3D-CNN without shape and infiltrate priors

To evaluate the significance of shape prior ($\mathbf{S_P}$) or infiltrate prior ($\mathbf{I_P}$) provided to CNNs as an auxiliary channel, we trained a 3D-CNN with only chest CT volumes as the only input channel (without $\mathbf{S_P}$ and $\mathbf{I_P}$). The 3D-CNN trained without $\mathbf{S_P}$ and $\mathbf{I_P}$ resulted in an AUC = 0.792; 95 % CI [0.77–0.82], AUC = 0.645; 95 % CI [0.60–0.68], AUC = 0.660; 95 % CI [0.62–0.69] and AUC = 0.589; 95 % CI [0.53–0.63] on D_1^{test}, D_2, D_3 and D_4 respectively. With respect to DeLong's test, CovSafeNet (with $\mathbf{S_P}$ and $\mathbf{I_P}$) performed significantly better than a 3D-CNN trained without $\mathbf{S_P}$ and $\mathbf{I_P}$ as auxiliary channels ($p < 0.0331$).

3.6.3. Comparison of CovSafeNet architecture with vision transformers

We further compared the best 3D-CNN architecture (3D-Densenet-121) with 3D vision transformers [37] (implementation from MONAI opensource package [38]). The CovSafeNet with 3D vision transformers architecture achieved an AUC = 0.697 on the combined test set (D_1^{test}, D_2, D_3 and D_4) showing no significant difference from the 3D-Densenet-121 architecture ($p = 0.4321$).

4. Discussion

While a number of previous studies have developed and applied artificial intelligence (AI) predictors for COVID-19 diagnosis and prognosis [14,15], there has not been, to our knowledge, a rigorous, comprehensive, and quantitative assessment of lung shape and deformation on account of COVID-19 and COVID-19 severity. Understand and predicting lung deformation and changes in lung shape could have long term implications for lung function. For instance, a study by Osanlouy et al. ([39], pp. 20–90) depicted that changes in lung shape could in turn affect lung function by causing aging. In this work AI based approaches were combined with statistical shape modeling based atlas approaches to (1) quantitatively capture lung morphological changes on account of COVID-19, (2) relate the changes in the shape and volume of the lung with severity of the disease and (3) combine deep learning with atlas based approaches to create integrated predictors of lung shape and lung infiltrates for COVID-19 severity prediction.

Previous studies [40] have shown that severe COVID-19 disease can have long-term sequelae such as subpleural reticulation and ground-glass opacities. Additionally, recent studies have also shown the mechanical deformations are introduced in the lungs due to severity of COVID-19 disease [41,42]. Furthermore, extensive and persistent lung damage was also observed in a study with postmortem samples of 41 patients [43] in patients with severe COVID-19 disease. These findings can be explained by the intense disruption of the normal lung parenchyma and interstitium with subsequent fibrosis. These damages caused to the lung may even lead to permanent loss of lung function [13]. However, as previously mentioned, a quantitative assessment of the nature, extent and precise location of the COVID-19 induced lung deformation and associated damage has not been previously attempted.

Previous studies have developed various population-atlas based models to quantify shape differences of different organs on imaging and have illustrated their association with severity of various diseases [24,44,45]. To our knowledge, this is the first study to explore the association of COVID-19 severity with population lung shape differences. With pulmonary fibrosis proven as a long

term sequelae of COVID-19 [46], there are several possible factors such as reticulations, traction bronchiectasis or honeycombing which might be associated with changes in lung shape and morphology. Additionally, a previous study also indicated increased pulmonary arterial medial wall thickness among patients who died from COVID-19 pneumonia ("[47]: a global perspective | Nature Reviews Cardiology," n.d.). A related previous study by Lin et al. [48] has showed that micro-alveolar ruptures were associated with COVID-19 disease. These data appear to suggest that micro-alveolar ruptures in the mediastinal surfaces of the lung could lead to preferential pleural defects affecting lung shape and morphology. However, these studies ([[46], [47], [48]]: a global perspective | Nature Reviews Cardiology," n.d.) have been mostly qualitative in nature. Our study represents the largest one to date in the use of AI and statistical atlas modeling for quantitative assessment of disease severity as also lung shape related changes using chest CT scans on account COVID-19.

The new study presented in this work revealed that majority of lung shape related differences between mild and severe COVID-19 patients were found at the mediastinal surfaces of both lungs. Furthermore, when lung shape differences were evaluated between a healthy control cohort and severely affected COVID-19 patients, lung shape differences were found to extend to basal surfaces (supplementary figure, Fig. S5) as also mediastinal surfaces. This can be explained due to the fact that bilateral lower lobes are more commonly involved with high occurrence of lung infiltrates in severe COVID-19 patients [49]. Additionally, it was also noted that initial shape differences (between control and mild COVID-19 patients) were more prominent compared to further worsening of the disease (between mild COVID-19 patients and severe COVID-19 patients). Therefore, early characterization of these physiological based changes can portend the possible long-term sequela of COVID-19 disease and help in better prognosis of COVID-19 patients.

Recently, a few previous studies have developed artificial intelligence (AI) based prognostic models to predict the severity of COVID-19 disease using convolutional neural networks (CNNs) [50] and radiomics based approaches [51]. Furthermore, some of the previous approaches have even integrated CT based biomarkers with clinical and biological variables such as prothrombin time, albumin, age, sex, neutrophil counts etc [14,52,53]. However, most of these studies have focused only on target regions of interest (ROIs) in lung parenchyma such as lung infiltrates (ground-glass opacities (GGOs) and consolidation regions) for characterizing severity of COVID-19 disease. The unique aspect of this study involved combining statistical population shape difference atlas models with deep learning for COVID-19 prognosis prediction. Additionally, the study was unique in the employment of novel combination of deep learning derived features from both lung shape differences as well as lung infiltrates. Furthermore, the study presented in this work represents the largest multi-institutional study ([52,54], p. 19) involving application of AI for COVID-19 severity estimation with N = 3230 patients from multiple sites. The presented framework, CovSafeNet yielded an AUC = 0.89 on D_1^{test}, a center on which the model was trained and AUC in the range of 0.66–0.77 on external test sets from other institutions**.** The study also demonstrated that the combined population-atlas and deep learning approach outperformed radiomics (M_R) and clinical based (M_C) machine learning models. Additionally**,** combining M_C with CovSafeNet resulted in a similar performance (AUC = 0.685) as CovSafeNet with no statistically significant difference (p = 0.6694). Although the study of post-acute sequelae of SARS CoV-2 (PASC), or "long COVID", was beyond the scope of the study, the findings from our experiment also holds relevance for this long term complication of COVID-19 disease in light of its rising incidence and major healthcare consequences [55]. Studies have implicated fibrosis as the inciting factor for lung disease in PASC, contributing to the long-term mortality. These findings support the results from our study [56].

We acknowledge that our study did have its limitations. No improvement in CovSafeNet's performance by integrating other clinical features may be attributed to the fact that many of the other important clinical factors such as lactate dehydrogenase (LDH), prothrombin time (PT), albumin (ALB) etc as indicated in the some of the previous studies [14,53] were not available across all the datasets (D_1^{test}, D_2, and D_4). Access to these additional clinical factors can further enhance the performance of CovSafeNet by providing valuable clinical information along with imaging biomarkers. Additionally, the drop in the performance of CovSafeNet on external test sets (D_2, D_3 and D_4) as compared to the test set $\mathbf{A_V}$ might be attributed to the fact that CT characteristics (mean intensities within the lung region) of mild COVID-19 patients from D_2, D_3 and D_4 which were found to be statistically different from D_1^{test} (Supplementary Fig. S7). Additionally, transfer learning techiniques such as fine-tuning or domain specific batch normalization[57] of CovSafeNet over a small subset of data from each of these independent test cohorts may also help in capturing distribution and CT characteristics across different institutions, and hence will be explored as part of future work. Additionally, this study mainly focuses on identifying the regions in CT scans with significant shape differences between mild and severe COVID-19 patients and further use the texture and intensity information from those regions via 3D CNN to predict severity of COVID-19 disease. However, characterizing the shape of the lung directly via shape features such as analyzing surface normals and curvature will be considered part of future work. Also, our study was retrospective in nature and to ensure the clinical practicality of CovSafeNet, the tool needs to be validated prospectively by following patients until discharge.

In conclusion, we demonstrated that there are significant differences in lung shape and morphology between COVID-19 patients with mild and severe disease. Further, we showed that AI based quantification of these lung shape differences can help in COVID-19 prognosis. Additionally, integrating these biomarkers with lung infiltrate-based biomarkers through a novel AI framework, we illustrated that COVID-19 prognosis can further be improved. Furthermore, we also showed that CovSafeNet was able to outperform other state-of-the-art approaches including radiomics and clinical based machine learning models. Lastly, fibrosis, as implied in this study is also the main pathophysiology behind PASC. Therefore, future studies would entail exploring the role of lung shape differences among COVID-19 patients as a biomarker in the context of long COVID.

Abstract

Background

Chronic lymphocytic leukemia (CLL) is the most common type of leukemia in Western countries. Although various patterns of lung involvement with CLL have been reported, data on clinicoradiologic presentation are sparse.

Methods

A computer-assisted search was conducted to identify patients encountered at Mayo Clinic from 1998 to 2022 and had leukemic pulmonary infiltrates (LPI) with CLL demonstrated on lung biopsy. Medical records and chest imaging studies were reviewed to identify clinical and radiologic features.

Results

Among 13 patients, median age was 77 years (range: 60–88) and included 10 men (77 %). All patients were known to have CLL with a median duration of 96 months (range: 50–408), and none were on treatment. Most common symptoms were dyspnea (62 %), cough (54 %), and fatigue (46 %); 2

patients (15 %) were asymptomatic. Dominant abnormality on CT consisted of single or multiple nodular/mass-like opacities in 10 patients (77 %), while diffuse centrilobular nodules, pleural mass, and diffuse bronchial wall thickening were each seen in one patient, respectively; intrathoracic lymphadenopathy was present in all.

After diagnosis of LPI, treatment for CLL was administered to 7 patients (54 %); 6 patients (86 %) exhibited improvement. During follow-up (median 41 months), 8 (62 %) patients died. Causes of death included progressive CLL or treatment-related complications (2 patients), pneumonia (1 patient), unrelated causes (3 patients), and unknown in 2 patients.

Conclusions

LPI in CLL is generally encountered in patients with known untreated CLL. The main imaging feature is single mass-like opacity or multiple nodular/mass-like opacities, associated with intrathoracic lymphadenopathy.

Introduction

Chronic lymphocytic leukemia (CLL) is the most common type of leukemia in Western countries and is defined by the presence of monoclonal B lymphocytes (5×10^9/L or more) that are mature but dysfunctional in the peripheral blood [1].CLL generally occurs in older adults, most of whom are asymptomatic at the time of diagnosis. As the disease evolves, however, many patients develop symptoms related to lymphadenopathy, hepatosplenomegaly, cytopenias, immunodeficiency, autoimmune diseases, and Richter transformation (transformation to high-grade lymphoma) [1,2]. The abnormal B lymphocytes of CLL commonly infiltrate lymph nodes and spleen but also various non-lymphoid organs including the liver, skin, central nervous system, kidney, gastrointestinal tract, heart, and lung.

When patients with CLL present with pulmonary symptoms or imaging abnormalities, most common cause of pulmonary disease is infection [3,4]. However, noninfectious pulmonary complications are also encountered including drug-related pneumonitis, pleural effusion, venous thromboembolism, lung cancer, and leukemic pulmonary infiltrates (LPI) [3,4]. While autopsy data suggest lung involvement in leukemias to be relatively common, descriptions of clinicoradiologic features associated with LPI occurring in CLL have been rather sparse [[4], [5], [6], [7], [8]]. For example, a retrospective study of 32 cases of CLL with bronchopulmonary leukemic infiltrate confirmed histopathologically did not provide detailed clinico-radiologic data [8].Herein, we describe clinical and imaging findings in 13 patients with LPI caused by CLL, confirmed by lung biopsy, along with treatment and clinical course.

Materials and methods

A computer-assisted search was conducted to identify patients with CLL seen at Mayo Clinic (Rochester Minnesota, Jacksonville Florida, and Scottsdale Arizona) and diagnosed to have lung infiltration with CLL demonstrated on lung biopsy over the period of 1998 through 2022. During this 25-year span, 8262 patients with CLL were seen. The study was approved by the Institutional Ethics Committee of Mayo Clinic, IRB: 23–003485. Medical records and chest imaging studies were reviewed to identify

Results

Among13patients with CLL and LPI confirmed by lung biopsy; 77 % were men and 77 % were never smokers (Table 1). Median age at diagnosis of lung involvement was 77 years (range, 60–88). All

patients were known to have CLL with a median disease duration of 96 months (range, 50–408) at the time of pulmonary evaluation, all of which occurred in the outpatient clinic. No patient was on treatment for CLL at the time of LPI diagnosis.

The most common symptoms were dyspnea (62 %) and cough (54 %) (Table

Discussion

In this study, we find leukemic pulmonary infiltrates (LPI) in CLL to be encountered in patients with known CLL but not under active treatment. Although most patients with LPI due to CLL were symptomatic (respiratory or systemic), some were not and presented for evaluation of abnormal chest imaging. Interlobular septal thickening has been reported to be the most notable chest imaging finding in patients with other forms of leukemias, but we found the single mass-like opacity or multiple

Conclusions

We conclude single mass-like opacity or multiple nodular/mass-like opacities to be the dominant abnormality that correlate with LPI in patients with CLL. Such imaging finding accompanied by intrathoracic lymphadenopathy in patients with untreated CLL should suggest the diagnosis of LPI, particularly in the absence of symptoms suggestive of ongoing infection.

Actinomycosis is a rare chronic suppurative granulomatous disease. Surgical biopsy is often performed in patients with chest actinomycosis because malignancy is suspected in most cases. A 62-year-old man presented to our hospital with fever and exertional dyspnea that had persisted for several months. Contrast-enhanced computed tomography showed an irregularly shaped mass with contrast enhancement in the anterior mediastinum and consolidation in the left upper lung lobe contiguous with this mass, as well as multiple nodules in both lungs. The pulmonary artery trunk was stenotic and surrounded by the mass, and the right heart system was enlarged. Thoracoscopic biopsy was performed but failed to yield a diagnosis. Contrast-enhanced computed tomography after one month revealed an increased mass and worsening right heart strain. ^{18}F-FDG (fluorodeoxyglucose) positron emission tomography/computed tomography and contrast-enhanced magnetic resonance imaging also suggested a malignant tumor, and an open chest biopsy was performed. No malignant cells were identified and actinomycetes were detected by histopathology and bacterial culture. The patient was treated with antibiotics, following which his contrast-enhanced computed tomography findings and general condition improved. Introduction

Actinomycosis is a chronic and rare infection caused by gram-positive anaerobic bacteria that can affect the cervicofacial area, chest, abdomen, and pelvis. It usually spreads contiguously to the adjacent tissues but can also spread hematogenously [1,2]. Diagnosing actinomycosis can be very difficult, and it is also frequently delayed owing to nonspecific clinical signs. In most cases, it is misdiagnosed as a malignant lesion despite being benign, and surgical biopsy or resection is often performed [3]. The definitive diagnosis is still based on histological or microbiological conformation [1].

Imaging can reveal some specific features of the actinomycosis, such as a highly infiltrative mass containing suppurative necrosis [1]. To our knowledge, little has been reported about thoracic actinomycosis involving the mediastinum in which contrast-enhanced magnetic resonance imaging was performed, and no studies in the literature that have mentioned the diagnostic findings of thoracic actinomycosis [4,5].

In this report, we have described a case of mediastinal abscess due to pulmonary actinomycosis, which was difficult to distinguish from malignancy and led to open chest surgery.

Case report

A 62-year-old man presented with a 4-month history of fever ranging from 37°C to 38°C and 2-week history of exertional dyspnea. He had also been presented with a low-grade fever a year earlier, and received symptomatic treatment and short-term oral antimicrobial therapy. He had a history of smoking 15 cigarettes per day for 20 years, and his medical history included chronic obstructive pulmonary disease. He worked as a hospital clerk. Physical examination revealed clear breath sounds and a systolic heart murmur. Oral hygiene was good, and there were no loose or carious teeth. Blood tests showed an inflammatory response, with a white blood cell count of 11,500/μL and C-reactive protein of 9.80 mg/dL. Tumor marker levels were not elevated except for a highly soluble interleukin-2 receptor level of 757 IU/mL. A left hilar mass and cardiac enlargement were observed in the chest radiograph, while the chest radiograph from one year prior showed aortopulmonary window opacification. Chest contrast-enhanced computed tomography (CT) revealed a 7 cm-sized irregularly shaped mass with a contrast enhancement in the anterior mediastinum. Contiguous with the mass, consolidation was observed in the medial upper lobe of the left lung and multiple nodules were present bilaterally in the lungs. The mass surrounded the pulmonary artery, and mild stenosis was observed in the main trunk of the pulmonary artery, along with mild enlargement of the right ventricle and right atrium (Fig. 1). An anterior mediastinal malignant tumor and multiple metastases to the lungs were suspected, and a thoracoscopic biopsy was performed; however, an accurate diagnosis could not be made.

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Fig. 1. Axial portal phase chest computed tomography (CT) image. (A) In the mediastinal window, an irregular shaped mass with contrast enhancement is visible in the anterior mediastinum (white arrow). The pulmonary artery trunk shows mild stenosis due to the mass (black arrow). (B) In the lung window, the consolidation adjacent to the mass in the left upper lobe (arrow) and mild emphysema can be seen. (C) Multiple nodules in the bilateral lungs (arrowhead).

^{18}F-FDG PET/CT (fluorodeoxyglucose positron emission tomography/computed tomography) showed high avidity, with a maximum standardized uptake value (SUVmax) of 19.0, from the anterior mediastinal mass to the left upper lobe consolidation, as well as high avidity in the multiple pulmonary nodules. High FDG accumulation was also observed in the right ventricular myocardium, suggesting right heart strain (Fig. 2). A contrast-enhanced CT after one month revealed an enlarged mass, progressive pulmonary artery stenosis, thrombus formation in the lumen, and worsening enlargement of the right ventricular system. Consolidation contiguous with the mass decreased, whereas a new consolidation appeared in the lingular segment. The bilateral lung nodules had partially decreased in size, but an increase in some lesions was also noticeable. In addition, left pleural effusion was observed (Fig. 3). Between the initial CT and contrast-enhanced CT the following month, no antibiotics were administered, except for a small intravenous dose of cefazolin (1 g) before and after thoracoscopic biopsy.

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Fig. 2. Axial fused 18-fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG PET/CT). (A, B) There is high avidity, with a maximum standardized uptake value (SUVmax) of 19.0, in the anterior mediastinal mass and consolidation in the left upper lobe (arrow). (C) Pulmonary nodules are also hyperintense (arrow). Increased FDG accumulation is seen in the right ventricular myocardium, suggesting right heart strain (arrowhead).

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Fig. 3. Axial portal phase chest computed tomography (CT) in the following month (each at the same level as Fig. 1). (A) In the mediastinal window, the mass is enlarged (white arrow), with progressive stenosis of the pulmonary artery trunk, along with a luminal thrombus (black arrow). (B) The consolidation contiguous to the mass is partially decreased (arrow). (C) A new consolidation has appeared in the lingular segment (arrow). Bilateral lung nodules are decreased, but an increase in some lesions is also noticeable (arrow). Left pleural effusion is seen (A-C, black arrowhead).

His general condition deteriorated, and blood tests showed a marked increase in the N-terminal pro-brain natriuretic peptide level to 5,714 pg/mL, indicating right heart failure.

Magnetic resonance imaging (MRI) showed an anterior mediastinal mass with mildly high signal intensity on T2-weighted image (T2WI) and equal signal intensity on T1-weighted image (T1WI) compared to the skeletal muscle, with heterogeneous high signal intensity on diffusion-weighted image (DWI) and decreased apparent diffusion coefficient (ADC). Fat-suppressed contrast-enhanced T1WI demonstrated heterogeneous enhancing effects, and pulmonary artery invasion was suspected. Multiple tiny areas with poor enhancement were observed inside the mass (Fig. 4).

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Fig. 4. Axial chest magnetic resonance imaging (MRI). (A) T2-weighted image (T2WI) shows that the anterior mediastinal mass is mildly hyperintense compared to the skeletal muscle. A small amount of fluid on the ventral side of the mass is seen (arrow). (B) Diffusion-weighted image (DWI) shows heterogeneous hyperintensity. (C) In fat-suppressed contrast-enhanced T1-weighted image (T1WI), the mass is accompanied by heterogeneous enhancing effects and seems to be invading the surrounding structures, including the pulmonary artery trunk. There are multiple tiny areas of poor enhancement in the mass.

An open chest biopsy was performed, and intraoperative rapid pathology did not identify any malignant cells. The mass showed multiple elongated discharges of a yellowish-white fluid that appeared to be pus. The mediastinal mass and the thickened and stiffened pericardium were resected as much as possible. Subsequently, pulmonary artery stenosis was relieved and the movement of the right ventricle improved. Histopathological examination revealed no malignant cells; however, sulfur granules, which are characteristic of actinomycetes, were observed. Grocott's staining revealed dark brown intertwined filamentous fungi forming a fungal mass (Fig. 5). In addition, *Actinomyces israelii and Actinomyces meyeri* were detected in bacterial cultures of the pus

collected intraoperatively. The patient was diagnosed with an anterior mediastinal abscess associated with pulmonary actinomycosis.

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Fig. 5. Intraoperative photograph and histopathology. (A) Pus-like yellowish-white fluid is observed to be draining from the mass (arrow). (B) Hematoxylin and eosin staining shows inflammatory findings and characteristic sulfur granules (arrow). (C) Grocott's stain shows dark brown filamentous fungi intertwining and forming a fungal mass.

He was treated with intravenous antibiotics (ampicillin/sulbactam 3 g QID) for 6 weeks, followed by oral antibiotics (amoxicillin/clavulanic acid 500 mg). The patient's clinical symptoms and inflammatory response gradually improved. Contrast-enhanced CT performed 2 months after surgery showed a decrease in the anterior mediastinal mass, consolidation, and pulmonary nodules; enlargement of the right heart had improved; and pleural effusion had disappeared. The patient recovered and was discharged from the hospital three months after surgery.

Discussion

Actinomyces belongs to the normal oral flora but also is a part of the gastrointestinal and genital flora of humans. Infections can affect every organ of the body, and cervicofacial actinomycosis is the most common type of infection [1,2]. Thoracic actinomycosis, which affects the lungs, mediastinum, pleura, and chest wall, accounts for approximately 10%-20% of all actinomycosis cases [1]. Inhalation of oropharyngeal secretions in patients with poor oral hygiene, seizure disorders, alcoholism, and a perforated esophagus is a risk factor [1]. In particular, mediastinal infections are considered rare, with the exception of infections in patients with medical devices such as esophageal stents [6].

It takes 1-12 months to diagnose thoracic actinomycosis, usually 3 months [1]. Diagnosis can be difficult due to prolonged nonspecific symptoms, such as cough, hemoptysis, chest pain, weight loss, and increased sputum production. In actinomycosis, cultures of clinical specimens are negative in >50% of the cases [1]. Therefore, histopathological examination of the infected tissue biopsies to identify sulfur granules is useful for diagnosis [1,2].

In this case, based on the findings of a plain chest radiograph taken one year prior, it was suspected that the patient had already been infected with actinomycetes, but more than a year had passed before the final diagnosis was made.

Thoracic actinomycosis usually results in the formation of masses (inflammatory pseudolesions) in the lungs and mediastinum, but can also present with extensive or localized pneumonia, pyothorax, and endobronchial masses [7]. In addition, mediastinal actinomycosis has been reported to cause pericardial effusions with or without pleural effusions, pericardial masses, and mediastinal masses, which can lead to various complications, including superior vena cava syndrome, Pancoast syndrome, and esophagotracheal fistulas [8].

In this case, the main lesion was a continuous mass in the left upper lung lobe and anterior mediastinum, with multiple small lesions in both lungs. There are two possible routes of infection. One is the spread of the infection to the mediastinum from pneumonia due to a respiratory tract infection. The second is hematogenous spread from other foci to the lungs and mediastinum.

Although this patient did not have a history of poor oral hygiene or a high risk of aspiration, we believe that a respiratory tract infection was the more likely route because no findings other than those in the lungs and mediastinum were suggestive of infection. Therefore, the consolidation adjacent to the anterior mediastinal mass in this case was considered actinomycotic pneumonia.

On reviewing the CT scans, we found that some of the lung consolidation adjacent to the mass had decreased before surgery, and some of the nodules suspected to be metastatic lesions had also reduced. Furthermore, the consolidation had a regional distribution, which was atypical for mediastinal tumor invasion of the adjacent lungs. Careful interpretation of the CT images led to a diagnosis of an inflammatory mass rather than a malignant tumor.

The common PET/CT finding in actinomycosis is severe hypermetabolism, comparable to that in malignancy; therefore, hyperintensity does not exclude actinomycosis [9]. The SUVmax of 19.0 in the anterior mediastinal lesion in this case indicated hyperintensity and did not aid in differentiation from malignancy. However, it became clearer that no other lesions were present, except for those in the thoracic region.

