Peer Review File

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Reviewer A

The authors presented imaging features of SMARCA4-UT. Because it is relatively new disease entity, little is known about imaging features. Contrast enhanced ultrasound is not commonly used, and it may have additional information to differential diagnosis of SMARCA4-UT.
There are several important points to be solved.

1. The authors compared the image findings between several diseases such as lymphoma or schwannoma. However, the most important differential diagnoses are SMARCA4-deficient NSCLC or other sarcoma with SMARCA4 deficiencies. They should be appropriately compared.

Reply 1: Thank you for your suggestion, as the WHO released its thoracic tumor classification system in 2021, which grouped previous SMARCA4-deficient thoracic sarcoma into a new entity, SMARCA4-deficient UT, and distinguished it from SMARCA4-deficient NSCLC, we likewise believe that differentiation of the two diseases is necessary. SMARCA4-deficient NSCLC accounts for 3-6% of all NSCLC and has also emerged as a distinct subgroup of NSCLC only in recent years, usually presenting as a primary solid lung mass, with a predominance of upper lobe and peripheral lung tumors. Compared to SMARCA4-intact non-small cell lung cancer, the tumors are found to be larger and have more adrenal and lymph node metastases, as well as pericardial, cerebral, and hepatic metastases when found. While, SMARCA4-UT (previously SMARCA4-deficient thoracic sarcoma) has been reported to present with mediastinal and pleural occupancy, aggressive growth, and common distant metastases to the adrenal glands, lungs, and bones, with emphysema in a significant proportion of patients, as discussed in the discussion on page 5, rows 255 to 265. Thank you again for your advice and guidance.

Changes in the text: SMARCA4-deficient NSCLC (non-small cell lung carcinoma), which has emerged as a unique subgroup of NSCLC only in recent years, is also characterized by highly aggressive growth, and SMARCA4-deficient NSCLC accounts for only 3% to 6% of all NSCLC compared to SMARCA4-intact non-small cell lung cancer, which is larger at the time of discovery, and more adrenal and lymph node metastases are present. However, SMARCA4-deficient NSCLC usually presents as a primary solid lung mass, with a prevalence of upper lobe and peripheral lung tumors, and is associated with pericardial, brain, and hepatic metastases, and emphysema has not been reported in many patients.

2. The findings the authors showed seem reflect highly necrotic nature of SMARCA4-UT. What do they reflect? It would be better to add some speculation.

Reply 2: Thank you for your question, due to the patient's clinical condition at that time is no longer suitable for surgical resection treatment, we would like to through the patient's puncture biopsy samples for pathological tissue and imaging manifestations of the control, but unfortunately the patient's subsequent transfer to the hospital will be the pathology section will be taken away to consult the follow-up treatment, and we through the summary of the relevant literature in recent years found that the cytology of SMARCA4-UT was characterized by

atypical round or polygonal cells that appear singly or in loose clusters, and some cells demonstrating rhabdoid morphology, with convoluted nuclei, prominent nucleosomes and binucleated and multinucleated forms set in a necrotic background. which reflects its highly malignant state, it is highly progressive and invasive, and the imaging manifestations in this case, such as the large tumor volume, showing infiltrative growth, extrusion destroying the surrounding tissues and organs and large necrotic areas inside the lesion, are also consistent with its histopathological features. Relevant literature has been added to the article on page 4 to 5, lines 208 to 232 for you and other reviewers to read.

Changes in the text: Meanwhile, in recent years researchers have found that the cytology of SMARCA4-UT was characterized by atypical round or polygonal cells that appear singly or in loose clusters, and some cells demonstrating rhabdoid morphology, with convoluted nuclei, prominent nucleosomes and binucleated and multinucleated forms set in a necrotic background. The above manifestations reflect its highly malignant state, it is highly progressive and invasive, and the imaging manifestations in this case, such as the large tumor volume, showing infiltrative growth, extrusion destroying the surrounding tissues and organs and large necrotic areas inside the lesion, are also consistent with its histopathological features.