Only a few cases of thoracic actinomycosis involving the mediastinum in which MRI was performed, have been reported. To our knowledge, contrast-enhanced MRI was performed in only two such cases, and there is no literature on the diagnosis of thoracic actinomycosis [4,5]. The multiple tiny areas with poor enhancement in the mediastinal mass on fat-suppressed contrast-enhanced T1WI in the present case were thought to correspond to the numerous elongated abscesses and fistulae observed during surgery. We named this MRI finding the “pound cake sign” because it resembles the cut surface of a pound cake. The “pound cake sign” is considered a characteristic feature of pyogenic granulomas formed by actinomycosis and may help to differentiate them from malignant tumors.

Malignant tumors arising in the anterior mediastinum that can be differentiated prior to open chest biopsy include high-risk thymomas, thymic carcinomas, and leiomyosarcomas. Common imaging features of these malignant anterior mediastinal tumors include a heterogeneous enhancement, infiltrative tendency, high signal on DWI, and increased FDG uptake [10], [11], [12], [13]. Furthermore, high-risk thymomas and thymic carcinomas also show marginal irregularities and severely reduced ADC, and lymph node and hematogenous metastases have also been reported in thymic carcinomas [14]. In fact, the mass in this case had several signs in common with the malignant tumors mentioned above, including a heterogeneous enhancement, infiltration of the pulmonary artery, high signal on DWI, decreased ADC, and high FDG uptake. Consequently, a malignant tumor was strongly suspected in this patient from the beginning and the images were not interpreted carefully, making preoperative diagnosis even more difficult.

The principal treatment of actinomycosis is 2-6 weeks of intravenous high-dose antimicrobial agents, followed by 6-12 months of oral benzylpenicillin or amoxicillin [1]. Surgical approaches are considered if abscesses, extensive necrotic tissue, or fistulas are present, antimicrobial agents are unsuccessful, or when the purpose is to exclude malignancy [1]. Our patient was successfully treated by removing the granulation tissue and abscess through open chest surgery, followed by the administration of antibiotics.

Conclusions

Pulmonary mediastinal actinomycosis is a rare infectious condition that often mimics malignancy. Diagnosis is often delayed owing to a lack of specific imaging findings. The present case showed multiple tiny areas with poor enhancement in the mediastinal mass on fat-suppressed contrast-

enhanced T1WI, which we named the “pound cake sign.” This finding is believed to be characteristic of actinomycosis, reflecting abscesses and fistulas in pyogenic granulomas, and it may be useful in differentiating actinomycosis from malignant tumors.

Abstract

A 70-year-old woman had been treated with methotrexate for rheumatoid arthritis by a rheumatologist who opened a clinic near our hospital. In January of a certain year, she had respiratory symptoms of cough, sputum, and fever. Laboratory test results showed a white blood cell count of 8600/μL (neutrophil count of 5330/μL, lymphocyte count of 2490 μ/L), C-reactive protein (CRP) of 3.30 mg/dL. Chest radiography showed multiple infiltrative shadows in the right middle and lower lobes. Bronchoalveolar lavage fluid (BAL) lymphocyte count was increased (65.1%), and histopathological findings were consistent with numerous bowl-shaped cryptococcus cells stained black by Grocott staining. Added measurement of serum cryptococcal antigen titers was 4096-fold. Treatment with fluconazole 400 mg/day was initiated, and her symptoms resolved; the shadows of the lung fields improved. When asked in detail, the cryptococcus infection route was suspected from swallow excreta. There have been no reported cases of pulmonary cryptococcosis suspected due to inhalation of swallow excreta presenting with multiple infiltrative shadows.

1. Introduction

Pulmonary cryptococcosis is an opportunistic infection that commonly occurs in immunocompromised patients. Pigeon excreta are the main route of pulmonary cryptococcosis, and swallow excreta are rare. On CT images, pulmonary cryptococcosis shows a single nodule, multiple nodules, infiltrative shadow, and ground-glass opacities, and multiple infiltrative shadows type is very scarce. We experienced a rare case of pulmonary cryptococcosis with multiple infiltrative shadows whose infection route was suspected from swallow excreta.

2. Case presentation

A 70-year-old woman had been affected by rheumatoid arthritis two years ago and started treatment with methotrexate one year ago by a rheumatologist who opened a clinic near our hospital. There were no complications of rheumatoid-related interstitial pneumonia or chronic lower respiratory tract infection. In January of a certain year, she underwent transurethral lithotripsy in the urology department due to right ureteral calculi and calculous pyelonephritis. She had respiratory symptoms of cough, sputum, and fever before surgery. The COVID-19 and Flu antigen tests were negative. A postoperative chest radiograph showed infiltrative shadows in her right middle and lower lung field. She was suspected of developing bacterial pneumonia, and tazobactam/piperacillin 13.5 g/day and levofloxacin 500 mg/day were administered. However, symptoms and infiltrative shadows did not improve. She visited the respiratory medicine department for an investigation.

A physical examination revealed a body temperature of 37.7 °C, and coarse crackles were heard in the right lower lungs. Laboratory test results showed a white blood cell count of 8600/μL (neutrophil count of 5330/μL, lymphocyte count of 2490 μ/L), C-reactive protein (CRP) of 3.30 mg/dL, serum rheumatoid factor (RF) of 25 IU/mL, and anti-cyclic citrullinated peptide (CCP) antibody of 856 U/mL. β-D-glucan, anti-mycobacterium avium complex (MAC) antibody, QuantiFERON (QFT), and HIV antibodies were negative.

Chest radiography showed multiple infiltrative shadows with surrounding consolidation of the right middle and lower lobes (Fig. 1). The administration of antibiotics was ineffective. On the 10th day, after consulting with our department, a bronchoscopy was performed, followed by bronchoalveolar lavage (BAL) and endobronchial ultrasonography with a guide sheath (EBUS-GS). The BAL lymphocyte count was increased (65.1%). Histopathological findings were consistent with numerous bowl-shaped cryptococcus cells stained black by Grocott staining. Cryptococcus cells produced granulomatous lesions, and the bacterial cells themselves are not stained and only appear missing with HE staining (Fig. 2). Added measurement of serum cryptococcal antigen titers was 4096-fold. When asked in detail, swallows had built nests at the patient's house entrance, and there had been a large amount of swallow excreta. She cleaned without putting on a musk and inhaled much excreta for one year before developing symptoms. Considering that the incubation period from being infected with Cryptococcus fungi to developing symptoms can range from 6 weeks to 2 years, there was a possibility of the infection route of Cryptococcus from swallow excreta. We consulted with a neurologist regarding the necessity of cerebrospinal fluid testing. However, since no symptoms or findings suggest meningitis, the neurologist decided that a cerebrospinal fluid test was unnecessary. Therefore, we considered that we did not need to perform a head MRI. Treatment with fluconazole 400 mg/day was initiated, and her symptoms resolved; the shadows of the lung fields improved, her blood CRP count decreased to normal, and serum cryptococcal antigen titers decreased to 4-fold. We finished the treatment in about a year (Fig. 3). No recurrence was observed after that.

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Fig. 1. Chest radiology showed multiple infiltrative shadows with surrounding consolidation of the right middle and lower lobes.

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Fig. 2. Histopathological findings were consistent with numerous bowl-shaped cryptococcus cells stained black by Grocott staining. (Grocott staining × 400). Cryptococcus cells produced granulomatous lesions, and the bacterial cells themselves are not stained and only appear missing with HE staining. (HE staining × 200).

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Fig. 3. The timeline from the exposure to the swallow excreta to finishing the fluconazole treatment.

3. Discussion

Cryptococcosis is an infectious disease with worldwide distribution and a wide array of clinical presentations caused by pathogenic encapsulated yeasts in the Cryptococcus genus. Currently, two species of Cryptococcus commonly cause disease in humans: *Cryptococcus*

neoformans and Cryptococcus gattii [1]. *C neoformans* are found worldwide and associated with excreta from certain birds, such as pigeons, and cause disease in immunocompromised hosts [1,2].

Mohammad et al. evaluated the isolation of *C. neoformans* from swallow excreta in two northern cities of Iran. Ninety-seven swallow excreta were assessed, and 498 yeast-like colonies were isolated and identified as *C. neoformans* (8.7%). *Cryptococcus neoformans* was isolated from 5/97 (5.2%) of collected samples. The results of this study demonstrate that swallow excreta may harbor *C. neoformans* [3]. Incubation periods of the Cryptococcus spp. can vary between 6 weeks and 2 years [4]. In our case, swallow excreta was suspected as the route of cryptococcal infection in this patient based on the medical history, and there were no other suspected causes of cryptococcus infection.

On CT images, pulmonary cryptococcosis shows a single nodule, multiple nodules, infiltrative shadow, and ground-glass opacities. Multiple infiltrative shadows type is very scarce, and there have been no reported cases of pulmonary cryptococcosis with multiple infiltrative shadows whose infection route was suspected from swallow excreta. Kohno et al. reported that serum cryptococcal antigen titers are likely higher in infiltrative shadows type than other types [5]. Therefore, pulmonary cryptococcosis should be considered in the differential diagnosis of infiltrative pneumonia in immunocompromised patients, and serum antigen titer measurement should be considered.

4. Conclusion

We experienced a case of pulmonary cryptococcosis suspected due to inhalation of swallow excreta presenting with multiple infiltrative shadows. Pulmonary cryptococcosis should be considered in the differential diagnosis of infiltrative pneumonia in immunocompromised patients, and serum antigen titer measurement should be considered.

Abstract

Physicians are facing a growing challenge in characterizing suspicious pulmonary lesions through biopsy. Video thoracoscopic surgery is crucial for conducting surgical biopsies of these nodules. However, accurately identifying small pulmonary nodules, tiny, subsolid, and deep ones, remains a significant challenge due to the absence of digital palpation. One proposed technique for localization involves using a harpoon, initially designed for mammary nodules but also applied to pulmonary nodules. In cases involving solitary pulmonary nodules, histologic characterization is often necessary also accurate descriptions through computed tomography and the patient's clinical and epidemiologic context allow for a presumptive diagnosis. In this case, during an abdominal CT scan, a 49-year-old female patient was serendipitously found to have a ground-glass infiltrate in the anteromedial segment of the lower lobe of her left lung. Despite presenting with normal lung auscultation on physical examination, the increasing prevalence of subsolid lung nodules, combined with the contemporary era of minimally invasive surgery, prompted the medical team to employ CT-guided harpoon marking for precise lesion localization. Subsequent pathology analysis confirmed the presence of lepidic pattern adenocarcinoma. This case underscores the efficacy of the CT-guided harpoon marking approach, which significantly enhances surgical precision. Such precision is paramount in formulating individualized treatment strategies and follow-up plans for patients with similar clinical presentations.

Keywords

Chest CT

Harpoon

Pure ground-glass opacity (p-GGO) infiltrates

Video thoracoscopic surgery (VAST)

Background

Physicians are confronted with an increasing number of suspicious pulmonary lesions that must be characterized by biopsy. Today, video thoracoscopic surgery (VATS) plays a central role in the surgical biopsy of suspicious pulmonary nodules [1]. However, the lack of digital palpation in small, subsolid, and deep nodules may make it impossible to identify small pulmonary nodules accurately. When lung nodules are not identified intraoperatively on VATS, the procedure should be converted to an open thoracotomy; therefore, there is an indication to preoperatively locate small nodules that are distant from the pleural surface [2,3].

One of the first localization techniques described suggested using a harpoon for mammary nodules [4]. Still, they have also been described in pulmonary (PN) using percutaneous placement [5], [6], [7], [8], [9], either fiber- or platinum-coated [10,11]. Possible complications include bleeding with hematoma formation, pneumothorax, hemothorax, and harpoon dislodgement, which may occur before surgery or during intraoperative placement [5,8,10]. Another localization technique involves a percutaneous injection of different substances, radiotracer [12], or intraoperative localization of nodules under ultrasound guidance.

In most cases of patients with solitary PN, histologic characterization is necessary; the behavior over time is an essential factor in predicting the etiology of the nodule; most benign processes resolve spontaneously or within weeks or months after adequate treatment. Malignant nodules may remain unchanged for a long time (2-3 years) until they present an increase in size or density, and during this period, they are usually entirely asymptomatic [13]. Initial evaluation and follow-up depends on 2 essential characteristics: size and tomographic density [14]. PNs are classified as solid or subsolid; the latter may correspond to pulmonary adenocarcinoma but have a good prognosis in noninvasive or minimally invasive lesions. This case report highlights a 49-year-old female patient with an incidental subsolid PN marked with a harpoon. The histopathologic diagnosis revealed mucinous adenocarcinoma with a “lepidic” growth pattern, exemplifying the significance of precise characterization in determining patient management.

Case report

A 49-year-old female patient with a medical history of obesity, hashimoto's thyroiditis, low risk nonalcoholic fatty liver disease, and chronic undertreated sinusitis with an incidental finding in abdominal CT of a ground-glass infiltrate in the anteromedial segment of the lower lobe of the left lung. One year later, she was redirected to thoracic surgery and pulmonology, who found the patient with an intermittent clinical presentation of nasal congestion, dysphonia, and cough with mobilization of secretions; physical examination with normal pulmonary auscultation and extension imaging studies were performed:

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Chest computed tomography (CT), 2018: In the anterior region of the apical segment of the left lower lobe, there is an area with increased density in ground-glass of 20 × 18 mm with a central cavitation with no continuity with a structure of the airway. A comparison with a prior extra-institutional study conducted in 2017 reveals an enlargement in the size of the ground-glass component along with an elevated density. It is worth noting that the entire previous study was not encompassed in this evaluation (Fig. 1A).

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Fig. 1. Lung computed tomography of (A) 2018: In the anterior region of the apical segment of the left lower lobe area with increased density in ground-glass of 20 × 18 mm with a central cavitation with no continuity with a structure of the airway and (B) 2021: Ground-glass lesion located in the anteromedial basal segment of the left lower lobe, which has increased in size compared with previous studies, probable neoplastic etiology (adenocarcinoma with lepidic pattern).

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Fibrobronchoscopy: Negative for malignancy and microorganisms in bronchioalveolar lavage.

- •

Chest computed tomography (CT), 2021: Ground-glass lesion located in the anteromedial basal segment of the left lower lobe, which has increased in size compared with previous studies, probable neoplastic etiology (adenocarcinoma with lepidic pattern). A histopathologic study is recommended. Subcentimeter nodule in the right lower lobe, possible intrapulmonary lymph node (Fig. 1B).

During the imaging follow-up, lesion growth was detected, so it was presented to the medical board with radiology and pathology, and a pulmonary wedge resection was indicated by left thoracoscopy with previous CT-guided marking of the lesion (Fig. 2). The patient was taken under general anesthesia, and the marking harpoon was found on the junction of the apical segment with the anteromedial (Fig. 3); without palpable lesions, the procedure was performed, and the samples were analyzed intraoperatively.

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Pathology report: Microinfiltrating lepidic pattern adenocarcinoma, with mucinous differentiation, tumor size 0.5 × 0.5 × 0.5 cm, well-differentiated (G1). Resection margin of the negative parenchyma and pleura. Lymphovascular invasion was not observed.

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Fig. 2. Chest CT scan in sagittal section showing pneumothorax after placement of the harpoon in the pulmonary nodule described.

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Fig. 3. (. A-C) Intraoperative thoracoscopic image of the harpoon and lung parenchyma; (D) resected lung segment with the harpoon anchored in the non-nodular lesion.

The surgical procedure was carried out without any complications, and the patient was subsequently discharged in an asymptomatic condition, displaying normal lung auscultation, and the absence of

dyspnea. Following discharge, the patient continued to receive outpatient follow-up care under the supervision of the thoracic surgery and pulmonology department. During the follow-up, the thoracic surgery team requested a control chest X-ray and CT scan, both of which revealed no evidence of consolidation or infiltrative-type lesions. Additionally, the pulmonology department recommended conducting a positron emission tomography (PET-CT) 6 months after the surgery. This PET-CT was aimed at assessing the potential presence of lymph node involvement that might necessitate further management by the oncology team.

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PET-CT, 2023: There was no evidence of hypermetabolic lesions indicative of a neoplastic process.

As of the latest update, the patient remains asymptomatic, with no evidence of new lesions detected during the ongoing check-ups.

Discussion

With the increasing availability and accessibility of radiological diagnostic techniques, coupled with the emergence of lung screening programs and the rising incidence of incidental findings of pulmonary nodules across various imaging modalities, the prevalence of pulmonary nodules (PN) detected via CT scans has surged to 31%, further escalating to 50% among patients with identifiable risk factors [15]. This phenomenon has driven an augmented demand for diagnostic and therapeutic thoracic procedures [16]. Consequently, it is imperative to offer image-guided biopsies or surgical interventions to obtain histologic information from these suspicious nodules and determine the appropriate course of action. In this context, the preoperative assessment of pulmonary lesions through thoracic tomography assumes a crucial role in defining the indication for surgical resection, in addition to facilitating the selection of patients who may benefit from preoperative localization techniques. These techniques encompass the use of metallic harpoons [17,18], methylene blue [19], injection of high-intensity substances such as lipiodol or barium [20], or colored collagen [21], along with other approaches like intraoperative detection with ultrasound [22,23]. Despite inherent limitations, these methods have proven to be highly beneficial in planning and treating patients with small pulmonary nodules [24]. In specific clinical scenarios, precise lesion marking is imperative to ensure successful targeting while preventing either inadequate excision/treatment or excessive removal of healthy tissue. In 1993, Kanazawa et al. [25] pioneered the development of a hook-wire/suture system designed to streamline the localization of lung lesions for subsequent thoracoscopy. Since then, various targeting systems have been developed, and the role of interventional radiology procedures within the treatment strategy algorithm has evolved significantly [26].

It is noteworthy that techniques for marking suspicious lesions with metallic harpoons have found successful application in breast cancer [27], soft tissue masses, and other oncologic lesions, including pulmonary metastases [28]. These minimally invasive surgical procedures enable less aggressive approaches [28], resulting in shorter hospital stays and expedited postoperative recovery. One of the minimally invasive surgical procedures is VATS, which has become a fundamental tool in the diagnosis and treatment of pulmonary nodules [2,29]; however, in some cases, it is impossible to locate them correctly at the time of the procedure, which, according to the authors, occurs in 7.5%-11% of cases [26,30] and on such occasions preoperative marking is necessary to perform complete and safe resection using VATS [31,32].

In cases involving lung lesions, sublobar lung resection is recommended for early-stage primary lung cancer and small size metastases. However, localizing these lesions can pose challenges, either due

to their deep-seated location or their presentation as nonsolid opacities, such as ground-glass opacity [26]. Potential complications of the procedure encompass bleeding leading to hematoma formation, pneumothorax, hemothorax, and harpoon dislodgement, which may manifest either before surgery or during intraoperative placement [5,8,10]. To overcome these challenges, multiple localization techniques have been proposed: percutaneous hook-wire placement [8,[33], [34], [35]]; localization with injection of dyes, ethiodized oil, or radioisotopes [36]; and lung nodule marking before stereotactic-body-radiotherapy (SBRT) [37].

This case report focuses on a female patient with intermittent upper respiratory symptoms and normal pulmonary auscultation with an incidental ground-glass lung lesion on chest CT, which had increased in size during her imaging follow-up. Depending on the tomographic density, such lung lesions can be classified as solid or subsolid [38]. The morphology of a solid PN refers to an area of increased attenuation due to airspace collapse, as opposed to subsolid PNs, which in turn include pure ground-glass opacity (GGO) and mixed nodules [38]. Our patient had a nodule in pure GGO.

Although CT cannot confirm the benign or malignant etiology of the lesion, it can make an approximation considering some characteristics of the lesion. For pure GGO nodules, a size >8 mm and the presence of lobulated borders are alarm factors, while if it measures <4 mm, it is considered benign. A pure GGO nodule can be malignant in up to 20%-40%, while the rest is due to fibrotic processes or inflammatory, hemorrhagic, or infectious foci [13]; in the case of our patient, the lesion was more prominent than 8 mm [14].

While CT-guided harpoon marking has been previously described for PN lesions with a placement success rate of up to 100% [18], we emphasize that every suspicious lesion, regardless of its size, warrants meticulous evaluation. This approach ensures accurate assessment, especially when considering thoracoscopic procedures with prior marking, which enhances control and facilitates precise adjustments in treatment strategies.

In the case of the patient's pathology, lepidic pattern adenocarcinoma is an infrequent entity that can present in the fifth decade of life, tends to be multifocal, and predominates in women, nonsmokers of Asian descent [39,40]. Adopting this diagnostic technique for non-nodular pulmonary lesions is necessary since pulmonary carcinoma remains one of the neoplastic lesions with the highest morbimortality worldwide.

Therefore, in the era of minimally invasive surgery, interventional radiology assumes a pivotal role in the localization and marking of diverse pathologies. With the rising incidence of subsolid lung nodules, CT-guided harpoon marking proves to be an effective method for guiding the resection of ground-glass nodules, offering superior surgical precision. This precision holds significant relevance in determining appropriate therapeutic strategies and follow-up plans for individual cases and future research should prioritize long-term follow-up studies to assess durability, including ground glass nodule recurrence rates and impact on patient quality of life.

Abstract

Invasive ductal carcinoma is the most common type of breast cancer and can affect any age group, predominantly females older than 55 years of age. We present a case of a female in her mid-30s complaining of a fungating mass in the upper outer quadrant of the left anterior chest wall. On workup of the patient, it was histopathologically found that the patient was affected by infiltrating ductal carcinoma of the left breast, which was causing tumoral thrombosis of the left axillary vein. Also, thrombosis of the right axillary vein, bilateral brachiocephalic veins, and superior vena cava with a focal hepatic hotspot sign were appreciated on contrast-

enhanced computed tomography scan. No such case of tumoral thrombosis of the axillary vein causing superior vena cava obstruction has been reported in recent literature.

Case report

A female patient in her mid-30s came to the general surgery department at a camp organized by our hospital in a rural area of central India, complaining of a large foul smelling, blood and pus-discharging ulcerated lesion in the upper outer quadrant of the left breast as shown in Fig. 1. Six months ago, the lesion was a small, pea-sized pus-discharging nodule in the upper outer quadrant of the left breast, which gradually increased in size, and then it burst, and a small ulcer was formed. With time, the ulcer increased and has grown to its current size.

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Fig. 1. A fungating mass lesion in the left half of the left breast.

The patient presented with a current weight of 61 kgs with a body mass index of 22.7 and complained of weight loss of more than 18 kilograms in the past 6 months without any significant exercise, leading to a suspicion of a malignant lesion.

The patient is a housewife and a mother of 2 children aged 9 and 6 years, both by normal vaginal delivery. She has a regular 30-day menstrual cycle with no history of oral contraceptive pills intake or any other hormone replacement therapy. She is a nonalcoholic and vegetarian by diet.

She did not visit any medical facility previously due to the lack of a hospital in the nearby area.

The patient complains of pain in the left shoulder and upper back, with a gradual increase in the circumference of the left arm compared to the right arm. Initially, the pain was associated with the movement and usage of the left arm, but in a few months, the pain was constant even without work and would limit her daily activities. There were no aggravating factors, and it would be relieved to some extent with the use of painkillers.

She complains of breathlessness while doing daily chores, leading to a suspicion of involvement of the respiratory system. The patient gives no history of any known systemic illness. No member of the family or a relative is known to be affected by any breast pathology.

Swelling was large, malodorous and fungating measuring approximately 6 × 4 cm with associated pus and blood discharge in the left anterolateral chest wall. The lesion was tender on touch. A swelling was noted in the left arm with a significant difference in the circumference of approximately 3.2 cm compared to the right arm, suspecting lymphedema.

A defect in the skin surface and subcutaneous tissues reaching up to the pectoralis muscle was noted in the outer half of the left breast along with heterogeneously enhancing soft tissue density spiculated mass lesions in the upper half of the left breast and the nipple areolar complex suggesting a neoplastic etiology as shown in Fig. 2.

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Fig. 2. Contrast enhanced computed tomography axial section early arterial phase (A), and venous phase (B) showing heterogeneously enhancing soft tissue density mass lesion with spiculated margins in the upper half of the left breast (orange arrow) with enlarged heterogeneously enhancing lymph node in the left axillary region (green arrow); and multiple venous channels showing enhancement in the early arterial phase (yellow arrows) noted.

Similar lesions were noted in the upper and lower half of the right breast suggesting metastatic spread of the tumor as shown in Fig. 3.

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Fig. 3. Contrast enhanced computed tomography axial section early arterial phase (A), and (B) showing heterogeneously enhancing soft tissue density mass with spiculated margins in the upper half (yellow arrow), and lower half (blue arrow) of the right breast; heterogeneously enhancing enlarged lymph node in the left axilla (green arrow); and a defect in the skin surface and subcutaneous tissue reaching up to the pectoralis muscle (white arrow).

Post contrast administration, heterogenous enhancement with a filling defect was noted in the left axillary vein suggesting tumor thrombosis as shown in Fig. 4.

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Fig. 4. Contrast enhanced computed tomography axial section early arterial phase (A), and venous phase (B) showing heterogenous enhancement of the left axillary vein post contrast administration with filling defect in all phases of imaging suggesting tumoral thrombosis (orange arrow); a filling defect in left brachiocephalic vein suggesting thrombosis (yellow arrow); and multiple venous channels showing enhancement in the early arterial phase (blue arrows) noted.

In the early arterial phase of contrast administration, multiple enhancing vascular collaterals were visualized in the subcutaneous and intermuscular planes of the right neck and thorax region with a dense blush of contrast in the inferior vena cava (IVC) and the portal vein. A dense blush of contrast is seen in segment IV of the liver demonstrating the focal hepatic hotpot sign suggesting superior vena cava obstruction (SVCO) as shown in Fig. 5.

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Fig. 5. Contrast enhanced computed tomography axial section early arterial phase demonstrating superior vena cava obstruction - (A) showing dense blush of contrast in the segment IV (orange arrow); dense opacification of inferior vena cava (yellow arrow) and portal vein (green arrow) suggesting focal hepatic hot spot sign; and (B) showing a filling defect in the superior vena cava (gray arrow) and the left brachiocephalic vein (black arrow) suggesting thrombosis.

Also, filling defects were observed in the bilateral brachiocephalic veins, the right axillary vein and the SVC in all the phases of imaging post contrast administration.

The lung parenchyma showed a few soft tissue density nodules. Osteolytic lesions were visualized in the thoracic vertebrae and the scapula as shown in Fig. 6.

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Fig. 6. Contrast enhanced computed tomography axial section early arterial phase (A), and (B) showing osteolytic lesions in the thoracic vertebrae (orange arrows); and the left scapula (green arrow); and multiple venous channels showing enhancement in the early arterial phase (blue arrows) noted.

A diagnosis of neoplastic etiology causing tumoral thrombosis of the left axillary vein with SVCO and lung and skeletal metastasis was compiled. A frozen section trucut biopsy of the mass lesion in the upper half of the left breast was carried out by general surgery and the tissue sample was sent to the pathology where a histopathological diagnosis of the invasive ductal carcinoma was achieved as shown in Fig. 7, Fig. 8, Fig. 9.

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Fig. 7. Core needle biopsy of breast using hematoxylin and eosin stain and magnification of 4x showing tumor cell nests invading surrounding parenchyma (blue arrows).

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Fig. 8. Core needle biopsy of breast using hematoxylin and eosin stain and magnification of 10x showing tumor cell nests forming solid sheets and focal cribriform pattern (blue arrow); and invasion of the tumor cells into the adjacent breast parenchyma (yellow arrow).

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Fig. 9. Core needle biopsy of breast using hematoxylin and eosin stain and magnification of 40x showing tumor nests composed of large irregular cells having abundant foamy to clear cytoplasm, nuclei with coarse chromatin and prominent nucleoli (blue arrow); and infiltrative borders of tumor with surrounding desmoplastic stroma (yellow arrow).

The patient was transferred to the medical oncology department to evaluate the role of chemotherapy or radiotherapy in managing the disease. The patient was advised of emergency

radiotherapy for superior vena cava obstruction, and the patient refused it due to financial constraints.

Discussion

Invasive ductal carcinoma is the most prevalent type of breast carcinoma and is reported to involve 70%-80% of the patients affected by breast carcinoma [1]. The cancer can metastasize through blood or lymphatic channels to various body parts [2]. The development of tumoral thrombosis in cases of invasive ductal carcinoma is extremely rare and is demonstrated in this case. On injecting the contrast intravenously; the contrast flows into the venous collaterals, which drain the portal vein and ultimately into the segment IV of liver. Internal thoracic, superior, and inferior epigastric veins form the collateral pathways, which drain into the left lobe of the liver after draining into the para-umbilical veins. The inferior and superior veins of Sappey are the chief paraumbilical veins having drainage into the liver parenchyma and portal vein branches giving the hepatic hotpot sign [3,4].

Breast carcinoma is the most prevalent carcinoma in females and also the leading cause of death [5]. The aetiology of breast cancer is very complex and multifactorial, but the role of hormones, diet, genetics, reproductive factors and environment is considered important [1]. There is an increased risk of development of breast carcinoma in patients who have first-degree relatives with breast carcinoma, which was absent in our case. Other risk factors include mutations in the BRCA1 and BRCA2 gene [1].

Tumor thrombosis is characterized by the invasion of tumor cells into a vessel, mostly a vein. It is important to identify tumor thrombosis because its presence can affect the staging, prognosis, and management of the disease. It is most often seen in renal cell carcinoma, Wilms tumor, hepatocellular carcinoma and adrenal cortical carcinoma and mostly affects the inferior vena cava. The infiltration of the tumor cells into the axillary vein has yet to be reported in the recent literature. It is important to differentiate bland thrombus from tumor thrombus, the latter showing the vessel's expansion with enhancement postcontrast administration. The most specific sign suggesting tumor thrombosis is vessel enhancement. Vessel expansion is another sign suggesting tumor thrombosis. On magnetic resonance imaging, the tumor thrombosis shows diffusion restriction [6]. In our case, the tumor was invading the left axillary vein, causing tumoral thrombosis. This further leads to disruption in the normal blood flow through the veins, encouraging venous stasis and later leading to thrombus formation in the other vessels.

The focal hepatic hotspot sign, also known as thehot quadrate sign is a pseudo lesion characterized by increased accumulation of iodinated contrast in the segment IV (medial segment of the hepatic lobe) due to superior vena cava obstruction (SVCO). In cases of SVCO, when the contrast is administered into an upper limb, it is diverted into the collateral pathways, including thoracic and epigastric veins, which communicate with the paraumbilical veins (superior and inferior veins of Sappey) carrying the blood to the left lobe of liver with direct perfusion of the liver parenchyma and also drainage of blood into the branches of the portal vein [3,4].

In the presented case, the cause of breathlessness was suspected to be due to the lesion's respiratory involvement, which was ruled out by imaging. The main cause of breathlessness in our patient was the presence of SVCO. It is believed that the SVCO causes a reduction in the blood volume of the heart due to an impairment of the venous return. As a result, the flow of the deoxygenated blood is impaired, causing a rise in the carbon dioxide levels in the affected veins [7]. Also, the cause of pain in the left shoulder and upper back was mainly due to the osteolytic lesions in the scapula and the upper thoracic vertebra.

Invasive ductal carcinoma of the breast is the most common type of breast carcinoma. The diagnosis is always made on histopathological evidence [1]. The treatment is dependent on the staging. The treatment guidelines include total mastectomy or lumpectomy with axillary staging ± dissection and breast reconstruction with radiation to the whole breast and the involved lymph nodes in cases where the metastasis to the adjacent bones and organs has not taken place. In cases of metastasis, surgical intervention is avoided, and selective radiation is provided to the metastatic lesions [8].

Tumor thrombus may lead to the development of bilateral axillary, brachiocephalic vein, and superior vena cava thrombosis, obstructing the superior vena cava, which shows an indirect sign known as focal hepatic hot spot sign in the early arterial contrast phase. CT plays an important role in diagnosing tumoral thrombosis and appears as increased vascular diameter, showing enhancement postcontrast administration. Infiltrative ductal carcinoma causing axillary vein thrombosis has not been reported in the recent literature and is a new learning point for readers.
However, histopathology is always the main modality for diagnosing the lesion.

Introduction and importance

Pleuropulmonary blastoma (PPB) is a rare primary malignant tumor in the chest that mainly occurs in children <6 years of age. Vascular extensions are even rarer, approximately 3 % of types II and III PPB, and have fatal complications. The patients of reported cases who had tumor extension to the heart are younger than three years old, whereas in this case, we reported an 11-year-old girl who was of school age. This case report aims to describe a rare case of a type III Pleuropulmonary Blastoma infiltrating the left heart of a school-age girl.

Case presentation

An 11-year-old girl presented at an emergency department with two months of progressive dyspnea with malnutrition. A fused mass was found in LA on an echocardiogram along with moderate MR, severe MS, and mild pericardial effusion. CT scan showed a massive pleural effusion with a solid mass in the left lung obstructing the left bronchial tree, accompanied by the expansion of the tumor mass into the left pulmonary vein and LA.

Clinical discussion

Total removal of the tumor was performed, aided by cardiopulmonary bypass. Type III PPB was confirmed histopathologically.

Conclusion

PPB is a rare, aggressive tumor that has three types. Various manifestations can occur in line with the presence of metastases. The treatment consists of aggressive surgery and chemotherapy. Because of its poor prognosis, prompt recognition of the involvement of the cardiac chamber and great vessels in type III PPB should be considered before surgery.

1. Introduction

Pleuropulmonary blastoma (PPB) is an uncommon primary malignant tumor but also the most common pediatric lung tumor (1,2). The majority of the patients are younger than six years old. Type III PPB typically occurs at 35 months and has the least favorable prognosis. Seven cases out of twelve reported vascular extension extending to the left heart (4,10). Six of twelve died from complications that may be related to vascular involvement (4,10).

We present a successful surgery in a school-age girl with extensive type III PPB that occludes pulmonary veins and infiltrates the left atrium. The surgery was done without adjunct chemotherapy. We consider this worthy of reporting because there is still no standard therapy for vascular extensions since it has a lower incidence.

2. Case report

An 11-year-old girl weighing 18 kg was presented at an emergency department with two months of progressive dyspnea, worsening two weeks before admission. Her dyspnea was relieved by sitting and bending forward. Dyspnea is accompanied by non-productive cough, pleuritic chest pain, fatigue, and malnutrition. She grew up with a poor appetite with continuous exposure to secondhand smoke. There is no history of hemoptysis, and her vital sign shows no fever. Decreased left lung sounds and pitting oedema were found on physical examination. Because of her symptoms, she was brought to a secondary hospital. A chest tube was inserted into the left hemithorax, and the patient was stabilized for seven days before she was referred to our hospital for further management (Fig. 1). A CT scan showed a massive pleural effusion with a solid mass in the left lung obstructing the left bronchial tree, accompanied by the expansion of the tumor mass into the left pulmonary vein and LA suspected PPB. A fused mass was found in LA on an echocardiogram with moderate MR, mild MS, and mild pericardial effusion (Fig. 2).

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Fig. 1. Echocardiography showed a mass at Left Atrium, moderate MR, mild MS, and mild pericardial effusion.

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Fig. 2. CT Scan showed a massive pleural effusion with a solid mass in the left lung obstructing the left bronchial tree, accompanied by the expansion of the tumor mass into the left pulmonary vein and LA.

A median sternotomy approach was performed, and tumor mass was easily identified in the left thorax after the opening of the visceral pleura. The tumor was attached to the hilum and adhered tightly to the pericardium and inferior left pulmonary vein. No gross adhesions were found at the interior of the thorax cavity, aorta, esophagus, and diaphragm. The heart appeared normal in size (Fig. 3).

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Fig. 3. The tumor is well defined and was attached to the hilum and adhered tightly to the pericardium and inferior left pulmonary vein.

The heart was temporarily stopped by antegrade cardioplegia to aid the removal of the intracardiac tumor, followed by pneumonectomy of the left lung. The inferior left pulmonary vein was resected, and the left atrium was reconstructed. Mitral valve function was normal on TEE evaluation without any additional procedure. All the affected nodules were removed. The aortic cross-clamp was 91 min, and the cardiopulmonary bypass was 113 min. Type III PPB was confirmed histopathologically (Fig. 4). Her symptoms significantly improved, and she was discharged on day 7.

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Fig. 4. Histopathologic examination showed pleuropulmonary blastoma type III with tumor cells extended to the left atrium.

3. Discussion

Pleuropulmonary Blastoma (PPB) is the most common lung primary tumor in pediatrics. They are also sporadic and occur in 25–50 cases annually in the United States. They have an aggressive nature that differs between the three types. Type I or cystic PPB have the most favorable outcome as they often present in the first year of life, unilateral, unifocal, and peripheral, which makes them easily resected utterly. Type I PPBs may progress to Type II or cystic/ solid PPBs, present at a median age of 35 months. Type III or purely solid PPB have the least favorable outcomes with a 53 % survival rate (2) as they may present at a more advanced age with a median age of 41 months and an advanced stage. (1,2) Pathogenic variants in the DICER1 gene have been shown in PPB as the hallmark neoplasm (2,3). About 80 % of the DICER1 germline pathogenic variants in PPB are inherited by a parent and nearly 20 % are de novo (4). DICER1 carriers are commonly found in cystic PPB and only a minority experience progression to type II or III PPB, given the rarity of these tumors (3).

The patient often manifests respiratory symptoms initially, as PPB involves the lung. The tumor may also arise from the diaphragm, mediastinum, and pleura. Mediastinal or pleural involvement was correlated with poorer survival. (5) Various manifestations can occur in line with the presence of metastases. The most common metastatic site is the brain, followed by the bone. (5) The most common metastatic site is the brain, followed by the brain (3). The other sites are lymph nodes, liver, pancreas, adrenal glands, heart and great vessels. Vascular extension is rare and is an essential complication in 3 % of type II and III PPBs. In our cases, the tumor extended into LA and PV is rare occurrence and only 11 cases have been published before. ([6], [7], [8]) Occluded pulmonary veins, left atrial tumor thrombus, great vessels occlusion, paraplegia, thromboembolism, insufficiency of the heart valve, and extensive intracardiac disease that requires removal and re-insert the heart are the expected complications in vascular extensions. (6,7)

Chest X-ray can be used as the first workup for PPB as respiratory problems are the most common, especially in endemic tuberculosis countries like Indonesia. It appears as a radio-opaque thorax mass with mediastinal shift and contralateral tracheal deviation. It may not be helpful in a significant tumor mistaken for massive pleural effusion. (9) Computed Tomography (CT) of the chest, abdomen, and head are useful in excluding other diseases, estimating tumor size, and locating metastases. Echocardiography should be done to assess the heart function and intracardiac anatomy if the tumor extends to the heart chamber. (2) Seeking germline mutation in the DICER1 gene might be helpful as it happens in 66 % -80 % of children with PPB (1,2,9), but it was unrelated to the outcome. (2,3) Histopathology must be done because the PPB type was the strongest outcome predictor.

(1,2,9) The gross lesion of our patient is a well-circumscribed white tan solid mass with necrosis and hemorrhage in the friable areas. The microscopic elements of our patient's lesion consist of extensive areas of necrosis with hyperplastic round oval to spindle-shaped cells that condense and group together and give a blastemal appearance, some form an adenomatoid image, and some form a fasciculus image. Some cells' cytoplasm is eosinophilic (rhabdoid) and pleomorphic, hyperchromatic, mitotic cell nuclei were found. These findings are aligned with type III PPB which has the least favorable outcome (10). PET scan to find malignant tumor cells in the body was not performed due to cost dilemmas from the patient's family and the patient's inability to lie still.

Patients with type I PPB can be treated by surgical resection with negative margins and no tumor spill with possible additional chemotherapy (11). Complete resection might be achieved since type I PBB has a definable margin. Depending on tumor location, tumor removal can be approached with either wedge resection, pulmonary lobectomy, or broad resection. (1) Type II and III PPB are both aggressive tumors. Surgical resection and systemic chemotherapy are important roles in the treatment. Cerebral events and cerebral metastases might happen in patients with large vascular extensions, and chemotherapy plays a significant role in preventing them (6). Chemotherapy can be given as a neoadjuvant or adjuvant. There are no standard chemotherapy regimens for PPB management. The regiments used in therapy are combinations of drugs (vincristine, actinomycin-D, ifosfamide, doxorubicin, carboplatin, etoposide, epirubicin, and cyclophosphamide) like CEVAIE, VAIA, IVA, VAC, VACA, VA, and IVAD. (1,5,9,12). Complete resection of the tumor with radical surgery should be applied for surgical treatment. Tumor size, location, regional metastases, and distant metastases should be considered. For this reason, patients with initially unresectable tumors should be treated with neoadjuvant chemotherapy to reduce the tumor burden and allow safe tumor resection (1,2,6). Radiotherapy for PPBs, which remains a debatable treatment. Literature that disagrees with radiotherapy argue that there is no significant difference in survival between patients who receive radiotherapy and those who do not (13), even poor outcome after radiotherapy has been applied to the thorax and mediastinum (12). However, Paolo et al. said that local radiotherapy after initial radical surgery is an effective adjunct therapy (3). Although the European Cooperative Study Group for Pediatric Rare Tumors (EXPeRT) recommends giving neoadjuvant chemotherapy before surgery (14), the urgency to relieve the circulatory obstruction and the possibility of worsening the malnutrition condition are the reason not to give neoadjuvant therapy like what was done in some cases (8,11,15) and immediate debulking surgery can be considered based on EXPeRT. (14) Recommendations from EXPeRT are expert opinions and standard-evidence-based therapy for rare tumors like PPB has not been established yet.

4. Conclusion

PPBs are extremely rare yet the most common primary lung tumor in childhood. Vascular extension of the tumor is rarer and has many complications. Combining clinical findings with imaging and histopathological examinations should help determine the PPB diagnosis. Radical resection and the absence of metastasis are promising and can be done safely with chemotherapy and possibly radiotherapy.

Background

Treatment with tumor-infiltrating lymphocytes (TILs) is rapidly evolving for patients with solid tumors. Following metastasectomy, TILs (autologous, intratumoral CD4+ and CD8+ T cells with the potential to recognize tumor-associated antigens) are isolated and non-specifically expanded *ex vivo* in the presence of interleukin-2 (IL-2). Subsequently, the TILs are adoptively transferred to the patients after a preconditioning non-myeloablative,

lymphodepleting chemotherapy regimen, followed by administration of high-dose (HD) IL-2. Here, we provide an overview of known cardiac risks associated with TIL treatment and report on seven patients presenting with cardiac symptoms, all with different clinical course and diagnostic findings during treatment with lymphodepleting chemotherapy, TIL, and HD IL-2, and propose a set of clinical recommendations for diagnosis and management of these symptoms.

Patients and methods

This single-center, retrospective study included selected patients who experienced TIL treatment-related cardiac symptoms at the Netherlands Cancer Institute. In addition, 12 patients were included who received TIL in the clinical trial setting without experiencing cardiac symptoms, from whom complete cardiac biomarker follow-up during treatment was available [creatine kinase (CK), CK-myocardial band, troponin T and N-terminal pro-B-type natriuretic peptide].

Results

Within our TIL patient population, seven illustrative cases were chosen from the patients who developed symptoms suspected of severe cardiotoxicity: myocarditis, myocardial infarction, peri-myocarditis, atrial fibrillation, acute dyspnea, and two cases of heart failure. An overview of their clinical course, diagnostics carried out, and management of the symptoms is provided.

Conclusions

In the absence of evidence-based guidelines for the treatment of TIL therapy-associated cardiotoxicity, we provided an overview of literature, case descriptions, and recommendations for diagnosis and management to help physicians in daily practice, as the number of patients qualifying for TIL treatment is rapidly increasing.

Adoptive cell therapy (ACT) with tumor-infiltrating lymphocytes (TILs) is a rapidly evolving treatment field for patients with solid tumors, which has recently shown promising results in patients with melanoma and non-small-cell lung cancer (NSCLC).[1,2] In TIL treatment, autologous, intratumoral CD4+ and CD8+ T cells with the potential to recognize tumor-associated antigens are used as a personalized treatment modality. Following metastasectomy, TILs are isolated and non-specifically expanded *ex vivo* in the presence of interleukin-2 (IL-2). These expanded TILs are then adoptively transferred to patients after a preconditioning non-myeloablative, lymphodepleting chemotherapy regimen, followed by administration of high-dose (HD) IL-2. The preconditioning chemotherapy regimen and HD IL-2 are known to have the potential to cause cardiac adverse events, while the risk of TILs driving cardiotoxicity is hypothetical.[3,4] Here, we provide an overview of known cardiac risks associated with TIL treatment and report on seven patients presenting with cardiac symptoms, all with different clinical courses and diagnostic findings, during treatment with lymphodepleting chemotherapy, TIL, and HD IL-2 at the Netherlands Cancer Institute (NKI, Amsterdam, The Netherlands), and propose a set of clinical recommendations for diagnosis and management of these symptoms.

Chemotherapy

The preconditioning chemotherapy regimen typically consists of a combination of cyclophosphamide, an alkylating agent, and fludarabine, a purine analog. The reduction of immunosuppressive cell populations and endogenous T cells before the cell infusion is thought to enhance the efficacy of TIL by creating a favorable immune environment, promoting their *in vivo* expansion.[5] However, next to the well-known hematological side-effects,

including leukocytopenia and thrombocytopenia, both agents have also been associated with cardiotoxicity.

Cyclophosphamide-induced cardiac toxicity has an incidence of 7%-33% and is thought to occur as a result of oxidative stress in combination with direct damage to the capillary endothelium, leading to extravasation of erythrocytes, proteins, and toxic metabolites, further damaging the myocardium and capillaries.[6, 7, 8] This in turn leads to interstitial hemorrhage, micro-thrombi formation, and edema, which can result in a spectrum of clinical manifestations, typically within 48 h of administration. These manifestations include acute heart failure, arrhythmias, refractory hypotension, (hemorrhagic) myocarditis, pericarditis, or death.[6,9,10] Risk factors include a higher total dose of cyclophosphamide administered, advanced age, pre-existing risk factors for ischemic heart disease, and history of radiotherapy to the mediastinum or left chest wall.[6] Thus far, one case of fatal cardiac failure following cyclophosphamide administration before TIL infusion has been reported.[11]

In addition to the direct cardiotoxic effects of cyclophosphamide, the concomitant hyperhydration given to patients to prevent hemorrhagic cystitis may cause left- or right-sided congestive heart failure.[12]

Cardiotoxicity caused by single-agent fludarabine is rare; however, there is evidence that the combination of fludarabine with other chemotherapeutics (i.e. melphalan) can result in cardiac dysfunction. In patients with hematological malignancies both fatal and non-fatal congestive heart failure,[13,14] acute severe left ventricular failure,[15,16] hypotension and chest pain,[17] and myocarditis[18] have been observed. None of the patients in these studies had prior cardiac dysfunction.

Of note, most information is based on experiences in pre-stem cell transplantation settings for patients with hematological malignancies, who typically have different prior treatments, disease dynamics, and chemotherapy doses in their pre-transplant regimen. Thus far, there is limited knowledge about the potential cardiotoxicities of cyclophosphamide and fludarabine in the ACT treatment setting for solid tumors.

TIL

Cardiotoxicity has been observed in TIL trials; however, these studies do not distinguish if this was related to chemotherapy, TIL, or IL-2. While there is currently limited information about the potential of TIL to induce cardiotoxicity, there are three hypothetical categories in which adoptively transferred T cells can induce cardiotoxicity: (i) 'on-target, on-tumor' effects causing cytokine release syndrome (CRS); (ii) 'on-target, off-tumor' effects resulting in direct cardiac injury because of shared antigens between the tumor and cardiac tissue, recognized by the T-cell product; and (iii) 'off-target, off-tumor' effects due to unexpected cross-reactivity of TILs with cardiac epitopes.[19] So far, none of these toxicities have been demonstrated to be caused by TIL infusion.

CRS is the result of *in vivo* antigen recognition, leading to T-cell activation, proliferation, and concurrent massive cytokine release.[20] It is characterized by three core symptoms (fever, hypotension, and/or hypoxia), often accompanied by constitutional symptoms, and, depending on the severity, dyspnea, coagulopathy, and organ dysfunction.[21,22] In patients treated with chimeric antigen receptor (CAR) T cells, another form of ACT, CRS has been identified as the risk factor for cardiovascular complications, including heart failure, myocardial infarction (MI), arrhythmias, cardiac arrest, and capillary leak syndrome (CLS).[23, 24, 25] The cytokine IL-6, a key mediator of CRS, is thought to play an important role in the pathophysiology of ACT-associated cardiotoxicity. IL-6 promotes oxidative stress, resulting in cardiomyocyte apoptosis, mitochondrial dysfunction,

and cardiac hypertrophy via activation of the gp130/STAT3 pathway.[24,26] Furthermore, elevated IL-6 levels have been associated with atrial fibrillation and fatal ventricular arrhythmias.[27] Moreover, other pro-inflammatory cytokines, including interferon-γ, IL-1β, IL-2RA, and tumor necrosis factor-α, can have negative inotropic and cytotoxic effects on cardiomyocytes, which can result in decreased myocardial contractility, hypotension, and cardiomyopathy.[28] Of note, these cytokines are also elevated in inflammation from other causes (i.e. sepsis). CAR-T-cell-treated patients who developed cardiotoxicities were typically older, and more likely to have a history of coronary artery disease or hyperlipidemia, or to use cardiac medication.[25] This is supported by Fradley and colleagues, who observed a markedly higher prevalence of hypertension and hyperlipidemia in melanoma patients developing cardiotoxicity after TIL treatment.[29]

'On-target, off-tumor' toxicity has been described in patients treated with TIL. In melanoma patients, rash or vitiligo, uveitis, and hearing loss have been observed due to shared antigens between melanoma cells and melanocytes.[1,30, 31, 32, 33] Likewise, it cannot be fully excluded that T cells in the infusion product could potentially recognize cardiac epitopes, leading to cardiotoxicity.