3. There is too much information of disease region in each modality, especially PET and MRI. If the most important point is the unique feature of SMARCA4-UT, the extent of disease is not so important that it would be better to shorten these findings.

Reply 3: Thanks to your comments, we have narrowed down the information on disease areas in MRI and PET, see page 3, lines 135 to 138, and page 3 to 4, lines 146 to 172.

Changes in the text: The T2-weighted image of MRI showed a tumor located in the posterior mediastinum with a maximum diameter of approximately 7.0×6.5 cm, which presented as a fusion of multiple masses encompassing and infiltrating the connective tissue and bony structures of the cervico-thoracic region (Fig 3A). T2 and T3 corresponded to spinal canal stenosis and spinal cord compression signal changes, with a length of about 3.6 cm and unclear borders, and local cerebrospinal fluid flow was blocked (Fig 3B). ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography (PET/CT) showed a left posterior mediastinum and intravertebral canal mass with irregular nodular growth along the posterior costal pleura and mediastinal pleura with unevenly increased uptake, with a maximum standardized uptake value (SUV) of 12.0 (Fig 3C-D). The mass encircled the left ribs and involved the thoracic vertebrae, with localized visible bone destruction. In addition, multiple nodules and masses with increased uptake in the bilateral neck, mediastinum, and left hilum, with a maximum SUV of 14.9, partially fused into a mass. Emphysema and multiple pulmonary alveoli were also seen in the right lung (Fig 4A).

Reviewer B

Thank you for providing this overview of a rare disease!

Reply: Thank you very much for recognizing this article!

Reviewer C

1. The paper reports for the first time on the ultrasound (US) and contrast-enhanced ultrasound

(CEUS) images in the chest for SMARCA4-deficient undifferentiated tumor (SMARCA4-UT). This indeed adds new knowledge to the field, contributing to its novelty.
Reply 1: Thank you very much for recognizing the novelty of this article.

2. The manuscript fails to compare the ultrasound findings with other malignancies, missing an opportunity to highlight the distinctiveness of SMARCA4-UT. Furthermore, there's no comparison with pathological images, which is a significant gap, as this would have strengthened the argument about the utility of ultrasound findings in diagnosis.
Reply 2: Thank you for suggesting the shortcomings of this article. Ultrasound is not a preferred modality for thoracic tumors due to the effects of air in the lungs and occlusion of the bony structures of the thorax, and therefore ultrasound has limited value for diagnostic imaging and differential diagnosis of mediastinal occupations and has little clinical use. This article mentions on page 7, lines 329 to 333 and 337 to 341, that a study summarized CEUS image features of mediastinal lymphoma and compared it with this case. We have also included a summary of the characteristics of transthoracic echocardiography for some categories such as pericardial cysts, thymic cysts, teratomas, and thymomas, as described on page 6 and 7, lines 316 to 329, and lines 335 to 337. In addition, we agree with you that there are no pathologic image deficiencies in this article, due to the patient's clinical condition at that time is no longer suitable for surgical resection treatment, we would like to through the patient's puncture biopsy samples for pathological tissue and imaging manifestations of the control, but unfortunately the patient's subsequent transfer to the hospital will be the pathology section will be taken away to consult the follow-up treatment, and we through the summary of the relevant literature in recent years found that the cytology of SMARCA4-UT was characterized by atypical round or polygonal cells that appear singly or in loose clusters, and some cells demonstrating rhabdoid morphology, with convoluted nuclei, prominent nucleosomes and binucleated and multinucleated forms set in a necrotic background. which reflects its highly malignant state, it is highly progressive and invasive, and the imaging manifestations in this case, such as the large tumor volume, showing infiltrative growth, extrusion destroying the surrounding tissues and organs and large necrotic areas inside the lesion, are also consistent with its histopathological features. Relevant literature has been added to the article on page 4 to 5, lines 208 to 232.
Changes in the text: Due to the effects of air in the lungs and occlusion of the bony structures of the thorax, ultrasound has limited value for diagnostic imaging and differential diagnosis of mediastinal occupations, and has little clinical use. Some studies have summarized the ultrasound characteristics of mediastinal occupations by transthoracic ultrasound, such as pericardial cysts and thymic cysts usually show anechoic occupations with good sound transmission, teratomas often show mixed echogenic masses, and benign thymomas often show round or oval hypoechoic masses with clear borders and homogeneous internal echogenicity, while malignant thymic carcinomas, etc., often show non-smooth membranes with unclear borders and inhomogeneous internal echogenicity. In contrast, malignant thymic carcinoma often shows a hyperechoic occupancy with an unsmooth periphery, unclear border and uneven internal echoes. In addition, mediastinal lymphoma is often a well-defined hypoechoic mass with multiple fusions on ultrasound. Regarding the CEUS presentation of mediastinal tumors, Pan et al found the specificity of CEUS was higher than that of contrast-enhanced MRI (CE-MRI), and the positive predictive value and diagnostic coincidence rate of CEUS were equal to