Finally, it is possible that T cells in the infusion product cross-react with epitopes expressed on cardiac cells. This has previously been observed in patients treated with T-cell receptor (TCR) T-cell products targeting MAGE-A3, resulting in unexpected fatal toxicities due to cross-reactivity with the cardiac protein titin.[34]

Interleukin-2

IL-2 is a cytokine mainly produced by activated CD4+ T cells in secondary lymphoid structures and serves as a growth factor for T lymphocytes.[35,36] It activates and promotes the proliferation of effector CD8+ T cells, natural killer (NK) cells, and immunosuppressive CD4+ regulatory T cells (Tregs).[35,36] Despite its pleiotropic functions, HD recombinant IL-2 (aldesleukin) became one of the first approved immunotherapies for metastatic melanoma and renal cell carcinoma because of its potent ability to stimulate cytotoxic T cells and NK cells.[37,38] However, its use is now limited because of the severe toxicities. Despite this, in current TIL treatment protocols, HD IL-2 is used both *ex vivo* during the cell production, as well as *in vivo*. HD IL-2 administration following TIL infusion has been shown to enhance T-cell persistence and expansion *in vivo* and is therefore a key component of commonly used ACT protocols.[4,39]

Administration of IL-2, either as monotherapy or as part of TIL treatment, is associated with an array of toxicities. The most profound effects of HD IL-2 treatment are CLS due to the binding of recombinant IL-2 to the high-affinity IL-2Rαβγ receptor expressed on endothelial cells, and toxicities resulting from immune cell activation and subsequent release of pro-inflammatory cytokines.[40] Clinical presentations include fever and chills, edema, hypotension, gastrointestinal symptoms, skin rash, and hematologic toxicities, which are typically dose-related and peak 4-6 h after administration.[40] The majority of these toxicities are reversible upon IL-2 discontinuation and can either be prevented using premedication, or effectively managed using supportive measures.[40]

A particularly challenging subgroup of IL-2-related side-effects are cardiotoxicities, as they can vary widely in presentation and severity, often making them difficult to diagnose. Documented effects on the cardiac system include hypotension, arrhythmias, angina pectoris, acute MI, and myocarditis.[37,40,41] However, real-world data on the presentation and management of these symptoms are scarce.

Patients and methods

Patients and clinical data

This single-center, retrospective study included patients treated with TIL at the NKI experiencing cardiac symptoms. Additionally, 12 patients were included who received TIL in the clinical trial setting without experiencing cardiac symptoms, from whom complete cardiac biomarker follow-up during treatment was available. The following patient and tumor characteristics were obtained: primary tumor type, age at time of treatment, sex, sites of metastases, prior treatments received, and relevant cardiac medical history. Furthermore, information on cardiac tests during screening, cardiac enzymes, cardiac symptoms, diagnostic tests carried out, symptom management, and follow-up was collected.

The study was conducted in accordance with the Declaration of Helsinki and all patients gave written informed consent.

TIL treatment protocol

TIL treatment protocols consist of 2 days of cyclophosphamide (60 mg/kg), 5 days of fludarabine (25 mg/m^2) and the TIL infusion, followed by intravenous HD IL-2 (600 000 IU/kg/dose) every 8 h, with the first dose ∼4 h after the TIL infusion, until the maximum dose number is given or stopping criteria are met.[1,41] Patients were hospitalized from the day before the chemotherapy until recovery of symptoms. Blood draws took place daily until the HD IL-2 period, and thereafter at least every other day until discharge from the hospital. During chemotherapy, creatine kinase (CK), CK-myocardial band (MBm), troponin T (TropT), and N-terminal pro-B-type natriuretic peptide (NT-proBNP) were determined before and after TIL infusion, daily during the HD IL-2 period, and at least every other day until discharge.

Laboratory results

CK, CK-MBm, TropT, and NT-ProBNP were measured on Roche Cobas Pro systems, and CK activity measurement was standardized against the International Federation of Clinical Chemistry (IFCC) reference method. At the NKI, the upper limit of normal for the laboratory results mentioned in this manuscript vary based on sex (CK and CK-MBm), or sex and age (NT-ProBNP). Reference values can be found in Supplementary Table 1, available at https://doi.org/10.1016/j.esmoop.2024.102383.

Statistical analysis

Figures were made using GraphPad Prism version 9.4.1 and Biorender. For the 12 asymptomatic patients, the mean and standard deviations were calculated and shown in the graphs.

Results

Within our TIL patient population consisting of 108 patients with melanoma and 12 patients with NSCLC, 7 illustrative cases of patients who developed symptoms suspected of severe cardiotoxicity were selected. With the exception of case 7, none of the selected patients had a history of thoracic radiotherapy. Here, we give an overview of their clinical course, carried-out diagnostics, and management of symptoms (Table 1, Figure 1). An overview of potential cardiac symptoms per stage of TIL treatment can be found in Figure 2.

Table 1. Patient characteristics and overview of clinical course

Empty Cell	**Case 1**	**Case 2**	**Case 3**	**Case 4**	**Case 5**	**C**
Sex	M	M	F	F	F	F
Age	73	65	50	66	54	6
Primary tumor	Melanoma	Melanoma	Melanoma	Melanoma	Melanoma	N
Metastatic sites	Adrenal gland, lung, subcutaneous, lymph nodes	Brain, lung, muscle, subcutaneous	Lung, bone, lymph nodes, subcutaneous	Lymph nodes, subcutaneous	Lung, muscle, subcutaneous	A b
Cardiac history	—	NSTEMI with PTCA of LAD, RCA and AL; pulmonary embolism	—	—	Small PFO	H at ta at ex
PreTx ECG	No abnormalities	Reduced R-wave anteroseptal leads; AP class 1	No abnormalities	Right bundle branch block	No abnormalities	A ex
PreTx EF	67%	62%	63%	67%	51%	7
Chemotherapy	No cardiac toxicities	No cardiac toxicities	No cardiac toxicities	Pulmonary congestion with hypoxia, edema, and tachycardia	No cardiac toxicities	P co w ar
TIL infusion	Irregular heartbeat shortly after infusion	No cardiac toxicities	No cardiac toxicities	Dyspnea and tachypnea	No cardiac toxicities	D ch d
IL-2	3x; fever, chills, CLS, hypoxia	3x; fever, chills, hypoxia	2x; fever, chills, CLS, dyspnea	1x; fever, chills, dyspnea	1x; fever, chills	1 d
Cardiac symptoms	Chest pain, hypoxia, hypotension	Chest pain	Hypotension with low urine output not responding to fluid challenge; chest pain	Respiratory failure, fluid retention, tachycardia	Rapid, irregular heartbeat; dyspnea	H ta
ICU admission	Yes (3 days)	Yes (3 days)	Yes (1 day)	Yes (5 and 8 days)	No	N

Empty Cell	**Case 1**	**Case 2**	**Case 3**	**Case 4**	**Case 5**	**C**
Diagnostics	ECG (non-specific STT changes); lab (TropT and NT-proBNP ↑, kidney function ↓); TTE (EF 45%-50% + wall motion disturbances)	ECG (1 mm ST-elevation in anteroseptal/anterior leads); lab (TropT ↑)	ECG (low voltage); lab (TropT and NT-proBNP ↑); TTE (small pericardial effusion without hemodynamic compromise)	ECG (micro-voltages); TTE (small pericardial effusion without hemodynamic compromise); lab (TropT and NT-proBNP ↑)	ECG (atrial fibrillation)	C (p co E o is (T P
Differential diagnosis	Acute coronary syndrome; type 2 myocardial infarction; peri-myocarditis	Acute coronary syndrome; type 1 myocardial infarction; myocarditis	Peri-myocarditis; myocarditis	Heart failure; peri-myocarditis; capillary leak syndrome; sepsis	Atrial fibrillation	C
Management	Hemodynamic monitoring; beta-blocker, furosemide	Hemodynamic monitoring	Hemodynamic monitoring	Non-invasive ventilation; ACE inhibitor, beta-blocker, aldosterone antagonist and furosemide	Beta-blocker	N vo
Final diagnosis	Cardiac MRI: myocarditis	MRI heart: myocardial infarction	Suspected peri-myocarditis	Decompensatio cordis + nephrotic syndrome due to pauci-immune glomerulonephritis	Atrial fibrillation	C
Follow-up	Fully recovered	Fully recovered	Fully recovered	Cardiac function recovered, progressive edema due to nephrotic syndrome	Fully recovered	Fu

ACE, angiotensin-converting enzyme; AL, anterolateral; AP, angina pectoris; CLS, capillary leak syndrome; ECG, electrocardiogram; EF, ejection fraction; F, female; ICU, intensive care unit; IL-2, interleukin-2; LAD, left anterior descending artery; M, man; MRI, magnetic resonance imaging;

NSCLC, non-small-cell lung cancer; NSTEMI, non-ST-elevation myocardial infarction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PFO, patent foramen ovale; PTCA, percutaneous transluminal coronary angioplasty; RBBB, right bundle branch block; RCA, right coronary artery; TIL, tumor-infiltrating lymphocyte; TropT, troponin T; TTE, transthoracic echocardiography; US, ultrasound.

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Figure 1. **Cardiac biomarkers.** Graphs show the course of cardiac biomarkers (CK, CK-MBm, Troponin T, and NT-proBNP) per case, as well as the mean and standard deviations for the 12 asymptomatic patients as reference. The upper limit of normal of TropT is indicated by the dotted line. Day 0 is the day of the TIL infusion. The gray box indicates the maximal period during which patients could have received IL-2, though the individual duration of treatment varies per patient.

CK, creatine kinase; CK-MBm, creatine kinase-myocardial band; IL-2, interleukin-2; NT-proBNP, N-terminal pro-B-type natriuretic peptide; TIL, tumor-infiltrating lymphocyte.

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Figure 2. **TIL cardiotoxicity timeline**. This timeline shows the cardiac toxicities that can be expected based on the treatment phase the patient is in (lymphodepletion, TIL infusion, or IL-2 administrations).

IL-2, interleukin-2; TIL, tumor-infiltrating lymphocyte.

Case 1. Myocarditis

A 73-year-old male presented with *NRAS*-mutated, metastatic melanoma progressive on first-line nivolumab, without history of cardiac disease. Pre-TIL-treatment cardiac screening showed normal electrocardiogram (ECG) and left-ventricular ejection fraction (LVEF). No significant toxicities were observed during lymphodepleting chemotherapy. Shortly after TIL infusion, an irregular heartbeat was noticed during regular checkups. In the absence of ECG abnormalities, the first HD IL-2 was administered. Patient developed fever, chills, and temporary hypoxia, for which broad-spectrum antibiotics, pethidine, and oxygen were given, respectively. On day +1, non-specific STT changes appeared on ECG, becoming more pronounced on day +2 after the third IL-2 dose, with the patient experiencing short periods of chest pain, and CLS with hypoxia, hypotension, and decreased kidney function. High-sensitive TropT levels and NT-proBNP were elevated. The patient was admitted to the intensive care unit (ICU) for hemodynamic monitoring. Transthoracic echocardiography (TTE) showed a decreased LVEF (45%-50%) and wall motion disturbances with hypokinetic anterolateral segments. Differential diagnoses comprised acute coronary syndrome, type 2 MI, and peri-myocarditis. A close wait-and-see approach with continuous monitoring was chosen; symptoms subsided and ST-segments normalized. As the patient was pancytopenic, no anti-coagulant or antiplatelet medication was given. Over the next days, a low-dose beta-blocker and furosemide were started. With this, cardiac biomarker levels dropped, and LVEF recovered; CK-MBm levels were never markedly elevated. After 2 days, the patient was discharged to the ward and

steadily recuperated. A cardiac magnetic resonance imaging (CMR) 19 days after TIL infusion, showing edema in basal anterior segments and a slightly decreased EF (51%), confirmed myocarditis (Figure 3). During outpatient clinic follow-up, the patient recovered completely (EF 60%).

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Figure 3. Cardiac imaging. Cardiac MRI of case 1, 19 days after TIL infusion: (A) MRI short-axis T2 SPAIR image—edema in the anterolateral wall of the most basal slice is shown by the orange arrow; (B) MRI short-axis T1 SPIR post-contrast (gadolinium) image—early enhancement is shown in the anterolateral wall of the basal slice by the orange arrow, indicating inflammation; (C) 2D short-axis late gadolinium (viability) image—no late enhancement is seen, indicating that no fibrosis is present, and thus no infarction. Baseline and on-treatment day +1 ECG from case 2 showing new ST-elevation in the anteroseptal/anterior leads indicative of myocardial infarction (D); (E & F) cardiac MRI late gadolinium image—late enhancement (LGE) subendocardial in the anteroseptal wall, which is typically for infarction (E is short axis, F shows the long axis). Baseline and on-treatment day +4 ECG from case 3 showing micro-voltages suspect of peri-myocarditis (G). Baseline and two on/post treatment ECGs from case 7 showing convex ST-segment in the precordial (septal) chest leads and an interventricular conduction disorder, incomplete right bundle branch block, and left anterior fascicular block on day +6, after which the T-wave in the anterior wall and septal precordial leads progressed to a negative T-segment on day +29 (H).

2D, two-dimensional; ECG, electrocardiogram; MRI, magnetic resonance imaging.

Case 2. Myocardial infarction

A 65-year-old man presented with *BRAF* V600E-mutated, metastatic melanoma progressive on first-line nivolumab, and a history of coronary artery disease: non-ST-elevation MI (NSTEMI) with a percutaneous transluminal coronary angioplasty (PTCA) of the left anterior descending artery (LAD), right coronary artery (RCA) and anterolateral (AL), and a recent pulmonary embolism. Pre-treatment ECG showed reduced R-waves in anteroseptal leads; EF on TTE was normal. The patient was asymptomatic (AP class 1). No significant toxicities were observed during the lymphodepleting chemotherapy or TIL infusion. After the first IL-2 dose, the patient developed fever, chills, and temporary hypoxia, for which broad-spectrum antibiotics, pethidine, and oxygen were given, respectively. The following day, the patient developed chest pain 2.5 h after the third HD IL-2. ECG showed 1 mm ST-elevation in the anteroseptal/anterior leads (Figure 3). TropT was elevated, while CK-MBm remained normal. The patient was admitted to the ICU for hemodynamic monitoring. Differential diagnoses of acute coronary syndrome, type 1 MI, and myocarditis due to TIL or IL-2 were considered. As the patient was thrombocytopenic [$24*10^9$/l (150-$400*10^9$/l)], no antiplatelet therapy was added to the low-molecular-weight heparin dosed for pulmonary embolism. At the time of ICU admittance, chest pain had subsided and cardiac biomarkers normalized over the next days. After 2 days, the patient was discharged to the ward where he steadily recuperated. At follow-up, TTE showed normal ventricular function without wall motion abnormalities. CMR 29 days after onset of symptoms showed minimal late gadolinium enhancement anteroseptal, confirming MI (Figure 3) with normal ventricular function (EF 60%). During outpatient clinic follow-up, the patient recovered completely.

Case 3. Peri-myocarditis

A 50-year-old woman presented with progressive, *NRAS*-mutated, metastatic melanoma, with a recently diagnosed immune-related insulin-dependent diabetes mellitus after two cycles of pembrolizumab. Pre-treatment ECG and LVEF were normal. No significant toxicities were observed during the lymphodepleting chemotherapy or TIL infusion. After the second HD IL-2, the patient developed CLS, with hypotension and low urine output unresponsive to fluid challenge; therefore, IL-2 was discontinued. On day +4, ECG showed low voltage (Figure 3), and TropT and NT-proBNP were increasingly elevated. On day +5, the patient developed chest pain worsening with coughing and movement of the left shoulder. TTE showed small pericardial effusion without signs of hemodynamic compromise. Perimyocarditis was suspected either induced by cyclophosphamide or HD IL-2. The patient was admitted to the ICU for continuous hemodynamic monitoring. She remained stable, and was discharged to the ward after 24 h. Her pain subsided and TropT levels and NT-proBNP decreased spontaneously.

Case 4. Heart and kidney failure

A 66-year-old woman presented with *BRAF* V600E-mutated, metastatic melanoma progressive on first-line nivolumab, without cardiac history. Pre-treatment ECG showed a right bundle branch block; LVEF was normal. On day −2/−1 of the treatment protocol, the patient developed pulmonary congestion with hypoxia, edema, and tachycardia, without evident chest pain. ECG showed microvoltages; TTE demonstrated minimal pericardial effusion, without hemodynamic compromise. NT-proBNP and TropT were markedly elevated. Several hours after TIL infusion, the patient became increasingly dyspneic with tachypnea, which partly resolved throughout the night. After discussing with the patient, the first IL-2 dose was administered. Unfortunately, the patient's condition subsequently deteriorated with respiratory failure, for which ICU admission for non-invasive ventilation was necessary. Differential diagnosis comprised decompensated heart failure, perimyocarditis, CLS, or sepsis. Repeated TTE showed normal left and right ventricular function, and minimal pericardial effusion. TropT and NT-proBNP remained high. Treatment with an angiotensin-converting enzyme inhibitor, beta-blocker, aldosterone antagonist, and furosemide were initiated. Recovery was slow. During follow-up cardiac function remained stable; however, peripheral edema prevailed and she suffered progressive renal failure. A diagnosis of nephrotic syndrome due to pauci-immune glomerulonephritis was confirmed.

Case 5. Atrial fibrillation

A 54-year-old woman presented with *BRAF* V600E-mutated, metastatic melanoma progressive on first-line nivolumab, without history of cardiac disease. Pre-treatment ECG was normal; transthoracic echocardiogram showed an LVEF of 51% and a small patent foramen ovale (PFO). No significant toxicities were observed during the lymphodepleting chemotherapy or TIL infusion. On a regular checkup after the first HD IL-2, fever and a rapid, irregular heartbeat were observed. ECG showed atrial fibrillation with a ventricular response rate of 150 beats per minute (BPM, Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2024.102383). She was treated with a cardio-specific beta-blocker, followed by a decline in heart rate to 96 BPM. Psychical examination and ECG the next morning confirmed sinus rhythm. She experienced slight dyspnea, persisting after conversion to sinus rhythm. No further IL-2 was administered. Patient recuperated clinically and was discharged. Follow-up showed normal cardiac function and sinus rhythm.

Case 6. Acute dyspnea

A 67-year-old woman presented with metastatic NSCLC progressive after treatment with atezolizumab/bevacizumab/paclitaxel/carboplatin, with a history of hypertension, and palpitations due to atrial tachycardia and atrial extrasystoles, for which she received treatment with a calcium channel blocker (amlodipine) and beta-blocker (metoprolol). Previous cardiac ultrasound demonstrated mild left ventricular hypertrophy with normal cardiac function. Pre-treatment ECG showed sinus rhythm with atrial extrasystoles; LVEF was 75%. On day −3, the third day of chemotherapy, she was mildly dyspneic. Physical examination demonstrated a tachypnea of 24/min, oxygen saturation of 86%, mild systolic murmur, and peripheral edema with a high positive fluid balance. Chest X-ray showed signs of pulmonary congestion. Treatment with furosemide was successful. Directly after TIL infusion, the patient experienced dyspnea and chest discomfort. ECG showed no signs of acute ischemia. TropT and NT-Pro-BNP were both elevated. Symptoms subsided spontaneously, and the first IL-2 infusion was administered 4 h later than originally scheduled. Several hours later, the patient experienced acute-onset severe dyspnea. By physical examination, hypoxia (82%), a respiratory rate of 44/min, bilateral crackles, hypertension, tachycardia, and fever were observed. Via a non-rebreather mask, 15 l of oxygen was administered, restoring oxygen saturation to 95%. Most symptoms spontaneously resolved within 1 h. ECG showed no signs of ischemia, and cardiac ultrasound demonstrated normal cardiac function. TropT and NT-proBNP were repeated and remained significantly elevated. Symptoms were attributed to CLS, with elevated cardiac markers secondary to possible subclinical ischemia, tachycardia, and inflammation. IL-2 was discontinued. Residual pulmonary edema resolved gradually within the following days. The patient experienced no specific cardiac symptoms during outpatient follow-up.

Case 7. Heart failure due to myocarditis or ischemia

A 55-year-old male presented with metastatic NSCLC with a history of radiotherapy (1x 8Gy) on the dorsal side of costa 6 on his left side, progressive after pembrolizumab/adagrasib and carboplatin/pemetrexed, without known cardiac history. Pre-treatment resting- and stress-ECG were normal; EF was 61%. On day −2, low oxygen saturation and edema were noted for which low-dose diuretics were administered. Shortly after TIL infusion and the first two IL-2 doses, low oxygen saturation (83%) was observed without other accompanying symptoms. Oxygen was supplied with satisfactory result (97%). After the third IL-2 dose, the patient experienced progressive dyspnea, low oxygen saturation (77%), and tachycardia without chest pain, accompanied with mild general edema and low-grade fever. On day +3, the patient remained dyspneic on exertion, tachycardia persisted, and oxygen supplementation was required. Chest X-ray showed a pre-existing pleural effusion without further signs of congestion. ECG showed sinus tachycardia. Cardiac markers were markedly elevated. Symptoms were attributed to CLS, with subclinical cardiac ischemia or myocarditis as differential diagnosis for elevated cardiac markers. A close wait-and-see strategy was followed. Antibiotics were started for persistent febrile neutropenia. Follow-up ECG showed a slightly convex ST-segment in the precordial (septal) chest leads. The following days, the patient remained stable but suffered persistent fluid retention and remained oxygen supplementation dependent. On treatment day +7 TropT levels decreased, but NT-proBNP levels remained high. A differential diagnosis of CLS or heart failure due to myocarditis or subclinical ischemia was reconsidered. ECG demonstrated an interventricular conduction disorder, incomplete right bundle branch block, and left anterior fascicular block. Bedside cardiac ultrasound showed a mildly reduced EF of 50% and a possible hypokinetic ventricular septum. The patient was admitted to the ICU for continuous hemodynamic monitoring and treatment for severe fluid retention with the suspicion of myocarditis or cardiac ischemia. He remained hemodynamically stable without progression of conduction abnormalities. The patient was successfully treated with intravenous diuretics and an aldosterone receptor antagonist. Repeated cardiac ultrasound confirmed globally normal LVEF with a mid-septal

hypokinetic segment. On repeated ECGs, the T-wave in the anterior wall and septal precordial leads progressed to a negative T-segment (Figure 3), possibly related to myocarditis or subclinical ischemia. The patient slowly recuperated and was discharged from the hospital. During outpatient clinic follow-up, a CMR was considered to complete the diagnostic work-up, but due to progression of the NSCLC and physical discomfort this was no longer possible.

Discussion

Treatment using TILs has shown impressive results in melanoma and NSCLC patients, likely leading to the first approved TIL products within the next few years. With the impending approval and the increasing number of clinical trials investigating TILs, the number of patients eligible for TIL treatment is rapidly increasing. This also means that rare toxicities, such as severe cardiotoxicity, will become more prevalent. In a recent comprehensive evaluation of 43 melanoma patients treated with TIL at the Moffitt Cancer Center, an overall cardiovascular toxicity rate of 41.9% was observed, with 14 patients (33%) experiencing hypotension requiring intravenous fluids and vasopressors, 6 (14%) experiencing atrial fibrillation, and 1 patient (2%) with primary TropT elevation.[29] This is much higher than the combined reported incidence of 4% in the meta-analysis by Dafni and colleagues,[4] possibly because cardiovascular toxicities are currently not systematically reported in most trials. Information on TIL treatment-induced cardiotoxicity is limited and data regarding their optimal management are lacking. Therefore, we have provided an overview of the current literature on cardiotoxicity associated with TIL therapy and have presented seven patients treated with TIL at the NKI for their metastatic melanoma or NSCLC who developed cardiotoxicity. Here, we will propose clinical recommendations for diagnosis and management of these symptoms (Figure 4).

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Figure 4. **Guidelines.** A summary of the guidelines, both focused on the clinical parameters and the corresponding management recommendation, from the initial screening of the patient until the follow-up after treatment.

CCU, coronary care unit; CLS, capillary leak syndrome; ECG, electrocardiogram; EF, ejection fraction; ESC, European Society of Cardiology; GLS, global longitudinal strain; ICU, intensive care unit; IL-2, interleukin-2; LVEF, left-ventricular ejection fraction; MRI, magnetic resonance imaging; TIL, tumor-infiltrating lymphocyte; TTE, transthoracic echocardiography.

General

Based on our experience, as described in the cases, completing the diagnosis of toxicities or following the standard guidelines for treatment is often challenging because of the patients' clinical conditions. Patients are often pancytopenic from the lymphodepleting chemotherapy, and may require close monitoring as a result of the toxicity. Symptoms, especially edema and dyspnea, can also be attributed to CLS, and extra attention should be paid to a possible infectious cause as patients are immunocompromised. The challenge sits in using available diagnostics and detecting risk factors while definite risk factors and screening strategies have not been established. Moreover, if the patient's condition does not permit treatment of toxicity, or when the treatment strategy will not change by carrying out additional diagnostic tests, we refrain from burdening the patient, as diagnostics may also be invasive or potentially harmful. In every case, diagnostics must be justified and proportional to the possible treatment. Likewise, if a patient develops signs of cardiotoxicity

early on in the treatment, such as during or after the lymphodepleting chemotherapy or TIL infusion, extra caution is necessary and the risk–benefit ratio of continuing with the treatment should be carefully considered.

Pre-treatment

The evaluation of patients who are potential candidates for TIL treatment should at minimum follow the latest European Society of Cardiology (ESC) Guidelines on cardio-oncology.[42] This starts with risk assessment and includes cardiac serum biomarkers [cardiac troponin (TropT/I), BNP, NT-proBNP], a 12-lead ECG, and a TTE (LVEF and global longitudinal strain). A functional test, such as an (exercise) stress test, may be considered when in doubt of physical performance and symptoms. When abnormalities are observed in these assessments, or if the patient has a history of cardiac disease or has a high risk of developing cardiotoxicity (see ESC Guidelines on cardio-oncology), the patient should be referred to a cardiologist for further examination and risk assessment.[42] Furthermore, all patients should be encouraged to maintain physical activity, and if applicable, quit smoking and restrict their alcohol consumption.[43] If the time between screening and TIL treatment is long, it is recommended to repeat cardiac serum biomarkers to identify subclinical cardiac injury during TIL treatment.[42]

Lymphodepleting chemotherapy

As the TIL infusion is preceded by a lymphodepleting chemotherapy regimen, the first cardiotoxicities to be expected are related to the chemotherapeutic agents and the concomitant fluid challenge to the cardiovascular system. Special attention has to be paid to cyclophosphamide-induced acute heart failure. Symptoms and signs suggesting this should prompt direct evaluation, consisting of physical examination, 12-lead ECG, cardiac biomarkers, and cardiac imaging.[42] Although CMR is the diagnostically superior tool, a transthoracic echocardiogram is usually preferred due to its availability, and the possibility to carry it out at the bedside. Depending on the severity of symptoms, continuous hemodynamic monitoring at the ICU or referral to a specialized cardio-oncology center with a cardiac care unit (CCU) may be required. In general, management should follow the corresponding ESC Clinical Practice Guidelines.[42] In patients with obesity, it can be considered to use the (adjusted) ideal body weight or capping the body surface area at 2.0 m^2 in order to potentially reduce the risk of chemotherapy-induced toxicities in this patient population.[44], [45], [46]

TIL infusion

During the TIL infusion, patients can experience shortness of breath as a result of TIL accumulation in the lung capillaries. This is a temporary sensation and can be managed effectively by oxygen supplementation. When patients experience symptoms potentially related to cardiotoxicity, it is important to keep in mind that patients are immunocompromised and are/will be thrombocytopenic at this point.

High-dose IL-2

Patients receiving HD IL-2 after the TIL infusion are at risk of developing CLS, characterized by extravascular fluid accumulation. This, in combination with the systemic inflammatory response after TIL infusion further triggered by IL-2, may evoke myocardial ischemia, infarction, arrhythmias, and peri- or myocarditis. When patients develop signs of pulmonary congestion, tachycardia, hypotension, or experience symptoms of chest pain or dyspnea, possible cardiotoxicity should be evaluated. At a minimum, a cardiology consultation including a TTE is recommended. Symptoms typically peak ~4-6 h after the HD IL-2 administration and are reversible upon IL-2 discontinuation in

most cases.[40] A factor complicating the diagnostics is the increase of cardiac enzymes observed in almost all patients on the days of IL-2 administrations (Figure 1), making this diagnostic tool unsuitable in the evaluation of possible cardiotoxicity. Experienced personnel, trained in the management of symptoms and who are familiar with the criteria when doses should be postponed, restarted or permanently discontinued, as described in the Proleukin (aldesleukin, IL-2) prescribing information, is essential.[41] With appropriate treatment and monitoring, a stable patient with non-life-threatening toxicity can proceed with treatment. In patients with severe cardiotoxicity, or potentially life-threatening toxicity such as MI or myocarditis, treatment should be interrupted and patients should be admitted to an ICU or CCU for continuous hemodynamic monitoring. In patients who develop acute coronary syndrome or arrhythmias and who are still thrombocytopenic, the benefits and risks of anticoagulation should be carefully considered. Of note, in contrast to symptoms of systemic inflammation mediated by IL-6 as seen in CAR-T cell treatments, tocilizumab, an anti-IL-6 receptor antibody, is not typically administered to treat IL-2-induced systemic inflammation.

Follow-up

For patients who develop new abnormalities on ECG, elevated cardiac serum biomarkers, or other cardiac symptoms during the TIL treatment period, a cardiac consultation after discharge is recommended to establish a follow-up plan.[42] Here, 12-lead ECG, repeated TTE, and serum biomarkers should be considered to guide diagnosis and treatment. However, if the prognosis is poor or their life expectancy is short, this follow-up consultation is not indicated.[42] Importantly, while the cardiotoxicities observed vary greatly in presentation and severity, they did not seem to significantly affect survival in our patients or in the patients described in the study by Fradley and colleagues.[29]

Conclusions

In the absence of evidence-based guidelines for the treatment of TIL therapy-associated cardiotoxicity, we provided an overview of literature, case descriptions, and recommendations for diagnosis and management. These recommendations are intended to help physicians in their daily practice as the number of patients qualifying for TIL treatment is rapidly increasing. Thus far, there are limited data available on the prevalence of cardiotoxicity in patients treated with TIL, as most studies have not reported these toxicities. To better understand TIL-induced cardiotoxicities and develop evidence-based guidelines, systematic registration and publication of these data is required.

Case Presentation

A 79-year-old woman was admitted to the hospital for progressive dyspnea and severe hypoxemia, requiring oxygen supplementation. The dyspnea started approximately 3 to 4 weeks before presentation and was slowly progressive throughout the following weeks. Her medical history mentioned an adenocarcinoma with an epidermal growth factor receptor (EGFR) exon 19 deletion of the lung with metastases to the bones and brain for which treatment with osimertinib was started 14 months earlier. Furthermore, she was treated with rivaroxaban for a first episode of a pulmonary embolism. In the months leading up to her current presentation there were no changes in medication and no use of antibiotics. She had no known exposure to toxic fumes or substances, she was a nonsmoker, and her family history was unremarkable for autoimmune disorders or interstitial lung disease (ILD).

Physical Examination Findings

On admission, the patient experienced moderate to severe respiratory distress and her vital signs demonstrated a respiratory rate of 26 breaths/min; oxygen saturation of 90% with supplemental oxygen, using a 4-L/min nasal cannula; and no fever. Auscultation revealed bilateral crackles. There were no signs of acute heart failure and the rest of her physical examination did not reveal any other abnormalities.

Diagnostic Studies

Arterial blood gas analysis showed the following: pH, 7.49; $Paco_2$, 32.3 mm Hg; Pao_2, 51 mm Hg; and bicarbonate, 24.7 mEq/L. Laboratory studies showed hemoglobin at 7.2 mM, blood eosinophils $< 0.1 \times 10^9$ L, C-reactive protein at 47 mg/L, and procalcitonin < 0.1 ng/mL. Renal function was normal (creatinine, 0.85 mg/dL), and N-terminal pro-brain natriuretic peptide (NT-proBNP) was not elevated (572 pg/mL). PCR testing for SARS-CoV-2 showed a negative result twice. Pharyngeal swab and sputum PCR

Discussion

Osimertinib is the current first-choice treatment for patients with advanced adenocarcinoma of the lung with an EGFR mutation. It is a third-generation EGFR tyrosine kinase inhibitor (TKI) that was first approved for first-line treatment of non-small cell lung adenocarcinoma in 2018. Determination of an EGFR mutation is required before treatment initiation. Exon 19 deletion or exon 21 Leu858Arg point mutations make up 85% of the EGFR somatic mutations and are positively correlated with

Clinical Pearls

- 1.

Osimertinib is a rare cause of severe drug-induced pneumonitis; however, the incidence may increase as it is increasingly prescribed.

- 2.

A high clinical suspicion for a drug-induced pneumonitis is therefore essential in patients presenting with dyspnea and hypoxia while receiving osimertinib treatment, and one should discontinue the offending agent to prevent further deterioration.

- 3.

Rechallenge with EGFR TKI is possible, although concurrent steroid treatment may be needed.

- 4.

Extensive patient counseling

Background

Adoptive cell therapy (ACT) with tumor-infiltrating lymphocytes (TIL) is a personalized immunotherapy. The efficacy of TIL-ACT has been demonstrated prospectively in patients with advanced melanoma but is not limited to melanoma patients. Many patients are refractory to TIL-ACT, however, or their cancer becomes resistant. Combining anti-programmed cell death protein 1 (anti-PD-1) with TIL-ACT to antagonize the immunosuppressive tumor microenvironment may synergize to enhance the antitumor potential.

Material and methods

We set up the *BaseTIL* trial (NCT04165967), a single-center investigator-initiated phase I trial, to test feasibility and safety of TIL-ACT followed by PD-1 blockade in patients with advanced cutaneous melanoma with disease progression after at least one line of anti-PD-1. TIL-ACT included tumor collection, *ex vivo* TIL expansion, lymphodepletion with cyclophosphamide and fludarabine, TIL transfer, and *in vivo* TIL stimulation with interleukin 2 (125 000 IU/kg, 10 days). TIL-ACT was followed by nivolumab treatment for a maximum of 2 years. Nine patients were planned for inclusion.

Results

Between 2020 and 2022, we enrolled 11 patients and 9 underwent a TIL transfer (median transfused cell number: 66.25×10^9). Two patients did not start lymphodepletion. Nine patients received at least 1 dose of interleukin 2 (median number: 10; range, 1-10), seven started nivolumab (median number: 5; range, 2-23). All patients had hematologic adverse events (AEs). Most common non-hematologic AEs were fever and cytokine release syndrome. No nivolumab-associated AEs of ≥ grade 2 occurred. The objective response rate to TIL-ACT was 22% (2/9, 2 partial remission).

Conclusions

TIL-ACT with nivolumab is feasible and safe. Larger trials are needed to further determine the efficacy of this combination.

Introduction

Adoptive cell therapy (ACT) with tumor-infiltrating lymphocytes (TIL) is a personalized immunotherapy that has been pioneered by Steven A. Rosenberg and colleagues at the National Cancer Institute (NCI).[1, 2, 3] TIL-ACT is based on the infusion of autologous CD4+ and CD8+ T lymphocytes that have been collected from tumor material and expanded *ex vivo* in the presence of interleukin 2 (IL-2). Preconditioning with lymphodepletion, most often with non-myeloablative chemotherapy, is an integral part of current TIL-ACT protocols, as well as *in vivo* TIL activation with IL-2 following TIL transfer. Different clinical trials have shown significant response rates in patients with advanced melanoma depending on the stage and patient selection, even after progression on immune checkpoint inhibitors (ICI).[4,5] Recently, a randomized phase III trial has demonstrated superior efficacy of TIL-ACT compared with ipilimumab treatment.[6] In this trial, TIL therapy achieved a 50% reduction in the risk of progression or death compared with ipilimumab in patients with treatment-refractory melanoma. The overall response rate (ORR) was 49% with the TIL therapy compared with 21% for ipilimumab. Activity of TIL-ACT has also been shown in other tumor entities.[7, 8, 9]

Many patients are still refractory to TIL-ACT, however, or their cancer becomes resistant over time. Long-term responses to TIL-ACT seem to be restricted to patients with complete remissions.[5,10] Combining anti-programmed cell death protein 1 (anti-PD-1) inhibitors with TIL-ACT to antagonize the immunosuppressive tumor microenvironment may be a promising strategy to enhance the antitumor potential. The rationale for incorporating a PD-1 inhibitor after TIL-ACT is substantiated by increased PD-L1 expression among tumor-reactive T cells following TIL therapy.[11]

We designed the *BaseTIL* trial (CA209-7H9, NCT04165967), a single-center investigator-initiated phase I trial at the University Hospital Basel, Basel, Switzerland, to investigate feasibility and safety of TIL-ACT followed by PD-1 blockade with nivolumab in patients with advanced melanoma.

Material and methods

Patient population

Patients were eligible if they were ≥18 years of age and had histologically confirmed, unresectable stage III or IV melanoma with disease progression after at least one anti-PD-1-based treatment line and additionally: a BRAF inhibitor in patients with BRAF V600 mutation; an accessible metastasis for tumor collection; measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1; and Eastern Cooperative Oncology Group (ECOG) performance-status score of 0 or 1 (on a 5-point scale, with higher scores indicating greater disability). Key exclusion criteria were non-cutaneous melanoma and active, untreated brain or leptomeningeal metastases.

Trial design

In this investigator-initiated phase I trial, patients underwent surgical excision of a metastasis for the generation of the TIL product (Figure 1). Nine patients were planned for TIL treatment in the *BaseTIL* trial. TIL production was carried out at the GMP Facility for Advanced Therapies of the University Hospital of Basel (Supplementary Figure S1 and Supplementary Table S1, available at https://doi.org/10.1016/j.iotech.2024.100728). If generation of the TIL product proceeded as intended, patients started lymphodepleting chemotherapy (LD) intravenously (i.v.) with cyclophosphamide at a dose of 60 mg/kg for day −7 and −6, and fludarabine daily at a dose of 25 mg/m^2 (maximum of 50 mg) for days −5 to −1. Patients received the TIL infusion on day 0, followed by daily subcutaneous administration of IL-2 (aldesleukin) at a dose of 125 000 IU/kg for 10 days, with a 2-day break after the fifth dose. On day 14, patients started i.v. nivolumab at a dose of 240 mg and were thereafter treated with the same dose every 2 weeks. Treatment with nivolumab continued until the occurrence of disease progression or a maximum duration of 2 years. Patients received support with granulocyte colony-stimulating factor (G-CSF) after TIL infusion and further supportive treatment during TIL-ACT as needed. Prophylaxis for herpes simplex virus and pneumocystis was given after TIL-ACT for the duration of 3 and 6 months, respectively.

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Figure 1. **Study design.** The *BaseTIL* trial a single-center investigator-initiated phase I trial to test feasibility and safety of TIL-ACT followed by PD-1 blockade in patients with advanced cutaneous melanoma. ACT, adoptive cell therapy; ECOG, Eastern Cooperative Oncology Group; IL-2, interleukin 2; TIL, tumor-infiltrating lymphocytes.

Endpoints and assessments

The primary endpoint was safety of the study intervention by assessment of adverse events (AEs). AEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. A data and safety monitoring committee (DSMB) met after TIL transfer in three patients. Key secondary endpoints were the assessment of the ORR, progression-free survival (PFS), and overall survival (OS). Tumor response was assessed by the investigators according to the RECIST version 1.1 at 1 month after TIL transfer and then every 3 months. Imaging assessment included computed tomography (CT) scans, [^{18}F]2-fluoro-2-deoxy-D-glucose-positron emission tomography (FDG-PET)/CT scans, and magnetic resonance imaging (MRI) scans of the brain at baseline and during the follow-up. PFS was defined as the time from the date of TIL transfer to the date of objective tumor progression or death due to any cause, whichever occurred first. OS was the interval from TIL transfer until death from any cause.

Trial oversight

The protocol and amendments were reviewed and approved by the local ethics committee (EKNZ, Basel, Switzerland; EKNZ Nr. 2019-01908) and the Swiss Agency for Therapeutic Products (Swissmedic, Bern, Switzerland). The trial was conducted in accordance with the International Council for Harmonization Good Clinical Practice guidelines and the principles of the Declaration of Helsinki. All patients provided written informed consent. The study was designed by the authors. Drug supply of nivolumab was granted by Bristol Myers Squibb, Steinhausen, Switzerland.

Statistical analyses

Patients' characteristics were summarized by median and range for continuous variables and by frequency and proportion for categorical variables.

Results

Patient population and treatment

Between 2020 and 2022, 11 patients were enrolled in the *BaseTIL* trial and 9 underwent a TIL transfer. Two patients did not start LD: one patient due to rapid disease progression and one patient due to bacterial contamination of the TIL product due to non-adequate starting material. Among the nine patients who had received the TIL infusion (full analysis set), five patients were male and four female (Table 1). The median age was 61 years (range, 35-66 years). Three patients had a *BRAF* mutation. The median number of prior systemic treatment lines, both in the adjuvant and the metastatic settings, was three (range, one to five). Prior systemic treatments included therapy with a PD-1 inhibitor in all patients, and treatment with a BRAF and MEK inhibitor in all three patients with a *BRAF* mutation as required by the study protocol. One patient underwent surgery of a newly diagnosed hemorrhagic brain metastasis and whole brain radiotherapy before the start of lymphodepletion. All patients had poly-metastatic disease (≥5 metastatic lesions) after tumor collection and most patients (5/9) had visceral organ involvement. The median sum of target lesion diameters before TIL treatment was 4.8 cm (range, 2.3-10.4 cm). Median level of blood lactate dehydrogenase at the start of lymphodepletion was 187 U/l (range, 145-265 U/l).

Table 1. Patient and disease characteristics and treatment aspects

Patient and disease characteristics	**Full analysis set[a] (*N* = 9)**
Median age at TIL transfer (range), years	61 (35-66)
Gender (female/male), *n*	4/5
BRAF mutation[b], *n* (%)	3 (33)
Prior systemic therapies, *n* (%)	
• •	3 (1-5)
Median number of therapies (range)	
• •	9 (100)
Anti-PD-1	

Patient and disease characteristics	Full analysis set[a] (*N* = 9)
• • Anti-CTLA-4	2 (22)
• • Combined anti-PD-1/anti-CTLA-4	6 (67)
• • BRAF + MEK inhibitor	3 (33)
• • Chemotherapy or interferon treatment	3 (33)
• • Experimental treatment	2 (22)
Median target lesion sum of diameters (range), mm	48 (23-104)
Presence of brain metastases[c] (yes/no), *n*	1/8
Median level of LDH (range), U/l	187 (145-265)

CTLA-4, cytotoxic T lymphocyte-associated antigen-4; LDH, lactate dehydrogenase; PD-1, programmed cell death protein 1; TIL, tumor-infiltrating lymphocytes.

a

The full analysis set includes nine patients who underwent TIL transfer. Overall, two patients did not start lymphodepleting chemotherapy: one patient due to rapid disease progression; one patient due to bacterial contamination of the TIL product due to non-adequate starting material.

b

BRAF mutations: V600E, V600E/D, V600E.

c

The presence of uncontrolled brain metastases was an exclusion criterion in the study protocol. One patient was diagnosed with brain metastases shortly after study enrollment and underwent surgery of a hemorrhagic brain metastasis and whole brain radiotherapy before the start of lymphodepleting chemotherapy. Stability of brain metastases was documented before start of lymphodepleting chemotherapy.

The median duration of the pre-rapid expansion protocol was 12 days (range, 10-19 days), and for the rapid expansion protocol the median duration was 16 days (range, 14-16 days) (Supplementary Table S2, available at https://doi.org/10.1016/j.iotech.2024.100728). All nine patients started LD as

planned. Eight of nine patients received the planned LD (Table 2). One patient had four of the five intended doses of fludarabine. The median absolute number of transfused CD3+ cells was 66.25 × 10^9 (range, 50-84 × 10^9). The composition of T cells in the final infusion product and the number of infused cells of every patient are summarized in Supplementary Table S3, available at https://doi.org/10.1016/j.iotech.2024.100728. All patients received at least one dose of IL-2 (*N* = 5 with 10 doses, *N* = 1 with 9 doses, *N* = 2 with 8 doses). One patient was discontinued from the trial after TIL transfer and one dose of IL-2 due to grade 4 cytokine release syndrome (CRS). Seven patients started nivolumab treatment. The median number of nivolumab cycles was 7 (range, 2-23). Two patients were unable to start nivolumab due to logistical reasons (place of residence outside Switzerland, no possibility of local sourcing of nivolumab within the trial).

Table 2. Treatment data

Treatment	**Full analysis set[a] (*N* = 9)**
Lymphodepleting chemotherapy, *n*	
• •	2 (2-2)
Median number of cyclophosphamide doses (range)	
• •	5 (4-5)
Median number of fludarabine doses (range)	
Median number of TILs infused (range), *n* (×10^9)	66.25 (50-84)
Median number of IL-2 doses (range), *n*	10 (1-10)
Median single dose of IL-2 (range), *n* (×10^6 IU)	10.25 (8.13-12.5)
Median number of nivolumab doses (range), *n*	4 (0-23)

IL-1, interleukin 2; TIL, tumor-infiltrating lymphocytes.

a

The full analysis set includes 9 patients who underwent TIL transfer.

Safety

Median duration of hospitalization for TIL-ACT was 25 days (range, 20-44 days). All patients experienced AEs in the period from start of LD (day −7) until 30 days after TIL transfer (Table 3). Hematologic AEs occurred as expected in all patients and were attributed to LD. Highest-grade events were grade 4 white blood count decrease (9/9 patients), grade 4 neutropenia (9/9 patients), grade 4 lymphopenia (9/9 patients), grade 4 platelet count decrease (7/9 patients), and grade 3 anemia (7/9 patients). The most common non-hematological AEs of grade 2 or higher in the above-mentioned period were fever (7/9) and CRS (5/9). One patient experienced a grade 4 CRS, classified as serious AE, which led to trial discontinuation. This patient experienced progressive signs of an acute respiratory distress syndrome (ARDS) resulting in respiratory failure and need of invasive respiratory support shortly after TIL infusion and the first IL-2 dose. Multi-organ failure occurred with cardiac,

gastrointestinal, and neurological involvement. The patient received anti-IL-6 targeted treatment (tocilizumab, siltuximab) and high-dose steroid treatment. She fully recovered from the episode but required prolonged hospitalization. The patient did not proceed with any study treatment and did not receive nivolumab. Among the other four patients with grade 2 CRS, three patients received tocilizumab. All patients received antibiotic treatment during TIL-ACT.

Table 3. **Adverse events of grade 2 or higher.** Shown are all adverse events (≥grade 2) (*n*, %) of the highest grade recorded for all TIL-treated patients (*N* = 9) from start of lymphodepletion (day −7) until 30 days after TIL transfer (day +30)

CTCAE preferred term, *n* (%)	**Any grade**	**Grade 2**	**Grade 3**	**Grade 4**
Non-hematologic adverse events				
Fever/chills	7 (78)	7 (78)	0 (0)	0 (0)
Cytokine release syndrome (CRS)	5 (56)	4 (44)	0 (0)	1 (11)[a]
Hypertension	4 (44)	2 (22)	2 (22)	0 (0)
Pain[b]	3 (33)	2 (22)	1 (11)	0 (0)
Liver enzyme elevation[c]	2 (22)	1 (11)	1 (11)	0 (0)
Skin alterations[d]	2 (22)	1 (11)	1 (11)	0 (0)
Hypotension	2 (22)	2 (22)	0 (0)	0 (0)
Nausea/vomiting	2 (22)	2 (22)	0 (0)	0 (0)
Pre-syncope/syncope	2 (22)	2 (22)	0 (0)	0 (0)
Creatinine increased	1 (11)	0 (0)	1 (11)	0 (0)
Wound infection	1 (11)	0 (0)	1 (11)	0 (0)
Atrial fibrillation	1 (11)	1 (11)	0 (0)	0 (0)
Cystitis noninfective	1 (11)	1 (11)	0 (0)	0 (0)
Fatigue	1 (11)	1 (11)	0 (0)	0 (0)
Headache	1 (11)	1 (11)	0 (0)	0 (0)
Mucositis oral	1 (11)	1 (11)	0 (0)	0 (0)

CTCAE preferred term, *n* (%)	Any grade	Grade 2	Grade 3	Grade 4
Peripheral sensory neuropathy	1 (11)	1 (11)	0 (0)	0 (0)
Pleural effusion	1 (11)	1 (11)	0 (0)	0 (0)
Hematologic adverse events				
White blood cell decreased	9 (100)	0 (0)	0 (0)	9 (100)
Lymphocyte count decreased	9 (100)	0 (0)	0 (0)	9 (100)
Neutrophil count decreased	9 (100)	0 (0)	0 (0)	9 (100)
Anemia	9 (100)	2 (22)	7 (78)	0 (0)
Platelet count decreased	9 (100)	0 (0)	2 (22)	7 (78)
Hemolysis	1 (11)	0 (0)	1 (11)	0 (0)

TIL, tumor-infiltrating lymphocytes.

a

One patient with a CRS (grade 4) with multi-organ failure (classified as serious adverse event).

b

One patient with back pain (grade 3), one patient with bone pain (grade 2), one patient with non-cardiac chest pain (grade 2).

c

Elevation of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and/or GGT.

d

One patient with maculopapular rash (grade 2), one patient with purpura (grade 3).

No nivolumab-associated AEs of ≥ grade 2 occurred. Hyperthyroidism (grade 1) and adrenal insufficiency (grade 1) was documented in one patient, skin hypopigmentation (vitiligo, grade 1) in another patient.

Efficacy

Of all nine patients, seven patients had a radiographic shrinkage of target lesions of any magnitude at the first imaging scan carried out ~1 month after TIL-ACT (Figure 2). Three out of these seven patients, however, developed new tumor lesions at their first imaging scan while regression in tumor size was observed in target lesions. Best radiographic response according to RECIST v1.1 was partial remission in two patients, resulting in an ORR of 22%. Stable disease was observed in three patients, while four patients had progressive disease at the first imaging assessment after TIL-ACT. At study end, all patients had experienced disease progression (Figure 3). Median PFS from TIL transfer was

2.2 months. Five patients received subsequent systemic therapies, including the use of combined PD-1/cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) inhibitors in three patients. Median OS from TIL transfer was 7.2 months, with five and three patients alive at 6 and 12 months after TIL transfer, respectively.

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Figure 2. **Best response to the study intervention (waterfall plot).** Shown is the best % change in target lesion sum of diameters from the baseline assessment according to RECIST v1.1 in all nine patients. Censoring occurred at time of progressive disease (PD). The best overall response was partial remission (PR, blue bars) in two patients, stable disease (SD, yellow bars) in three patients, and PD (red bars) in four patients. Shrinkage of target lesions of any magnitude was observed in seven patients. Among the four patients with PD at their first imaging scan, three patients had a shrinkage of target lesions while new lesions were discovered at the same time.