those of CE-MRI, which means CEUS can be used as an effective alternative and complementary examination for patients who cannot undergo CE-MRI. Kong et al. summarized the ultrasonographic features of mediastinal lymphomas and found that thymomas often show homogeneous late enhancement with a low rate of internal necrosis. In contrast, late inhomogeneous centripetal enhancement features were more common in thymic carcinomas with a high rate of internal necrosis. And 57.6% of the enhancements of lymphomas began after 10 seconds, and 93.3% of them showed small areas of necrosis. In contrast, CEUS in the present case showed that tumor enhancement also began after 10 seconds, but presented a distinct ring-like enhancement with large internal necrosis.
Meanwhile, in recent years researchers have found that the cytology of SMARCA4-UT was characterized by atypical round or polygonal cells that appear singly or in loose clusters, and some cells demonstrating rhabdoid morphology, with convoluted nuclei, prominent nucleosomes and binucleated and multinucleated forms set in a necrotic background. The above manifestations reflect its highly malignant state, it is highly progressive and invasive, and the imaging manifestations in this case, such as the large tumor volume, showing infiltrative growth, extrusion destroying the surrounding tissues and organs and large necrotic areas inside the lesion, are also consistent with its histopathological features.

3. The suggestion to include Maximum Intensity Projection (MIP) images is valid, as it would provide a more comprehensive view of the disease's extent. The current presentation of images lacks clarity on what specific aspects each image is intended to highlight.
Reply 3: Thanks to your suggestion, we provide axial, coronal and sagittal MIP images centered on the lesion, which enable a more comprehensive view of the location of the disease and its relationship with the surrounding tissue structures, see page 4, lines 172 to 174, Figure 4(B-D) and related figure notes.
Changes in the text: Emphysema and multiple pulmonary alveoli were also seen in the right lung (Fig 4A). The axial, coronal, and sagittal MIP images centered on the lesion provided a more complete picture of the location of the disease and its relationship to surrounding tissue structures (Fig 4B-D).

4. The relevance of the patient's heavy smoking history is well noted in the paper, but the suggestion to include CT imaging of the lung to show the extent of emphysema could provide further valuable context, especially given the smoking-related nature of the tumor.
Reply 4: Thank you for your valuable comments, we have added image about emphysema in the CT of the patient's lungs in Figure 4(A) and illustrated in the figure notes.
Changes in the text: Emphysema and multiple pulmonary alveoli were also seen in the right lung (Fig 4A).

5. The manuscript incorrectly uses the term "T2 weighted phase" instead of "T2 weighted image." This is a technical error and should be corrected for accuracy.
Reply 5: Thank you for pointing out the problem. The term "T2-weighted image" has been corrected in the text (see Page 3, lines 135 and annotate in Fig 3).
Changes in the text: The T2-weighted image of MRI showed multiple nodules and masses visible in the deep cervical chain bilaterally, supraclavicular region bilaterally, left posterior

cervical triangle, left the internal mammary region, various regions of the mediastinum left pleura, and left posterior back muscles, fused into a mass with a maximum cross-section of approximately 7.0×6.5 cm, encircling the peripheral vessels of the lower neck and large mediastinal pericardial vessels, invading multiple ribs on the left, soft tissues around the left posterior upper back, T2, T3 vertebrae and their accessory bones (Fig 3A).