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Figure 3. **Time response to the study intervention (swimmer plot).** The bars indicate the time on the study for every patient from the time of TIL transfer until the end-of-study. Radiographic response according to RECIST v1.1 at the first imaging assessment (first response) is described for every patient, including the timepoint of the first imaging assessment. Disease progression is described for every patient, including the time point of disease progression. Best overall response was progressive disease in four patients. The arrow bars indicate the time from the end-of-study until death. Two patients deceased more than 15 months after TIL transfer (dashed arrow bars). ACT, adoptive cell therapy; PR, partial remission; SD, stable disease; TIL, tumor-infiltrating lymphocytes. ∗End-of-study for patient UPN-03 was on the day of TIL transfer. Data cut-off for survival: 15 November 2023.

Discussion

The *BaseTIL* trial was one of the first clinical studies that investigated TIL-ACT followed by a PD-1 blocking agent in melanoma patients who experienced disease progression on a previous line of PD-1-based treatment. Because upfront treatment with ICI, most often combined PD-1/CTLA-4 blockade, is standard in patients with advanced melanoma, the question arises as to the optimal therapy for those patients who do not respond to ICI or experience disease progression. TIL therapy has been shown to be an effective treatment option in this disease setting.[6] Whether the addition of an ICI after TIL therapy is a valid strategy for overcoming resistance to immunotherapy and whether the combination is possibly more effective than TIL therapy alone is currently unclear. The design of the phase I *BaseTIL* trial was aimed at testing feasibility and safety of this combination. With the current study with nine patients including seven patients with the combination of TIL-ACT and nivolumab (two patients did not receive nivolumab for logistical reasons as outlined), we were able to show that feasibility is given. The safety profile of TIL-ACT was in line with that of other clinical trials,[4] including a pivotal phase III trial.[6] Toxicities were related mostly to LD and to a lesser extent to IL-2. Importantly, patients in the *BaseTIL* trial received low-dose IL-2 and most patients did not experience dose-limiting toxicities due to IL-2. The rationale for the use of low-dose IL-2 in our trial is based on

the assumption of lower toxicity compared with high-dose IL-2, although the results are not clear in this respect.[4] Importantly, the present study was designed before publication of the results of Rohaan and colleagues,[6] which established the use of high-dose IL-2 as the standard after TIL transfer. One grade 4 CRS was attributed to the TIL product as has been described in the literature.[12] Due to the initially predominant pulmonary symptoms (ARDS) and the origin of the expanded TIL product from a pulmonary metastasis, recognition of 'self-antigen' expressed by normal lung tissue may be an alternative explanation of the event. Importantly, no higher-grade immune-related AEs occurred during nivolumab treatment. Only seven out of nine patients started nivolumab, however, and the drug exposure was rather short in most patients due to disease progression. In summary, the safety profile of TIL-ACT followed by nivolumab was consistent with the established profiles of the individual treatments, and no new safety signals emerged.

Parameters of efficacy were secondary endpoints in this trial. Interestingly, most patients had some degree of tumor response in the first imaging assessment following TIL-ACT. Apart from one patient who had a long-lasting response of more than 12 months, we did, however, not observe durable responses in our study population. Secondary resistance in our patient with long response was due to selection of a preexisting tumor cell clone due to a mixed histology of epithelioid/desmoplastic melanoma.[13] The response rate in our study is identical to the ORR of 22% (2/9 patients) in a phase II study of 12 patients with advanced melanoma who received TIL-ACT with a low-dose IL-2 regimen (125 000 IU/kg over 12 days).[14] Consistent with our results, partial remissions were also not durable in this trial, and no patient achieved a complete remission. Conversely, in the phase I study by Ellebaek and colleagues[15] at the Center for Cancer Immune Therapy, Denmark, investigating a low-dose IL-2 regimen (2 000 000 IU over 14 days), a sustained complete response to TIL-ACT was seen in two of six patients, indicating that complete remissions can also be achieved with low-dose IL-2. In recent years, however, various studies have increasingly advocated the use of high-dose IL-2. While a meta-analysis of eight TIL studies had already indicated that a higher ORR can be achieved with high-dose (≥720 000 IU/kg) compared with low-dose (<720 000 IU/kg) IL-2 (43% versus 35%), the results of the pivotal phase III study of TIL-ACT versus ipilimumab by Rohaan and colleagues[6] shifted the standard of care of *in vivo* IL-2 administration towards a high-dose regimen.[4,6] From the seven patients in our study who received nivolumab following TIL-ACT, no conclusions can be drawn about the efficacy of this combination. Differences in response rate and duration of response might also be related to the patient selection. Our trial included a population of heavily pre-treated patients with high tumor burden.

The limitation of this trial is the small number of the target population and the heterogeneity of the included patients in terms of previous treatments and treatment lines. Thus, our results contribute to the question of feasibility and safety of the combination of TIL-ACT and PD-1-based treatment, but conclusions about the effectiveness of this intervention are only preliminary. Other clinical trials have investigated TIL therapy in combination with PD-1 blockade.[9,16] Both trials concluded in line with our findings that the addition of ICI to TIL-ACT was safe and feasible.

In conclusion, we demonstrated feasibility and safety of TIL-ACT with nivolumab. Larger trials are needed to further determine the efficacy of TIL-ACT with ICI, and possibly explore new combination approaches.

Section snippets

Case 1

A 59-year-old woman presented with a 2-month history of fatigue, productive cough, and dyspnea on exertion. She had grown up in India and emigrated to the United States in 1990 but had spent several months in India 1 year earlier to seek an evaluation for the symptoms. The examination was notable for expiratory wheezing and basilar crackles. Absolute eosinophil count (AEC) was 11,123/mL and the purified protein derivative skin test was negative. Chest radiography demonstrated an infiltrate in

Case 2

A 64-year-old woman with a history of asthma and allergic rhinitis presented with fever and productive cough unresponsive to antibiotics. Born and raised in India, she had emigrated to the United States in 1985, traveling back periodically. The examination was significant for fever, diffuse wheezing, and decreased breath sounds. Laboratory results included AEC 7,434/mL, positive rheumatoid factor, and negative antifilarial antibody testing. The purified protein derivative skin test was

Case 1

Tropical pulmonary eosinophilia. Antifilarial antibody levels were markedly elevated. The patient was treated with a 2-week course of diethylcarbamazine with full resolution.

Case 2

Eosinophilic granulomatosis with polyangiitis (EGPA). Repeat bronchoscopy with biopsy revealed necrotizing eosinophilic vasculitis. Remission was attained with high-dose glucocorticoids and maintained with low-dose daily prednisone.

Discussion

Pulmonary infiltrates with hypereosinophilia can result from exposures (medications or radiation), hypersensitivities (allergic bronchopulmonary aspergillosis or hypersensitivity pneumonitis), infections (parasitic and other), malignancy, vasculitides, rare hypereosinophilic syndromes, and other idiopathic disorders.[4] As demonstrated by the featured cases, the initial presentation and findings may be consistent with multiple etiologies. A thorough history, including a time line of symptoms,

Abstract:

A woman with known type 2 severe asthma and previous pneumonia complained of severe cough with sputum, breathlessness, and wheezing. Symptoms had started 4 weeks earlier and did not respond to antibiotics. She was found with mild respiratory failure and admitted to the pulmonology guard. She had a significant peripheral eosinophilia and an increase in the indices of inflammation. Chest computed tomography-angiography excluded pulmonary embolism and detected bilateral opacities in the upper lobes. A bronchoscopy with bronchoalveolar lavage (BAL) led to the diagnosis of eosinophilic pneumonia (eosinophils 74% at cell differential count of BAL fluid). The patient had a prompt response to systemic corticosteroids, the eosinophils and inflammation indices returned to normal, and the pulmonary opacities resolved in 10 days. Long-term oral corticosteroid therapy was planned to prevent relapse and further investigations were carried out. In addition to asthma with eosinophilia and non-fixed pulmonary infiltrates, peripheral neuropathy, sinusitis, and esophageal extravascular eosinophils were found. The patient largely met the criteria for eosinophilic granulomatosis with polyangiitis and was offered treatment with mepolizumab 300 mg every 4 weeks (three times the dosage used for

severe eosinophilic asthma). The chapter addresses the diagnostic workup of recurrent pulmonary infiltrates in patients with eosinophilic asthma. Insights into the diseases associated with eosinophilia are also present, particularly those with respiratory manifestations such as acute and chronic eosinophilic pneumonia.

Abstract

Metastatic tumors (MTs) may show different characteristics of the immune microenvironment from primary tumors (PTs) in non-small cell lung cancer (NSCLC). The heterogeneity of immune markers in metastatic NSCLC and its associated factors has not been well demonstrated. In this study, 64 surgically resected specimens of paired PTs and MTs were obtained from 28 patients with NSCLC. Multiplex immunofluorescence (mIF; panel including programmed death-ligand 1 (PD-L1), Cytokeratin, CD8, and CD68) was performed on whole sections. The heterogeneity of the immune contexture of PD-L1 expression, infiltrating lymphocytes, and immune-to-tumor cell distances was quantified via digital image analysis. In a quantitative comparison of MTs and corresponding PTs, MTs showed higher PD-L1 expression levels, lower density of CD8+ cytotoxic T lymphocytes (CTLs), and longer spatial distance between CTLs and tumor cells. Subgroup analysis, which associated clinical factors, revealed that the heterogeneity of immune markers was more obvious in extrapulmonary, metachronous, and treated MTs, while fewer differences were observed in intrapulmonary, synchronous, and untreated MTs. In particular, MTs showed significantly higher PD-L1 expression and lower lymphocyte infiltration in metastatic NSCLC with EGFR mutations. Prognosis analysis showed that an increased density of CD8+ CTLs in MTs was associated with better overall survival (OS). Therefore, significant discrepancies in PD-L1 expression and lymphocyte infiltration in metastatic NSCLC are most likely associated with temporal heterogeneity with a history of anti-treatment and correlated with EGFR mutations. The detection of immune markers in re-obtained metastatic specimens may be required for immunotherapy prediction in these patients with metastatic NSCLC.

Introduction

Immune checkpoint inhibitors (ICIs) have been approved as first-line and/or second-line treatments for advanced non-small cell lung cancer (NSCLC). The expression of programmed death-ligand 1 (PD-L1) plays a critical role in immune escape mechanisms, and the tumor proportion score (TPS) of PD-L1 expression on tumor cells (TCs) has been widely recommended as a predictive biomarker for ICI immunotherapy in NSCLC [1, 2, 3]. In addition, characteristics of immune cells (ICs), such as subtypes, functional polarization, and spatial distribution through the tumor, have also been shown to influence the prognosis of cancer patients and correlate with the response to PD1/PD-L1 target immunotherapy [4, 5].

Based on current detection strategies for immune markers, the response to ICIs is still heterogeneous. Some patients with advanced NSCLC have non-detected metastases at the time of initial diagnosis and eventually develop metastases during the progression of the disease after surgical operation. Patients with metachronous metastases often present with multiple distant metastases, and the majority of them have been managed with systemic treatment, such as tyrosine kinase inhibitors (TKIs), chemotherapy, or radiotherapy (RT) before initiating ICI therapy. Emerging studies have indicated that a therapeutic regimen could affect both PD-L1 expression and characteristics of tumor immune contexture in NSCLC patients receiving neoadjuvant therapy [6, 7, 8, 9, 10, 11]. The clinical challenges of tumor heterogeneity in PD-L1 expression and the diversity of immune infiltrates have been a concern in the context of immunotherapy [12, 13, 14, 15].

The dynamic evolution of the tumor immune microenvironment (TIME) may occur during tumor metastatic progression. A discrepancy in PD-L1 expression has been noted between a certain proportion of metastatic tumors (MTs) and corresponding primary tumors (PTs) [16, 17, 18, 19, 20]. The heterogeneity of TIME of NSCLC in PTs and MTs is vital to address the heterogeneous response of multiple metastases to ICIs. However, the rule of dynamic changes in TIME and its associated clinical factors causing significant metastatic heterogeneity have not been revealed. Research still needs to elucidate what kind of cases may be more likely to exert a significant discrepancy of immune markers in MTs compared to PTs, and it is important to explain under which clinical conditions metastatic samples need to be re-obtained for immune markers' detection in patients with metastases. It remains unclear whether this is related to the metastatic sites, time interval, treatment status, or driving genes of metastasis.

Evaluating the tumor immune contexture of MTs in comparison with corresponding PTs may reveal changes in immune markers during NSCLC metastasis. One major challenge is that such studies have been limited by the actual clinical process in which biopsy samples may not show a satisfactory panoramic view of the immune microenvironment, which may result in deviations in the intratumoral heterogeneity between biopsies and surgical specimens [21, 22, 23]. In this study, we collected paired surgically resected primary and metastatic samples to avoid potential heterogeneity within the biopsies. We utilized multiplex immunofluorescence (mIF) and digital image analysis to quantitatively identify PD-L1 expression and tumor-infiltrating lymphocytes (TILs) in NSCLC. This study provides beneficial information regarding the heterogeneity of immune markers in metastatic advanced NSCLC and contributes to the current PD-L1 detection strategies.

Materials and methods

Patients and samples

We collected 28 NSCLC cases with available specimens of paired PTs and MTs at Peking University Cancer Hospital and Tianjin Medical University Cancer Hospital from June 2013 to October 2018. The cases were enrolled according to the following criteria: (1) both the PTs and MTs were surgically resected and tissue samples were formalin-fixed paraffin-embedded (FFPE); (2) tumor size was ≥0.5 cm; (3) PTs did not undergo preoperative neoadjuvant treatment; (4) the clinical information and follow-up data were available; and (5) focus was on extrapulmonary distant metastases, but samples of intrapulmonary metastases were also selected as comparative studies. In this study, 64 matched resected specimens were obtained from 28 patients with NSCLC, including eight patients with two resected metastases. Fourteen metastatic samples were intrapulmonary, while 22 metastatic samples were extrapulmonary, including seven brain samples, five adrenal samples, five soft tissue samples (two chest wall samples and three upper limb samples), and five distant lymph node samples.

Histological evaluation

The original histological diagnosis was confirmed in archival hematoxylin and eosin (H&E)-stained slides, and histopathological features, such as histologic subtype and tumor size, were collected. All tumors were pathologically staged according to the TNM classification of the 8th AJCC [24]. Pathological evaluation of multifocal lung cancer was performed using clinical and pathologic criteria, as defined by the International Association for the Study of Lung Cancer (IASLC) Lung Cancer Staging Project to distinguish intrapulmonary metastases from multiple primary lung cancer lesions [25]. All pathologic materials were reviewed by two expert pathologists using the 2015 World Health Organization classification.

Multiplex staining and scanning

Slides were stained with the UltiMapper Kit (Ultivue™ Inc., Cambridge, MA, USA) using the DNA-tagged mIF method. We validated the high comparability of this kit with conventional immunohistochemistry (IHC) of PD-L1 (22c3) and IC quantification in our previous study [26]. Briefly, mIF staining was performed on 4 µm sections of FFPE tissues using BOND RX. FFPE tissue slides were dried at 60–65 °C for 30 min before loading on the BOND RX. BOND RX was set up for the UltiMapper assay. Five markers were stained simultaneously on one slide using the mIF kit. Reference multiplex images of the UltiMapper® Kit included a panel of PD-L1 (clone 73-10), CD8 (clone C8/144B), CD68 (clone KP-1), cytokeratin (CK, clone AE1/AE3), and 4,6-diamidino-2-phenylindole (DAPI). Digital immunofluorescence images were scanned at 200× magnification in the fluorescent mode. Fluorescent images of DAPI (blue), FITC (CK, Cyan), Cy5 (PD-L1, red) Cy3 (CD8, green), and Cy7 (CD68, orange) for each section were captured in the corresponding channels.

Quantified analysis of multiplex images

The digital images were analyzed using HALO™ software (version 3.0; Indica Labs, Corrales, NM, USA). Before the analysis, the tumor areas were annotated in each digital whole-slide image. The intratumoral cell populations and densities for the phenotypes of interest (PD-L1+ cells, CK+ TCs, CD8+ cytotoxic T lymphocytes (CTLs), CD68+ macrophages, and PD-L1+/CK+ double-positive phenotype) were counted within the tumor area. TPS was quantitatively calculated as the proportion of intratumoral PD-L1+/CK+ TCs and CK+ TCs (Fig. S1). In addition to cell populations, digital image analysis on mIF enabled the localization of individual cells across whole sections, and subsequently determined the spatial distance of each type of IC with respect to adjacent TCs. Digital images were analyzed with cell segmentation to identify stained cells and map their *x*–*y* coordinates. The cell data are plotted, and then the *x*–*y* coordinates are used to quantify the average distance between the ICs and adjacent TCs using the proximity algorithm in HALO system (Fig. S2).

Statistical analysis

The Wilcoxon signed-rank test was used for the quantitative comparison of paired PTs and MTs, and the Mann–Whitney test was used for non-paired PTs and MTs. Cohen's κ coefficient of agreement was evaluated using the dichotomized TPS, and the value of κ was categorized as poor (<0.40), moderate (0.40–0.8), or excellent (≥0.8). Kaplan–Meier curves were used to assess the impact of biomarkers on overall survival (OS). The significance of biomarkers was determined by univariate and multivariate analyses using the log-rank test and Cox proportional hazards models. Statistical significance was set at $p < 0.05$. Statistical analyses were performed using SPSS Statistics software, version 22 (IBM Corp., Armonk, NY, USA).

Results

Patient demographics and clinicopathological characteristics

The characteristics of the 28 patients with NSCLC are summarized in Table 1. The median age was 57.5 years (range: 52–70 years); 18 patients were men and 10 were women. Smokers accounted for 60.71% (17/28) of the participants. Twenty-two patients were in AJCC 8th stage IB-IIB at the first surgical operation, while six patients were at stage IIIA-IV. Metachronous metastases were defined based on the time interval between tumor lesions of more than 6 months after the diagnosis. In our study, patients developed both synchronous metastases (14 samples) and metachronous metastases (22 samples). Among the collected metastatic samples, 16 samples were not treated, while 20 out of 22 metachronous metastases samples were subjected to anti-tumor treatments. Pathologic

examination revealed that lung adenocarcinoma (LADC, 23/28, 82.1%) was the major histological type. Squamous carcinoma (LSQC, 4/28, 14.3%) and large cell carcinoma (LCC, 1/28, 3.6%) were also confirmed. Fourteen of 28 cases (50%) were found to harbor an *EGFR* mutant, and the remaining 14 cases were *EGFR* wild-type. None of the cases harbored ALK or ROS-1 rearrangements. The follow-up time ranged from 335 to 2603 days, with a median of 1364 days. Thirteen patients died during the follow-up period due to lung cancer.

Table 1. Demographics of the included patients and samples.

Total patients (*n* = 28)	**Variables**	Empty Cell	**Variables**	Empty Cell
Age (year)	Mean	57.5	Range	52–70
Sex	Male	18	Female	10
Smoker	Yes	17	No	11
Primary tumor				
Location	Right lobe	18	Left lobe	10
AJCC 8th stage	IB-IIB	22	IIIA-IV	6
EGFR	Mutant type	14	Wild type	14
Metastatic tumor	Tumor 1	*n* = 28	Tumor 2	*n* = 8
Metastatic sites	Intrapulmonary	9	Intrapulmonary	5
	Chest wall	2		
	Brain	7		
	Adrenal	3	Adrenal	2
	Soft tissue	3		
	Distant LN	4	Distant LN	1
Time interval	Synchronous	14	Metachronous	22
Adjuvant treatment	With treatment	20	Without treatment	16
	Chem	15		
	Chem + R	1		

Total patients (*n* = 28)	**Variables**	Empty Cell	**Variables**	Empty Cell
	EGFR-TKIs	1		
	Chem + TKIs	3		
Histopathologic type	Primary		Metastases	
	LADC	23	LADC	23
	LSQC	4	LSQC	4
	LCC	1	LCC	1
Survival	Survival	15	Death	13
Follow-up time (days)	Range	335–2603	Median	1364

Chem chemotherapy, *Chem + R* combined chemotherapy and radiotherapy, *EGFR-TKIs* epidermal growth factor receptor tyrosine kinase inhibitors, *Chem +* TKIs combined chemotherapy and EGFR-TKIs, *LN* lymph node, *LADC* lung adenocarcinoma, *LSQC* lung squamous carcinoma, LCC large cell carcinoma.

Heterogeneity of PD-L1 expression and TILs in primary and metastatic NSCLC

Representative images of mIF stains of each cell subset in primary and metastatic NSCLC tumors are shown in Fig. 1A. We first compared PD-L1 expression and infiltrating lymphocytes in non-paired PTs and MTs using the Mann–Whitney test (Table 2). The results showed that MTs generally had a higher PD-L1 expression level in PD-L1+ cell populations ($p = 0.042$) and a higher TPS (median: 29.16% vs. 18.50%; average: 42.34% vs. 35.91%, $p = 0.096$) (Fig. 1B). A lower density of CD8+ CTLs ($p = 0.022$) in MTs was statistically significant (Fig. 1C), but no statistical discrepancies in the spatial distance of ICs to TCs were found (Fig. 1D).

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Fig. 1. Multiplex immunofluorescence (mIF) staining on immune markers in primary and metastatic tumors.

A The represented images of mIF staining on PD-L1 expression, tumor cells (TCs), and immune cells (ICs) in primary tumors (PTs) and metastatic tumors (MTs). Comparison the tumor proportion score of PD-L1 expression (**B**), ICs infiltration (**C**), and immune-to-tumor cell distances (**D**) in non-paired PTs and MTs, Mann–Whitney test.

Table 2. Heterogeneity of PD-L1 expression, lymphocytes infiltration and immune-to-tumor cell distances between primary and metastatic samples in non-small cell lung cancer.

Immune markers	Heterogeneity		Paired	Non-paired
Empty Cell	**PT**	**MT**	p^a	p^b
TPS (average, score %)	35.91%	42.34%	0.046*	0.096
PD-L1+ (average, cell/mm^2)	859.63	1391.86	0.029*	0.042*
CD8+ (average, cell/mm^2)	736.90	436.75	0.040*	0.022*
CD68+ (average, cell/mm^2)	776.18	592.01	0.068	0.102
CD8+ to TCs (average, μm)	55.24	80.26	0.038*	0.304
CD68+ to TCs (average, μm)	41.62	46.68	0.285	0.507

[a]Wilcoxon signed-rank test.

[b]Mann–Whitney test; *$p < 0.05$, considered statistically significant. *PT* primary tumor, *MT* metastatic tumor, *TPS* tumor proportion score, *TCs* tumor cells.

The heterogeneity of the quantitative immune markers between paired PTs and MTs was identified using the Wilcoxon signed-rank test (Table 2). Generally, paired PTs and MTs showed a significant change in the density of PD-L1+ cells ($p = 0.029$) and a significant heterogeneity in TPS ($p = 0.046$) (Fig. 2A). Among all 36 paired samples of 28 patients, 25 paired samples demonstrated an increase in TPS, only 11 paired samples showed a decrease in TPS in MTs compared with PTs (Fig. 2B). Dichotomizing the samples using cutoffs of 5% and 50%, respectively, TPS was consistent in the 28 paired lesions (77.8%) and inconsistent in eight samples (22.2%). A moderate agreement of TPS was achieved ($\kappa = 0.448$) at a cutoff of 5%, while an excellent agreement of TPS was observed at a cutoff of 50% ($\kappa = 0.869$) (Fig. 2C and Table S1).

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Fig. 2. Heterogeneity of immune markers in paired primary and metastatic tumors.

A Heterogeneity in the tumor proportion score (TPS) of PD-L1 expression between paired primary tumors (PTs) and metastatic tumors (MTs). **B** Circos figure shows the increase and decrease in the continuous quantified value of TPS in different sites of metastases. **C** Circos figure shows the agreements of categorical TPS in MTs. **D** Heterogeneity in CD8+ cytotoxic T lymphocytes (CTLs) infiltration between paired PTs and MTs. **E** Circos figure shows the increase and decrease of CTLs infiltrating in MTs. **F** Heterogeneity in the spatial distance of CTLs and tumor cells (TCs) between paired PTs and MTs. **G** Schematic diagram illustrates the spatial distribution of the immune-to-tumor cell distances.

MTs showed significant changes in the density of CD8+ CTLs ($p = 0.040$), but this was not statistically significant for CD68+ macrophages ($p = 0.068$) (Fig. 2D). More paired MT samples showed a decrease in the density of ICs, while a few cases showed an increase in IC infiltrates (Fig. 2E). The spatial

relationship was analyzed in paired PTs and MTs, and the results showed that the average spatial distance between CD8+ CTLs and TCs was significantly longer in MTs than in PTs ($p = 0.038$) (Fig. 2F, G).

Spatial heterogeneity of PD-L1 expression and TILs in primary and metastatic NSCLC

In addition to the total samples, the spatial heterogeneity of immune contexture in paired primary and metastatic NSCLC was analyzed to show the possible discrepancies in different locations of metastases. Subgroups of extrapulmonary MT (eMT) and intrapulmonary MT (iMT) were compared (Fig. 3 and Table S2). In paired eMTs and corresponding PTs, the changes in the density of PD-L1+ cells and TPS were not significant (both $p > 0.05$), while immune infiltrates decreased in most samples, with significant discrepancies in the density of CD68+ cells ($p = 0.039$). A significant increase in the average distance between CD8+ CTLs and TCs was also observed ($p = 0.046$). In contrast, in paired iMTs and corresponding PTs, significant discordance was not observed in PD-L1 expression, infiltrating lymphocytes, or spatial relations between ICs and TCs.

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Fig. 3. Spatial heterogeneity of immune markers in metastatic tumors.

The heterogeneity of PD-L1 expression, immune cell infiltration, and immune-to-tumor cell distances in subgroups of extrapulmonary metastatic tumors (eMTs) and intrapulmonary metastatic tumors (iMTs).

As for TPS with dichotomized results at a cutoff of 5%, there was poor agreement in paired eMTs ($\kappa = 0.154$), whereas there was good agreement in paired iMTs ($\kappa = 0.837$). At a cutoff of 50%, a high agreement of TPS was observed ($\kappa \geq 0.8$) in both subgroups of eMTs and iMTs (Table S1).

We also compared PD-L1 expression and infiltrating lymphocytes between eMT and iMT samples, and no significant discrepancies were observed.

Temporal heterogeneity of PD-L1 expression and TILs in primary and metastatic NSCLC

The heterogeneity of immune contexture in metachronous MT (mMT) and synchronous MT (sMT) in NSCLC was analyzed (Fig. 4 and Table S3). In paired mMTs and corresponding PTs, the heterogeneity of the density of PD-L1+ cells, and the changes in TPS were not significant, while IC populations decreased in most samples with significant discrepancies in the density of CD8+ ($p = 0.008$) and CD68+ ($p = 0.003$). A significant increase in the average distance of CD8+ ICs and CD68+ ICs to TCs was observed ($p = 0.003$ and $p = 0.024$, respectively). In contrast, in paired sMTs and corresponding PTs, no significant discrepancies were observed.

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Fig. 4. Temporal heterogeneity of immune markers in metastatic tumors.

The heterogeneity of PD-L1 expression, immune cell infiltration, and immune-to-tumor cell distances in subgroups of metachronous metastatic tumors (mMTs) and synchronous metastatic tumors (sMTs).

As for TPS with dichotomized results at a cutoff of 5%, there was poor agreement in paired mMTs (κ = 0.304), whereas there was moderate agreement in paired sMTs (κ = 0.553). At a cutoff of 50%, a high agreement of TPS was observed (κ ≥ 0.8) in both subgroups of mMTs and sMTs (Table S1).

We compared PD-L1 expression and infiltrating lymphocytes between the mMT and sMT subgroups. Significant differences were not observed in PD-L1 expression and immune infiltrates, while a longer average distance of CD8+ ICs to TCs was found in mMTs (p = 0.011).

Heterogeneity of PD-L1 expression and TILs in treated and untreated metastatic NSCLC

To assess the possible impact of postoperative adjuvant treatment on MTs, the heterogeneity of immune contexture in treated MT (tMT) and untreated MT (uMT) was analyzed (Fig. 5 and Table S4). In paired tMTs and corresponding PTs, significant heterogeneity with a higher density was observed in PD-L1+ cells (p = 0.047) and changes in TPS (p = 0.040). Most samples showed a decrease in cell populations with significant discrepancies in the density of CD8+ (p = 0.019) and CD68+ (p = 0.001) ICs. A significant increase in the average distance of CD8+ and CD68+ ICs to TCs was also observed (p = 0.004 and p = 0.017, respectively). In contrast, in paired uMTs and the corresponding PTs, no significant discrepancies were observed.

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Fig. 5. Heterogeneity of immune markers according to treatment status.

The heterogeneity of PD-L1 expression, immune cell infiltration, and immune-to-tumor cell distances in subgroups of treated metastatic tumors (tMTs) and untreated metastatic tumors (uMTs).

As for TPS with dichotomized results at a cutoff of 5%, there was poor agreement in paired tMTs (κ = 0.216), whereas there was moderate agreement in paired uMTs (κ = 0.500). At a cutoff of 50%, a moderate agreement was observed in tMTs (κ = 0.792), while a high agreement of TPS was observed in uMTs (Table S1).

PD-L1 expression and infiltrating lymphocytes between the tMT and uMT subgroups were compared. A higher density of PD-L1+ cells (p = 0.013) and higher TPS (p = 0.033) were observed in tMTs. A longer average distance of CD8+ ICs to TCs was also found in tMTs than in uMTs (p = 0.014).

Heterogeneity of PD-L1 expression and TILs in EGFR-mutated primary and metastatic NSCLC

The heterogeneity of immune contexture between primary and metastatic NSCLC cases was analyzed according to EGFR mutation status (Fig. 6 and Table S5). In the EGFR-mutant subgroup, significant heterogeneity with a higher density of PD-L1+ cells (p = 0.004) and an increase in TPS (p = 0.002) were observed in paired MTs and corresponding PTs. Most samples showed a decrease in cell populations with significant discrepancies in the density of CD8+ ICs (p = 0.006). A significant increase in the average distance of CD8+ ICs to TCs was also observed (p = 0.019). In contrast, in the EGFR-wild subgroup, no significant discrepancies in immune markers were observed in the paired MTs and corresponding PTs.

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Fig. 6. Heterogeneity of immune markers according to EGFR mutation status.

The heterogeneity of PD-L1 expression, immune cell infiltration, and immune-to-tumor cell distances according to EGFR mutation status in non-small cell lung cancer.

As for TPS with dichotomized results at a cutoff of 5%, there was poor agreement in paired MTs in EGFR-mutant cases (κ = 0.271), whereas there was an obvious higher agreement in paired MTs in EGFR-wild cases (κ = 0.765). At a cutoff of 50%, a moderate agreement was observed in EGFR-mutant cases (κ = 0.773), while a high agreement of TPS was observed in EGFR-wild cases (κ = 0.875) (Table S1).

Prognostic significance of the immune biomarkers in metastatic NSCLC

We analyzed the correlations between immune markers and OS in PTs and MTs separately. In PTs, none of the immune markers significantly correlated with OS, while in MTs, a higher density (higher than the median) of CD8+ CTLs significantly correlated with a better outcome (p = 0.0183). In particular, we compared the change in status (decrease or increase) of the quantitative immune markers in MTs compared to PTs. Our results showed that an increase in TPS was marginally correlated with a poor outcome (p = 0.0625), while an increase in the density of CD8+ cells (p = 0.0273) was significantly correlated with a better outcome. Multivariate analyses incorporating clinical parameters revealed that an increase in CD8+ ICs in MTs compared to corresponding PTs was an independent factor associated with a low risk of cancer-related death in metastatic NSCLC (p = 0.0385, hazard risk (HR): 0.1151, 95% CI [0.0149–0.8917]) (Fig. 7 and Table S6).

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Fig. 7. Prognostic significance of immune biomarkers in metastatic tumors.

A Survival analysis of immune biomarkers in metastatic tumors (MTs) and its change status with overall survival (OS). **B** Forest tree showing the risk of the heterogeneity of immune markers in metastatic NSCLC.

Discussion

The evolution of TIME and the intertumoral heterogeneity of immune markers in MTs are a concern in immunotherapy practice. In this study, we demonstrated the quantitative heterogeneity of immune contexture, including PD-L1 expression, lymphocyte infiltrates, and spatial relations between ICs and TCs in surgically derived whole sections of NSCLC via digital image analysis. We also revealed the possible factors associated with significant heterogeneity.

The TPS of PD-L1 expression is widely used to predict the response to PD1/PD-L1 inhibitors in advanced NSCLC. The inconsistency of TPS in a proportion of metastatic NSCLC has been reported, this may be due to the sample selection, intratumoral heterogeneity of biopsy samples, and inter-variability among observers [16, 17, 18, 19, 20]. Accurate assessment of PD-L1 scoring is a major

challenge for pathologists, and only semi-quantitative scores can be evaluated on current IHC slides. Compared with conventional IHC, digital mIF images enable multiplexed, quantitative analysis of tissue specimens for co-expressed PD-L1 on TCs and ICs. Thus, a digital mIF image method enables the quantitative assessment of TPS discrepancies between MTs and PTs.

Our quantitative results of TPS on surgically resected samples showed that significant heterogeneity of PD-L1 expression was observed in MTs compared to corresponding PTs, and more cases showed a higher level of PD-L1+ cell populations with an increased TPS, suggesting that PD-L1 expression is more commonly increased in metastatic NSCLC. The ATLANTIC trial reported that the expression of PD-L1 was significantly higher in tumor samples that received chemotherapy or radiotherapy before sampling than in tumor samples that did not receive treatment [27]. Similarly, we used paired samples and observed higher PD-L1 expression mainly in treated MT samples, suggesting that PD-L1 expression more commonly increased in metastases with a history of traditional anti-tumor treatment than in samples with natural tumor progression. This is helpful for the immunotherapy strategy of following clinical treatment in patients with NSCLC who develop distant metastases.

The characteristics of the immune microenvironment play a vital role in anticancer immunity. Mansfield et al. reported that brain metastases may lack either PD-L1 expression, lymphocyte infiltration, or both, in contrast to primary lung cancer samples [28]. In our cohort of paired samples, most MTs had lower CD8+ CTL infiltrates with varying degrees, indicating that most MTs possibly have a lower ability to recruit ICs against TCs after metastatic progression, especially in distant metastases. In addition, the multiplex strategy enabled us to provide an in situ map with spatial cell distribution. A closer spatial distance between ICs and TCs is commonly considered as a possible effective immune-to-tumor cell interaction [29, 30, 31]. Therefore, an increase in the spatial distance between infiltrating CD8+ cells and TCs in MTs indicated that the role of CTLs in TCs possibly decreased in most metastases.

Since advanced-NSCLC patients commonly develop several distant metastases, there is clinical confusion regarding the use of primary lesions or metastases for detecting immune markers. Exploring the main clinical factors causing the significant heterogeneity of immune markers in metastatic NSCLC may be helpful in addressing this challenge. Subgroup analysis of the associated clinical factors showed that the discrepancies in PD-L1 expression and TILs were mainly observed in subgroups of extrapulmonary, metachronous, and treated MTs, but not for intrapulmonary, synchronous, and untreated MTs. In particular, postoperative adjuvant therapy had a significant influence on tumor immune markers (Fig. S3). These results are consistent with those of a study on brain metastases in which the time is associated with CD8 expression discordance [32]. This means that in patients who developed metachronous MTs with adjuvant treatment, the MTs are suggested to be re-obtained for the detection of immune biomarkers as a significant change in comparison with PTs. It is important to predict the heterogeneous response of ICIs to multiple distant metastases in patients with advanced disease. In contrast, for intrapulmonary and synchronous metastasis, both PTs and MTs are suitable for the detection of immune markers.

EGFR mutation is the most common driver gene in East Asian NSCLC patients and is usually negatively correlated with PD-L1 expression [33]. Studies have shown that NSCLC patients with EGFR mutations commonly have a poor response to ICI immunotherapy [34, 35, 36, 37, 38]. We evaluated immune biomarkers in EGFR-mutant and wild-type NSCLC cases. Interestingly, we observed significant heterogeneity of immune contexture only in EGFR-mutant NSCLC, and nearly most samples showed an increase in PD-L1 score in paired MTs in the EGFR-mutant subgroup. The activation of the EGFR signaling pathway in NSCLC may upregulate the expression of PD-L1

and immunosuppressive factors, thereby promoting the immunosuppressive effect in TIME. Analyzing the mechanism of EGFR in the regulation of PD-L1 expression during NSCLC metastatic progression may be of interest in future research.

In this cohort, an increase in immune infiltrates was correlated with a better outcome. Similarly, Camy et al. found that CD8 expression in brain MTs was marginally associated with a better prognosis for brain metastatic lung cancer [32]. Considering the role of immunomodulation in tumor progression, the changing status of ICs may represent a positive or negative trend in the immune response, and an increase in the density of CTLs in MTs predicts a better outcome. Thus, when samples of both MTs and PTs are available, detection and comparison of changes in the status of immune infiltrates may be clinically useful for predicting the OS in patients with metastatic NSCLC.

Our study has some limitations. The major drawback of this study was the small number of samples, because the resected samples were unusual in patients with distant metastases. Some subgroup analyses on clinical factors, such as the impact of different therapies, were not performed. The impact of clinical factors on the heterogeneity of immune markers requires a larger sample size to be verified. In addition, the impact of the heterogeneity of immune markers in response to immunotherapy remains to be investigated in subsequent studies.

In conclusion, there was heterogeneity in PD-L1 expression and infiltrating lymphocytes between primary and metastatic NSCLC samples. Significant discrepancies were mainly observed in long-term metastases with a history of traditional adjuvant treatment and were correlated with EGFR mutations. MTs in these patients are recommended to be re-obtained as predictive samples because of the significant heterogeneity of immune biomarkers from PTs. In future studies, whether the heterogeneity of PD-L1 expression and TILs during NSCLC metastasis exerts an impact on the heterogeneous response to immunotherapy needs further verification.

Cavitary lung cancer is a rare type of lung cancer. Generally, the relationship between cavitary lung adenocarcinoma (LUAD) and specific immune checkpoints remains unknown. In this study, we aimed to detect the expression of programmed cell death ligand-1(PD-L1) and the density of CD8-positive ($CD8^+$) tumor-infiltrating lymphocytes (TILs) to evaluate their clinicopathological significance in the case of patients with cavitary LUAD. This study included 65 patients with cavitary LUAD. Patient specimens were obtained from surgery. The expression of PD-L1 protein and $CD8^+$ TIL status was detected by traditional immunohistochemistry and multiplex quantitative immunofluorescence technology. The correlation of PD-L1 expression and $CD8^+$ TIL status was assessed along with clinicopathological parameters. This included evaluation of overall survival in cavitary LUAD patients based on the follow-up data. High expression of PD-L1 protein was detected in the cancerous tissues of cavitary LUAD patients, whose positive rate was recorded at 44.6% (29/65). PD-L1 expression level was significantly related with the lymph node ($P = 0.001$), TNM stage ($P = 0.024$), and $CD8^+$ TIL status ($rs = -0.272$, $P = 0.025$). High PD-L1 expression indicated high mortality rate ($P < 0.001$); however, the $CD8^+$ TIL group showed better survival in cavitary LUAD patients ($P = 0.011$). This phenotype, with high PD-L1 expression and low $CD8^+$ TILs, can predict poorer overall survival of patients with cavitary LUAD compared with the other phenotypes. Moreover, $CD8^+$ TIL level was an independent good prognosis factor. Initially, we reported that PD-L1 is upregulated in cavitary LUAD patients and that high expression of PD-L1 negatively correlates with $CD8^+$ TIL status. High PD-L1 expression and low $CD8^+$ TILs can predict decreased overall survival of patients with cavitary LUAD. 1. Introduction

Lung cancer is the most common type of cancer diagnosed in both males and females combined. In addition, lung cancer is the leading cause of cancer death worldwide in 185 countries [1]. Cavitary lung cancer is a solitary and thin-walled tumor that is particularly unique and seldom reported [2].

Cavitary lung cancer occurs in 8% of all lung cancers [3], but other researchers reported an incidence rate of 1.00%–2.07%. Cavitation in a tumor nodule is previously thought to be more prevalent in patients with lung squamous cell carcinoma [4]. Following an increase of lung adenocarcinoma (LUAD), cavitary LUAD has been reported with an incidence of 5.7%–14.9% in patients with LUAD [5]. Cavitary lung cancer is a rare type of lung cancer, which has an irregular cystic wall, wall nodule with septa, or standard uptake value raised on positron emission tomography (PET) [6]. It is not easy for this type of cancer to be accurately diagnosed by clinicians via radiological measures. In addition, compared with noncavitary lung cancer, this cancer has a worse prognosis for affected patients due to a high TNM stage [7–11]. Since computed tomography (CT) can offer reliable messages about the morphology and density of lesions, this is the best method to noninvasively distinguish malignant and nonmalignant cavities [11]. Cavitary lung cancer is significantly associated with elderly, male patients that have tumors with larger maximum diameters, solid nodules, larger pT size, and advanced pTNM stage in multivariable analysis [12]. The biological features of the underlying cavity are still poorly understood, and it remains unclear whether immune checkpoints, for example, programmed cell death ligands 1 (PD-L1), have a different expression pattern.

Immunotherapy is a novel choice in the treatment of a variety of cancers with poor prognosis [13]. The development of immune checkpoint inhibitors has altered the therapy of non-small cell lung cancer (NSCLC) [14]. As one of the typical checkpoint inhibitors, programmed cell death 1 (PD-1) is an inhibitory cell-surface receptor that is expressed on activated T-cells and other immune cells. Anticancer immunotherapy that targets immune checkpoints with antibodies to PD-1 and its ligand PD-L1 is an established treatment modality for NSCLC [15–17]. One of these important mechanisms is the ability of anti-PD-L1 monoclonal antibodies to restrain lymphocyte inhibition by binding to the PD-1 receptor. This prevents PD-1 from binding with its ligands, PD-L1 or PD-L2. In addition, it allows T cells to maintain their tumor cell killing function [18,19]. NSCLC patients with PD-L1-positive had a higher chance of achieving an objective response, when treated with anti-PD-L1 monoclonal antibodies [20,21].

Although these most heartening results, the overall response of lung cancer patients to immune checkpoint treatment is still unsatisfactory [14]. Thus, more and better biomarkers need to be found. The predictable biomarkers of the next area of concern will be detected in the tumor microenvironment. Tumor-infiltrating lymphocytes (TILs) also play a vital role in predicting tumor progression in different kinds of cancers [22]. As the most studied component of the tumor-associated immune response, the expression of cytotoxic CD8-positive ($CD8^+$) T cell could forecast better prognosis of breast or ovarian cancer patients [23,24]. However, the correlation between cavitary lung cancer and immune checkpoints remains unknown. In this study, we take advantage of human specimens to assess PD-L1 expression pattern along with $CD8^+$ TIL density and investigate the prognostic significance of these results in the setting of cavitary LUAD.

2. Materials and Methods

2.1. Cavitary LUAD Sample Collection

A total of 65 patients who were diagnosed with cavitary LUAD between September of 2005 and October of 2015 in the General Hospital of Central Theater Command Hospital, People's Liberation Army of China, were included in the research. These patients had no chemotherapy and/or radiotherapy before surgical resection. All patients underwent a 64-row spiral CT scan with a slice thickness of 1.25 mm, 1.5 mm, or HRCT. Two radiologists (J. Liu and Y. Xue) independently examined and confirmed the imaging features of these samples. In accordance with previous studies, tumor

cavitation was defined as an air-filled space with a wall thickness of ≤4 mm along its circumference [5,11,25,26] (Fig. 1).

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Figure 1. Chest CT scanning presentation of patients with solitary cavitary lung cancer. A and B: The posterior segment of the upper lobe of the right lung showed an irregular and shallowly lobulated mass of 40 × 46 mm^2 that was adjacent to pleura traction and depression with irregular cavity. Multiple nodules of varying sizes were observed. C and D: The anterior segment of the upper apex of the left lung showed a 46 × 55 mm^2 soft tissue density shadow with eccentric cavity, which was lobulated and pulled by the adjacent pleura

Formalin-fixed, paraffin-embedded (FFPE) tissues of these 65 patients with cavitary LUAD were collected. Two pathologists (Q. Wang and Y. Ren) independently confirmed the histopathologic features of each sample. Clinicopathological data were retrieved from clinical records and histopathology reports. We followed up the patients with cavitary LUAD from the date of surgery or biopsy and ended in October 2018. The median follow-up was 45 months with a range of 1–115 months. We defined overall survival from diagnosis to death or the end of follow-up. We classified cavitary LUAD specimens according to the 8th edition of TNM classification by UICC/AJCC (2017) [27].

2.2. Immunohistochemical Analysis

After samples were deparaffinized and rehydrated, antigen retrieval was applied in citrate (10 mM, pH 6.0) at 95°C for 15 min by microwave (Meidi, China). PD-L1 and CD8 expression in FFPE tumor sections was performed by IHC using a primary rabbit anti-human PD-L1 polyclonal antibody (1:100 dilution, E1L3N, Cell Signaling Technology, USA) and a mouse anti-human CD8 monoclonal antibody (ready-to-use, C8/144B, Dako, Agilent, USA) that were incubated at 4°C overnight. The sections with PD-L1 and CD8 were incubated with horseradish peroxidase (HRP) conjugated to the goat anti-mouse/rabbit second antibody (Dako REAL EnVision Detection System, Agilent, USA) at 37°C for 30 min. After this, the sections were mixed with 3,3'-diaminobenzidine (DAB) (Dako, Agilent, USA) as the chromogen. Lastly, hematoxylin was used for nuclear counterstaining.

2.3. Evaluation of Immunohistochemistry

We observed the intensity of immunostaining using light microscopy (Olympus BX-53 with CCD DP73). Two independent pathologists (Q. Wang and Y. Ren) scored the PD-L1 and CD8 protein results, whose were blinded to the clinicopathological parameters of cavitary LUAD.

The immunohistochemistry characteristics and cutoffs of PD-L1 being considered as positive vary in different research studies. In the current study, the cutoff of PD-L1 protein expression in tumor cells was defined as 5%. PD-L1 ≥ 5% was regarded as high expression. This was consistent with many types of cancers [28–30].

For the evaluation of CD8, the $CD8^+$ TIL number was counted on each slide at ×200 magnification. The mean of these three counts was calculated for each case, and the cutoff value of high or low expression was decided based on the median number of total counts [24,31].

2.4. Multiplex Immunofluorescence Staining

We performed manually multiplex immunofluorescence (mIF) staining on 4-μm FFPE sections of cavitary LUAD with the use of the Opal 4-Color IHC Kit (Akoya Biosciences, USA) [30]. Three markers included PD-L1 (1:200 dilution, E1L3N, Cell Signaling Technology, USA), CK (AE1/AE3) and CD8 (C8/144B), whose were ready-to-use antibodies (1:2 dilution, Agilent/DAKO, California, USA). According to the kit protocol and the reference [30], we completed all the steps. In this study, we selected human tonsil tissues both with and without primary antibody as positive and negative controls, or autofluorescence control, respectively. We scanned the mIF-stained slides by a Vectra 2.3 multispectral microscope system (Akoya Biosciences, USA) from 420 to 720 nm at 20-nm intervals with the same exposure time. Lastly, we analyzed all three markers and gained the significant immune phenotypes using InForm 2.4 software (Akoya Biosciences, USA).

2.5. Statistical Analysis

Data were expressed as frequencies for categorical variables and mean ± SD for numeric values. All statistical analyses were completed by SPSS 21.0 (Chicago, IL, USA). We carried out Chi square test or Fisher exact test in order to evaluate the relationship between PD-L1 expression and clinicopathological parameters of cavitary LUAD patients. We used Spearman correlation analysis to explore the correlation between PD-L1 and $CD8^+$ TILs. The survival analysis was assessed using the Kaplan-Meier curve and log-rank test to decide statistical differences. At last, we performed COX proportion hazard regression model to complete univariate and multivariate analysis of survival, and evaluate the independent prognostic values. *P*-values <0.05 were considered statistically significant.

3. Results

3.1. Patient Characteristics

The cavitation in the lungs was continually evaluated with the use of chest CT scans. Our results found solitary and thin-walled cavities, which localized at pulmonary periphery, and all these cavitary lesions demonstrated suspected signs of malignancy (Fig. 1).

As shown in Tab. 1, among the 65 cavitary LUAD patients, 36 (55.4%) were male and 29 (44.6%) were female, with a mean age of 58 years old (range 48–71). Thirty-one (47.7%) of these patients were alive, and 34 (52.3%) had died by the end of follow-up. The data of the T, N, M, and TNM stages of cavitary LUAD for these patients are also shown in Tab. 1.

Table 1. Patient characteristics

Characteristic	**Sub-characteristic**	**Cavitary LUAD (%)**
Age		58 (range 48–71)
Sex	Male	36 (55.4)
	Female	29 (44.6)
Survival status	Death	34 (52.3)
	Survival	31 (47.7)
T	T1	5 (7.7)

Characteristic	Sub-characteristic	Cavitary LUAD (%)
	T2	50 (76.9)
	T3	8 (12.3)
	T4	2 (3.1)
N	N0	39 (60.9)
	N1-3	26 (39.1)
TNM stage	I	28 (43.1)
	II	31 (47.7)
	III	6 (9.2)
Total		65

3.2. PD-L1 and CD8 Protein Expression

As shown in Figs. 2 and 3, PD-L1 and CD8 proteins were expressed in cavitary LUAD tissues. In cancer tissues, PD-L1 protein was localized on the membrane and the cytoplasm of cancer cells. In some cases, the cytoplasm of immune cells showed negative PD-L1 in the cancer cells (Figs. 2A–2H). Different $CD8^+$ TIL statuses were observed both inside and outside the cancer nest of different cavitary LUAD patients (Figs. 3A–3F). Using mIF, we observed that PD-L1 could be expressed in the cancer cells ($PD\text{-}L1^+CK^+$) and TILs ($PD\text{-}L1^+CD8^+$) (Fig. 4).