6. The manuscript does use some abbreviations without proper introduction or explanation. This can make the paper less accessible to readers who are not familiar with these specific terms.
Reply 6: Thanks to you and the editors for pointing out the shortcomings, we have fully described the relevant abbreviations when they first appear, see page 1, lines 34 and lines 39, page 2, lines 60, page 2, lines 67 and page 5, lines 254.
Changes in the text: SMARCA4-deficient undifferentiated tumor (SMARCA4-UT) is a class of high-grade malignant tumors that has only been described in recent years, with an undifferentiated or rhabdoid morphology and genetic deletion of SMARCA4 (BRG1), a subunit of the BRG1-associated factors (BAF) chromatin remodeling complex.
Herein, we report a 51-year-old man who came to our hospital with multiple enlarged lymph nodes in the chest after a computed tomography (CT) examination at another hospital.
In 2015, Le Loarer et al.(1) first described aggressive thoracic tumors with defects in SMARCA4, the ATPase subunit encoding the BRG1-associated factors (BAF) chromatin remodeling complex, which plays an important role in transcription, differentiation, and DNA repair.
Thus, the term “Thoracic SMARCA4-deficient undifferentiated tumor (SMARCA4-UT)” has been added to other epithelial tumors in the World Health Organization (WHO) Classification of Thoracic Neoplasms, 2021 edition.
A multicenter study of 21 patients found that the majority of SMARCA4-deficient thoracic sarcomas presented on CT as compressive and infiltrative chest masses extending from the mediastinum to multi-compartment extension of lung apex, pleura, or neck with ill-defined necrotic lymph nodes, and primary tumors exhibit strong flurodeoxyglucose (FDG) avidity on PET-CT scan.

7. Intraspinal invasion in SMARCA4-UT is notable but lacks a comprehensive differential diagnosis. It misses the importance of correlating ultrasound findings with clinical progression, laboratory data, and multimodal imaging. Differential diagnoses such as small cell carcinoma, especially with elevated NSE and invasive growth, EBV-positive smooth muscle tumors, and NUT carcinoma for rapid progression should be included. The case's context of a heavy smoker and young patient with a high-grade tumor suggests SMARCA4-UT as a differential diagnosis. However, the case presentation's incompleteness hinders a thorough discussion on differential diagnosis.
Reply 7: Thank you for your careful observation and for pointing out the problem. In the article, we mainly carried out the differential diagnosis by imaging with the disease of mediastinal prevalence, and we really did not provide more detailed information about the patient's clinical data. The patient was examined for viral markers before the puncture biopsy in our hospital and was found not to be infected with HIV, syphilis, hepatitis B or C, and there was no history of previous surgeries or genetic disorders, which have been added to the text on page 4, lines 178-

181, and these results are of great value in the differential diagnosis of some of the diseases that you have subsequently proposed, such as in the case of the equally rare Epstein-Barr Virus-Associated Smooth Muscle Tumor, which typically occurs in immunodeficient or post-transplant individuals, and is also common in HIV-infected children with a clear correlation between the two, whereas in this case the patient had no prior history of any of these viral infections and no surgical history, and therefore was not included in the differential diagnosis as a disease of primary consideration. In the case of highly aggressive NUT carcinoma, it has been previously reported to be more commonly seen in young adults, with a median age at diagnosis of 16-30 years and roughly equal incidence in both sexes. Imaging findings often involve the patient's midline structures, such as the mediastinum, upper airway, and upper gastrointestinal tract are often involved, in which those with chest involvement often present with symptoms of cough, dyspnea, or bone pain due to bone metastases, which are similar to some of the symptoms in this case. However, patients with NUT carcinoma usually do not have a significant history of smoking, and there is no evidence that patients are often accompanied by emphysema. Immunohistochemical detection of NUT protein has a high sensitivity and specificity for the diagnosis of this disease, and therefore relatively extensive immunohistochemical testing is still needed to aid in its differential diagnosis from SMARCA4-UT. Relevant content has been added on page 6, lines 304 to 315. For the differential diagnosis of SCLC and NSCLC, including SMARCA4-intact NSCLC and SMARCA4-deficient NSCLC, we have added lines 255 through 265 on page 5 and lines 295 to 304 on page 6 for you and other reviewers to read.

Changes in the text: The patient was screened for viral indicators before undergoing a puncture biopsy at our hospital, and was not found to be infected with human immunodeficiency virus (HIV), syphilis, or hepatitis B and C viruses, and there was no history of previous surgeries or genetic disorders. Subsequently, pathological findings were obtained from the largest lymph node in the left supraclavicular region and the posterior mediastinal mass by ultrasound-guided histological biopsy.

SMARCA4-deficient NSCLC (non-small cell lung carcinoma), which has emerged as a unique subgroup of NSCLC only in recent years, is also characterized by highly aggressive growth, and SMARCA4-deficient NSCLC accounts for only 3% to 6% of all NSCLC compared to SMARCA4-intact non-small cell lung cancer, which is larger at the time of discovery, and more adrenal and lymph node metastases are present. However, SMARCA4-deficient NSCLC usually presents as a primary solid lung mass, with a prevalence of upper lobe and peripheral lung tumors, and is associated with pericardial, brain, and hepatic metastases, and emphysema has not been reported in many patients. It has also been shown that tumor necrosis is more pronounced in SMARCA4-UT than in SMARCA4-deficient NSCLC, and the same performance is also observed in the image of our case.

In addition, for the equally rare and highly aggressive nuclear protein in testis (NUT) carcinoma, it has been reported that it is more common in young people, with a median age at diagnosis of 16-30 years old, and a roughly equal incidence in both sexes(31). Imaging findings often involve the patient's midline structures, such as the mediastinum, upper airway, and upper gastrointestinal tract, with those with chest involvement often presenting with symptoms of cough, dyspnea, or bone pain due to bone metastases, which are similar to some of the symptoms in this case. However, patients with NUT carcinoma usually do not have a significant

history of smoking, and there is no evidence that patients are often accompanied by emphysema. Immunohistochemical detection of NUT protein has a high sensitivity and specificity for the diagnosis of this disease, so relatively extensive immunohistochemical testing is still needed to aid in its differential diagnosis from SMARCA4-UT.

In SCLC, which is also prevalent in heavy smokers, the most common imaging manifestation is a large hilar mass with massive mediastinal lymph node enlargement, characterized by a central airway infiltration of the submucosal layer and progressive narrowing of the bronchial lumen through outward or endobronchial spread, with a particular tendency for SCLC to spread to the liver, adrenal glands, bone, bone marrow, and brain. Microscopically, it showed a characteristic pike or oatmeal shape, diffusely distributed or lamellar, with cells about twice the size of lymphocytes, which could be differentiated from the present case on the basis of imaging features and histopathologic manifestations.

Overall Assessment: Despite its novelty and contribution to understanding SMARCA4-UT, the paper has several areas where improvement is needed. These include better comparative analysis with other tumors, more explicit imaging explanations, addressing terminology inaccuracies, and expanding on differential diagnosis considerations.

Reply to overall assessment: We are very grateful to you for your valuable comments and suggestions. We believe that these revisions have significantly improved the manuscript and hope to meet your recognition.

Reviewer D

I noticed that the abstract mentions "an undifferentiated or transverse myeloid morphology and genetic deletion of SMARCA4 (BRG1)". However, it should be noted that the correct term for this condition is rhabdoid morphology, not myeloid. SMARCA4-UT is characterized by rhabdoid morphology, which is in accordance with the classification of thoracic tumors by WHO (5th edition).

Reply: Thanks to your careful observation, we have changed transverse myeloid morphology to rhabdoid morphology in SMARCA4- UT in the abstract (see Page 1, line 33).

Changes in the text: with an undifferentiated or rhabdoid morphology and genetic deletion of SMARCA4 (BRG1)