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Figure 2. Different pattern of PD-L1 expression in cavitary LUAD. A, B, C, and D: high expression of PD-L1 in the cancer cells; E and F: positive expression of PD-L1 in the immune cells of stroma, negative in the cancer cells. G and H: a few immune cells showed positive PD-L1, but negative in the cancer cells (Positive signal was brown, original magnification A, C, E, G × 100; B, D, F, H × 200)

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Figure 3. $CD8^+$ TILs in cavitary LUAD. A and B: low expression of $CD8^+$ TILs; C and D: high expression of $CD8^+$ TILs in the stroma and cancer islets. E: low expression of $CD8^+$ TILs in different cases; F: high levels of $CD8^+$ TILs in the stroma of different cases (Positive signal was brown, original magnification A, C × 100; B, D, E, F × 200)

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Figure 4. Co-expression of PD-L1 and CD8, CK proteins detected by mIF in cavitary LUAD. A: CK (cyan); B: PD-L1 (orange); C: CD8 (red); D: co-expression of PD-L1 and CK (white arrow) was observed; E: co-expression of PD-L1 and CD8 (white arrow) was observed; F: unmixed composite image for PD-L1, CD8 and CK (original magnification of all images × 400)

Of the 65 cases of cavitary LUAD tissues that were studied, 55.4% (n = 36) of cases showed low PD-L1 expression, and 44.6% (n = 29) of cases were found to have high PD-L1 expression. Among the 65 cases of cavitary LUAD patients, 48 (73.9%) cases were low in $CD8^+$ TILs, and 17 (26.1%) were high in $CD8^+$ TILs.

3.3. PD-L1 Expression and Clinicopathological Parameters in Cavitary LUAD Patients

The relationship between tumor PD-L1 expression and clinicopathologic variables in cavitary LUAD was analyzed by Chi square test. Tab. 2 showed high expression of PD-L1 protein was significantly associated with lymph node metastasis (N) ($P = 0.001$) and the TNM stage ($P = 0.024$). Negative correlation was detected between PD-L1 and $CD8^+$ TIL status (rs = −0.272, $P = 0.025$; Figs. 5A–5D); but PD-L1 expression was not statistically correlated with age, sex, or tumor size in cavitary LUAD patients.

Table 2. The relationship between PD-L1 expression and clinicopathological parameters of patients with cavitary LUAD

Characteristic	Empty Cell	**PD-L1**		***P*-value**
Empty Cell	**n**	**Low (%)**	**High (%)**	
Sex				
Male	36	19 (29.2)	17 (26.2)	0.638
Female	29	17 (26.2)	12 (18.4)	
Age				
<=58	31	17 (26.2)	14 (21.5)	0.933
>58	34	19 (29.2)	15 (23.1)	
Tumor size				
T1 + T2	55	32 (49.2)	23 (35.4)	0.321
T3 + T4	10	4 (6.2)	6 (9.2)	

Characteristic	Empty Cell	**PD-L1**		***P*-value**
Empty Cell	**n**	**Low (%)**	**High (%)**	
Lymph node				
N0	39	28 (43.1)	11 (17.8)	**0.001**
N1-3	26	8 (12.4)	18 (27.7)	
TNM stage				
I	28	20 (30.6)	8 (12.4)	**0.024**
II + III	37	16 (24.7)	21 (32.3)	
CD8 status				**0.025**
Low	48	23 (35.4)	25 (38.6)	Rs = −0.272
High	17	13 (20.0)	4 (6.2)	

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Figure 5. Relation between PD-L1 expression and $CD8^+$ TILs in the same case of cavitary LUAD. A and B: low expression of PD-L1 protein; C and D: high expression of $CD8^+$ TILs in stroma and tumor islets (positive signal was brown, original magnification A, C × 100; B, D × 200)

3.4. Prognostic Value of PD-L1 Expression and $CD8^+$ TILs in Cavitary LUAD Patients

Survival analysis was determined by Kaplan–Meier curve, while log-rank test was used to explore the prognostic value of PD-L1 expression and $CD8^+$ TIL status in cavitary LUAD patients. High expression of PD-L1 was found to predict poor survival and a high mortality rate in cavitary LUAD patients (Fig. 6A, $P = 0.004$). The $CD8^+$ TIL group had better survival rates of cavitary LUAD patients (Fig. 6B, $P = 0.001$). In addition, patients with high PD-L1 expression and low $CD8^+$ TILs demonstrated poorer overall survival than those with other phenotypes (Fig. 6C, $P = 0.001$).

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Figure 6. Correlation of PD-L1 and CD8 expression level and OS of patients with cavitary LUAD. A: Patients with low PD-L1 expression demonstrated better OS than those with high PD-L1 expression ($P < 0.001$). B: Patients with high $CD8^+$ TILs demonstrated better OS than those with low $CD8^+$ TILs

(P = 0.010). TILs: tumor-infiltrating lymphocytes. C: Patients with high PD-L1 and low CD8 expression demonstrated poorer OS than those with the other phenotypes (P < 0.001)

In univariate analysis, both PD-L1 and $CD8^+$ TIL levels were observed to be significantly correlated with the overall survival (OS) of LUAD patients (HR: 2.670, and 95% CI: 1.334–5.345, P = 0.006; HR: 1.995, and 95% CI: 0.991–0.998, P = 0.002, respectively, Tab. 3). In addition, the phenotype of high PD-L1 expression and low $CD8^+$ TILs had higher risk of short OS than the other phenotypes (HR: 2.999, and 95% CI: 1.518–5.923, P = 0.002). Simultaneously, significant correlation was detected between the TNM stage and the OS of cavitary LUAD (Tab. 3). A multivariate COX proportional hazard model on OS was carried out to analyze whether the above univariate analysis as an independent prognostic factor. This multivariate analysis model included the two protein expressions and the clinicopathological parameters of cavitary LUAD. The results confirmed that only $CD8^+$ TILs were an independent prognosis parameter in OS of cavitary LUAD patients (HR: 0.655; and 95% CI: 0.007–0.401, P = 0.004, Tab. 3).

Table 3. COX proportional hazard models on OS of patients with cavitary LUAD

Factors	**Univariate analysis**		**Multivariate analysis**	
	***P*-value**	**HR (95% CI)**	***P*-value**	**HR (95% CI)**
Sex				
Male *vs.* female	0.769		0.594	
Age				
<=58 *vs.* >58	0.961		0.253	
PD-L1 expression				
Low *vs.* high	**0.006**	2.670 (1.334–5.345)	0.055	
Co-expression of PD-L1 and CD8				
High PD-L1 and low CD8 *vs.* the other				
	0.002	2.999 (1.518–5.923)	0.180	
CD8 expression				
Low *vs.* high	**0.002**	0.995 (0.991–0.998)	**0.004**	0.655 (0.007–0.401)
Tumor size (T)				
T1, T2 *vs.* T3, T4	0.449		0.730	

Factors	Univariate analysis		Multivariate analysis	
	***P*-value**	**HR (95% CI)**	***P*-value**	**HR (95% CI)**
Lymph node metastasis (N)				
N0 *vs.* N1, N2	0.127		0.434	
TNM stage				
I, II *vs.* III	**0.039**	2.052 (1.035–4.068)	0.239	

4. Discussion

This study is among the first to investigate high expression of PD-L1 protein in patients with cavitary LUAD. High expression of PD-L1 protein significantly correlated with lymph node metastasis, TNM stage, and $CD8^+$ TIL status. Interestingly, our study showed that high PD-L1 expression and low $CD8^+$ TILs could predict poorer overall survival of those patients with cavitary LUAD. $CD8^+$ TILs were an independent predictor of LUAD prognosis.

Imaging studies like chest radiography and CT are commonly utilized for the clinical diagnosis of lung cancer. Radiographic features of cavities that indicate malignancy including multiple holes, a nodular ill-defined inner or outer wall, and an eccentric excavation with irregular margins [11]. Patients with cavitary lung cancers often have a poor rate of survival compared with noncavitary NSCLC patients [5,26]. This is because of advanced tumor stage, as well as the vascular, lymphatic, or pleural invasion of cavitary LUAD [5,32]. Based on this finding, cavitary and noncavitary LUAD should be considered separate entities [32]. Currently, there is a lack of cognition concerning the onset and progression of cavitary LUAD. This rare type of cancer is subject to misdiagnosis and missed diagnosis as well [2]. Necrosis may cause a solitary cavity because of primary cancer overgrowth. Tumor growth leads to bronchial obstruction and vascular invasion, which provides an environment of ischemia and hypoxia. This results in tumor necrosis. Furthermore, the autophagy of neoplastic cells can also induce cavitary lesion [7,10].

Immune checkpoint inhibitors targeting the PD-1/PD-L1 axis have previously demonstrated encouraging results in patients with NSCLC. Overexpression of PD-L1 is associated with poor, recurrence-free survival, and OS [33]. In addition, the FDA has approved pembrolizumab as a first-line treatment for advanced PD-L1 positive NSCLC patients [34]. In the current study, we found that high expression of PD-L1 was detected in 44.6% (29/65) cases of cavitary LUAD. This is significantly increased compared with the assessment of patients with noncavitary LUAD, which found that 22.5%–41.2% of cases had high PD-L1 expression [35–38]. Our results demonstrated that tumor necrosis correlates with higher PD-L1 expression in LUAD. Similar results have been reported in previous studies [39]. Recent papers have identified that high expression of PD-L1 has also positive relation with mutations in KRAS, TP53, and MET, which is negatively associated with mutations of EGFR and STK11 in cases of LUAD [17]. In addition, these mutations may be involved with different clones of the PD-L1 antibody and may affect the criteria of a positive rate of PD-L1 protein. In our study, high expression of PD-L1 was correlated with high TNM stage and was able to predict poor prognosis of patients with cavitary LUAD. The above-mentioned results confirmed that high expression of PD-L1 might promote malignant progression, and PD-L1 can be regarded as a cancer

immunotherapy target of cavitary LUAD. Because of this, we had to further the specific molecular features of cavitary LUAD to analyze the mechanism of malignant progression and poor prognosis.

Inflammation is a notable feature of cancer that can lead to tumor progression [40]. Cytokines also have an anti-tumor immune effect, and IFN-α, IFN-γ, and TNF-α can all increase the expression of PD-L1 in a variety of cancers [41]. In addition, $CD8^+$ TILs correlate with PD-L1 expression and participate in the inflammation of the anti-tumor immune response [42,43]. Our results also confirmed there is negative correlation between high expression of PD-L1 and $CD8^+$ TIL status in cavitary LUAD. In addition, this immune phenotype of high expression of PD-L1 and low $CD8^+$ TILs could predict poorer overall survival of patients with cavitary LUAD compared with the other phenotypes such as low PD-L1 and high $CD8^+$ TILs. Moreover, we found that $CD8^+$ TILs were an independent marker to predict prognosis of cavitary LUAD. Comprehensively, these results suggested that inflammation or necrosis induced by the dysregulation of tumor growth activates the cytokines secretion and leads to the formation of thin-walled cavity lesions in the development of LUAD. In response to this, cytokines increase the expression and activities of PD-L1, which contributes to tumor cells escaping from immune surveillance and further promotes the malignant progression of cavitary LUAD. $CD8^+$ TILs are involved in the inflammation and immune processes described above. In addition, a negative correlation was observed between PD-L1 and $CD8^+$ TILs in the same cavitary LUAD case. Further studies are required to better understand the mechanism of high expression of PD-L1 and its relationship with inflammation or necrosis in cavitary LUAD cases.

In conclusion, this research demonstrated that PD-L1 expression is upregulated in cavitary LUAD patients and that high expression of PD-L1 negatively correlates with $CD8^+$ TIL status. High PD-L1 expression and low $CD8^+$ TILs can predict poorer overall survival of patients with cavitary LUAD. These results demonstrated that PD-L1 is a pivotal immune checkpoint and can improve our mechanistic understanding of cavitary LUAD.

Case Presentation

A 15-year-old girl presented to her local hospital with a 4-month history of fatigue, anorexia, and a 6-kg weight loss. She also reported fever, productive cough, and chest pain on the left lower chest posteriorly for 4 days before admission. Her medical history and systemic review were unremarkable for any respiratory or other organ disease. The patient was taking iron and multivitamin supplements. At her local hospital, she was febrile; chest radiography showed anemia and a left lower lobe infiltrate. She received a transfusion and was started on empiric antibiotics that were continued for 10 days without improvement. Subsequently, CT scan of the chest and upper abdomen showed a lung abscess and left renal mass that led to a referral to our center.

Physical Examination Findings

The following vital signs were recorded on admission: temperature, 37.6 °C; BP, 105/70 mm Hg; pulse rate, 113 beats/min; respiratory rate, 19 breaths/min; oxygen saturation, 98% on room air; and weight, 42.4 kg. Examination of the chest showed decreased breath sounds and dull percussion noted on the left base posteriorly. Systematic examination was otherwise unremarkable.

Diagnostic Studies

The following blood levels were noted on laboratory evaluation: WBC count, 22.67×10^9/L; hemoglobin level, 78 g/dL; and platelets, 689×10^9/L. Electrolytes, renal function test, and liver function test were all within normal ranges. Midstream urine culture grew *Escherichia coli*.

Imaging of the chest (chest radiography and CT scan) showed cavitary lesions with surrounding consolidation on the left lower lobe that measured 4 cm × 8 cm that extended to the posterior chest wall with the involvement of posterior intercostal space and a pleural effusion. Images of the upper abdomen showed a left renal mass that measured 8 cm × 13.5 cm that invaded the perinephric fat and the posterior pararenal space and through the left hemidiaphragm reaching into the pleural space. Additionally, multiple lymph nodes at the level of the left renal hilum were noted (Fig 1).

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Figure 1. A, Chest radiograph and B, coronal reconstruction of CT scan shows left lower lobe cavitary lesions surrounded by airspace consolidation (arrows) with left pleural effusion. C, Sagittal and D, axial reconstruction of CT scan show the tract of fistula (arrow) with the left renal mass lesion.

Bronchoscopy was performed to rule out malignancy or TB, which is endemic in the patient's region. There was no endobronchial abnormality; microbiologic and cytologic studies of BAL were unremarkable. Transbronchial biopsy specimen showed residual alveolar spaces with a collection of foamy macrophages and chronic inflammation that was composed of lymphocytes and plasma cells (Fig 2). Renal biopsy was also performed and showed a similar histopathologic picture.

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Figure 2. A, A lung biopsy shows residual alveolar spaces with a collection of foamy macrophages (hematoxylin and eosin stain; original magnification, ×40). B, A lung biopsy shows chronic inflammation composed of lymphocytes, plasma cells and many foamy macrophages (hematoxylin and eosin stain; original magnification, ×20).

What is the diagnosis?

Diagnosis: Xanthogranulomatous pyelonephritis

Discussion

Xanthogranulomatous pyelonephritis (XGP) is an invasive chronic infection of the kidney that is characterized by cellular infiltrates of lipid-laden mononuclear macrophages, which leads to the destruction of the renal parenchyma. Pathogens that are commonly incriminated in XGP include *E coli* and *Proteus mirabilis* and usually occur in association with calculi, congenital urinary anomalies, and recurrent urinary tract infections (UTIs). The inflammatory process may extend to the neighboring structures such as the colon, the skin, or rarely to the lung by fistula formation that leads to a lung abscess. It is believed that the inflammation spread superiorly through the lumbocostal trigone, which is a weak area in the diaphragm, into the thoracic cavity via a nephrobronchial fistula. In the latter situation, recognition of the subdiaphragmatic source is essential, especially if the respiratory presentation is dominating.

Reports of XGP complicated with nephrobronchial fistula and a lung abscess are rare. Terms such as "uroptysis" and "uriniferous" odor were used to describe clinical features in some of these reports. When the clinical presentation is predominantly respiratory without any history of UTIs and no

evidence of calculi or ureteric obstruction, the primary renal source may be overlooked. Only a handful of cases were reported in the literature without any renal symptoms, and it may take a lengthy time for the renal source of a lung abscess to be discovered. Conversely, however, the urinary symptoms may dominate, and the lung abscess may be found incidentally

The definitive treatment is nephrectomy, which may be open or laparoscopic. In some of the reports, resection of the involved lobes through a thoracoabdominal approach was performed. However, extensive surgery to include thoracic disease, which might increase morbidity and death, may not be required in all cases. Control of the primary abdominal source through the abdominal approach and adequate drainage can be sufficient for the resolution of the abscess.

Clinical Course

The patient underwent left radical nephrectomy, splenectomy, resection and repair of the posterior part of the diaphragm, and insertion of temporary drains. Postoperatively, a CT scan of the chest showed minimal change in abscess. However, the patient was improving clinically; drains were removed, and she was discharged 12 days after surgery. There was progressive improvement; when last seen 11 months after surgery, she reported no respiratory or urinary symptoms, and she had gained 15 kg in weight. The final chest radiograph showed significant resolution with only minimal atelectasis of the left base (Fig 3)

Case Presentation

A 48-year-old woman presented to the ED with a nonproductive cough, shortness of breath, and stridor. She was otherwise healthy and had never used tobacco. The patient was mildly tachycardic but otherwise hemodynamically stable, afebrile, and saturating well on room air. She did not display any signs of increased work of breathing at rest. Although auscultation of her thorax indicated good air entry bilaterally without any adventitious sounds, stridor was elicited with forced expiration.

Clinical Discussion

The clinical manifestations of blastomycosis are quite varied. Described as the “great pretender,” its clinical overlap with many commonly diagnosed entities contributes to both delayed and erroneous diagnoses.1, 2, 3 Because of the inhalation of aerosolized conidia that subsequently geminate into yeast in the human host, most patients present with signs and symptoms of pneumonia.[2,4] This includes both acute presentations of fevers, chills, dyspnea, malaise, and a productive or nonproductive

Conclusion

This was a rare case of tracheal blastomycosis initially diagnosed and treated as squamous cell carcinoma. Blastomycosis is often mistaken for community-acquired pneumonia or malignancy based on its clinical and radiographic presentation. Blastomycosis does not usually elicit granulomatous inflammation as do other endemic fungal yeasts, and it might present as a pseudoepitheliomatous reaction that mimics a squamous cell carcinoma. A high index of suspicion based on recent travel to endemic

SESSION TITLE: Lung Pathology Case Reports Posters (G)

SESSION TYPE: Case Report Posters

PRESENTED ON: 10/08/2024 10:20 am - 11:05 am

INTRODUCTION: Although frequently identified during autopsies, clinical documentation of leukemic pulmonary infiltration is seldom confirmed in practice. While pulmonary complications in leukemia patients are commonly attributed to infection, hemorrhage, or drug toxicity [1], it's vital to recognize the potential of pulmonary leukemic infiltration, a non-infectious complication associated with poor prognosis. We describe a rare case of leukemic pulmonary involvement in a patient whose leukemia was diagnosed following an assessment for acute respiratory failure.

CASE PRESENTATION: A 66-year-old male patient, known to have diabetes mellitus and atrial fibrillation, presented to the hospital with progressively worsening exertional dyspnea over the preceding seven days. Prior to this, he had no respiratory symptoms and denied cough, sputum, fever, or chest pain. On admission, he exhibited tachycardia and tachypnea, with an initial oxygen saturation of 85% on room air, which improved to 97% with high-flow nasal cannula (HFNC). Sinus tachycardia was evident on electrocardiography (EKG), and chest x-ray revealed fullness in the hilum and increased vascularization bilaterally. Further evaluation via computed tomography (CT) of thorax demonstrated bilateral diffuse perihilar ground-glass opacities, interlobular septal thickening, bilateral pleural effusions, and enlarged mediastinal and bilateral hilar lymph nodes. Laboratory investigations revealed hyperleukocytosis (WBC: 162.9 x10^3/mm3), thrombocytopenia (73K/mm3), and acute kidney injury (AKI). Empiric antibiotic therapy, which consisted of azithromycin and ceftriaxone, was started.An extensive infectious workup, including respiratory viral panel, pneumonia panel, blood and sputum cultures, interferon-gamma release assay (IGRA) for TB, human immunodeficiency virus (HIV) antibodies, hepatitis panel, (1-3)-β-d-glucan assay for fungal infections, urine legionella, and s. pneumoniae antigens returned negative. Transthoracic echocardiogram was unremarkable, with normal ejection fraction (EF) at 65%. Peripheral smear examination revealed numerous blasts with granules in the cytoplasm, indicative of myeloid cells, prompting therapeutic leukapheresis. All-trans retinoic acid (ATRA) was initiated based on a presumptive diagnosis of acute promyelocytic leukemia (APML); however, subsequent bone marrow pathology confirmed acute myelomonocytic leukemia. Throughout hospitalization, the patient's respiratory status showed improvement, albeit with residual interstitial edema observed on repeat chest x-rays. Ultimately, the patient was transferred to another facility for further management of his leukemia.

DISCUSSION: Pulmonary leukemic infiltrates should be included in the differential diagnosis of respiratory complications in leukemia patients, especially when presenting with deteriorating respiratory function, bilateral interstitial infiltrates on imaging, and markedly elevated white blood cell counts exceeding 100,000/mm3 [1]. Nevertheless, acute respiratory failure attributed to pulmonary leukemic infiltration can occur even in patients with a peripheral leukocyte count of less than 50,000/mm3, indicating the involvement of qualitative rather than quantitative mechanisms [2]. Prompt treatment is crucial, and the therapeutic approach should prioritize the elimination of leukemic blasts through leukapheresis and cytoreduction. Patients with monocytic leukemia warrant an exceptionally high index of suspicion for leukemic pulmonary infiltration [3]. Leukemic cells typically affect the perilymphatic interstitium, resulting in the smooth thickening of the bronchovascular bundles and interlobular septa; however, imaging is usually nonspecific.

CONCLUSIONS: Pulmonary leukemic infiltration should be suspected in leukemia patients with abnormal chest x-rays and CT thorax findings, negative cultures, and significantly elevated blast counts.

SESSION TITLE: Diffuse Lung Disease Case Reports Posters (K)

SESSION TYPE: Case Report Posters

PRESENTED ON: 10/09/2024 12:30 pm - 01:15 pm

INTRODUCTION: Fever, cough, and radiographic pulmonary infiltrates often lead the clinician to a presumed diagnosis of infectious pneumonia. Not all pneumonia is infectious, however, and it is important to maintain a broad differential diagnosis. The following is a case initially thought to be fungal pneumonia, but eventually diagnosed as pulmonary limited hyper-eosinophilic syndrome.

CASE PRESENTATION: The patient is a 24-year-old male never smoker with hereditary spherocytosis necessitating a splenectomy in 2007. He previously received all post-splenectomy vaccinations. He presented with two days of dyspnea, fever, and cough. He worked as a landscaper in Ohio and had recently traveled to South Carolina. Computed tomography of the chest revealed diffuse bilateral septal thickening with increased prominence at the bases and ground glass opacities (Figure 1). The patient underwent a bronchoscopy with bronchoalveolar lavage (BAL) of the right middle lobe. Rare broad-based budding yeast forms were observed on fungal stain, thought to be blastomycosis. Amphotericin B was initiated. He did not receive any glucocorticoids. His absolute eosinophil counts increased during his hospitalization from 500/µL to 7,500/µL (0-500/µL). A previous complete blood count obtained 10 months earlier–when the patient was asymptomatic–showed an absolute eosinophil count of 1,000/µL. Confirmatory testing including urine Blastomyces antigen, fungal antibody panel, and BAL fungal polymerase chain reaction (PCR) later returned negative.He was readmitted a few weeks later, again with fever and dyspnea. Imaging revealed a right lower lobe dense infiltrate. Bronchoscopy was repeated. BAL fluid cell count showed 249 wbc/µL, of which 20% were eosinophils. Transbronchial biopsy showed reactive cellular changes with mixed neutrophilic and eosinophilic inflammation. The clinical scenario was consistent with acute eosinophilic pneumonia with suspicion of Hypereosinophilic syndrome (HES). Neogenomics Eosinophilia Fluorescence In Situ Hybridization (FISH) testing was negative for rearrangements of PDGFRA, PDGFRB, and FGFR1. Peripheral blood flow cytometry was negative for any aberrant B or T cell phenotype. He was treated with a long taper of oral prednisone with marked subsequent clinical and radiographic improvement. He was subsequently started on Mepolizumab as steroid-sparing agent for treatment of FIP1L1–PDGFRA–negative HES.

DISCUSSION: The typical presentation of acute eosinophilic pneumonia (AEP) involves fatigue, dyspnea, fevers, or weight loss.[1] Rarely cases resolve spontaneously and there may be a waxing and waning course.[2] Systemic glucocorticoids are the cornerstone of treatment.[3] The high level of peripheral eosinophilia, recurrent fevers, and pulmonary involvement was felt to be consistent with organ-restricted HES. Secondary causes of eosinophilia were ruled out as detailed above and the final diagnosis was consistent with FIP1L1–PDGFRA–negative HES. Given two admissions within a few weeks of each other, mepolizumab was initiated as per the recent randomized controlled trial showing efficacy in reducing the frequency of flares.[4]

CONCLUSIONS: This case highlights the need for maintenance of a broad differential diagnosis. The fungal stain drove the presumed diagnosis, but it was incongruous with the patient's remaining clinical, radiographic, and laboratory data. Although the level of BAL eosinophilia was below the traditional threshold of 25%, the clinical picture was most consistent with AEP and the patient responded well to treatment.