alpha,beta6 targeted molecular PET/CT imaging of lung post SARS-CoV-2 infection

Running Title: PET/CT Imaging in COVID-19

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**Contributions:** JLS, RAD, and SHH developed and prepared the 18F-α,β6-Binding Peptide. JLS and CCF designed the clinical protocol. JLS consented the patients, supervised and managed data acquisition, and prepared the manuscript. CCF performed PET data analysis and interpretation.

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ABSTRACT

The true impact and long-term damage to organs such as the lungs following severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection remain to be determined. Non-invasive molecularly targeted imaging may play a critical role to aid in the visualization and understanding of the systemic damage. We have identified αvβ6 as molecular target; an epithelium-specific cell surface receptor that is low/undetectable in healthy adult epithelium but up-regulated in select injured tissues, including fibrotic lung. Herein we report the first human positron emission tomography (PET)/computed tomography (CT) images using the integrin αvβ6 binding peptide (18F-αvβ6-BP) in a patient 2 months after the acute phase of infection. Minimal uptake of 18F-αvβ6-BP was noted in normal lung parenchyma, with elevated uptake in the lung corresponding to areas of opacities on CT. This case suggests 18F-αvβ6-BP PET/CT is a promising non-invasive approach to identify the presence and potentially monitor the persistence/ progression of lung damage.
INTRODUCTION

As Coronavirus disease 2019 (COVID-19) continues to spread through the global population, the burden of diseases such as fibrotic lung post-acute COVID-19 is unknown and close follow up of patients is critical (1). The integrin subtype αvβ6 is an epithelial-specific receptor found at low and generally undetectable levels in healthy adult epithelium, is known to be up-regulated during tissue remodeling, is a potent activator of transforming growth factor beta 1 (TGF-β1), and plays a key role in the progression of numerous fibrotic diseases including lung, liver and kidney fibrosis (2-5). We and others have developed radiolabeled peptides to non-invasively image integrin αvβ6 expression (6-8). A recent study by Lukey et al. evaluated [18F-FB-A20FMDV2 Positron Emission Tomography (PET) in healthy and fibrotic lung and concluded that lung uptake of 18F-FB-A20FMDV2 was markedly increased in subjects with pulmonary fibrosis as compared with healthy volunteers (8). In our study using 18F-αvβ6-BP PET/CT (NCT03164486) in patients with cancer, immunohistochemical analysis of biopsy specimen confirmed high expression of integrin αvβ6 for tissues showing high uptake by 18F-αvβ6-BP PET (7). Taken together, given the role of the integrin αvβ6 as a potent activator of TGF-β1 and its role in the progression of numerous fibrotic diseases including those of lung, liver and kidney, we propose to extend the use of the demonstrated integrin αvβ6 imaging agent 18F-αvβ6-BP to assess lung damage in patients following SARS-CoV-2 infection. Here we present the first molecularly targeted 18F-αvβ6-BP PET images obtained in a patient two months post SARS-CoV-2 infection and correlate to CT images.

MATERIALS AND METHODS

This study protocol was approved by the UC Davis Institutional Review Board (FWA00004557), and prior written informed consent was obtained from the patient. This is the first case report of a patient included in the 18F-αvβ6-BP PET/CT COVID-19 imaging trial (ClinicalTrials.gov NCT04376593). The study was conducted following U.S. Common Rule. The primary objective of this study is to determine the safety and feasibility of 18F-αvβ6-BP PET to detect the presence and monitor the regression/progression of lung
damage in patients post SARS CoV2 infection. Up to 10 patients with a prior diagnosis of SARS CoV2 infection and have since tested negative will be recruited to the study. Each participant will undergo up to 3 $^{18}$F-$\alpha_v\beta_6$-BP PET/CT scans over a 6-month timeframe. The specific aims are; to acquire $^{18}$F-$\alpha_v\beta_6$-BP PET/CT images in patients diagnosed with SARS CoV2, to demonstrate $^{18}$F-$\alpha_v\beta_6$-BP accumulation in lung damage, to establish that $^{18}$F-$\alpha_v\beta_6$-BP accumulation correlates with the regression or progression of lung damage over time and to correlate $^{18}$F-$\alpha_v\beta_6$-BP accumulation in the lung to lung damage as indicated on CT. $^{18}$F-$\alpha_v\beta_6$-BP was manufactured in compliance with current good manufacturing practice (cGMP) under the guidelines of USP Chapter <823> as previously described (7).

Subject history

A 71-year-old male with a prior history of hypertension developed respiratory symptoms, tested positive for SARS-CoV-2 (RT-PQR nasopharyngeal swab) and was subsequently admitted to the hospital. He was treated for hypoxia and superimposed bacterial pneumonia and received supplemental oxygen and antibiotics but was not intubated. The patient’s chest X-ray at admission to the hospital showed diffuse pulmonary opacities in the mid and peripheral lungs bilaterally (FIGURE 1, left ) consistent with diagnosis of SARS-CoV-2 associated pneumonia. The chest CT scan of the thorax 4 days later showed moderate to severe bilateral central and peripheral patchy areas of ground glass and consolidative changes throughout the lungs (FIGURE 1, middle and right). After testing negative twice for COVID-19 (approximately 2 months after initial positive test) the patient was enrolled on the $^{18}$F-$\alpha_v\beta_6$-BP PET/CT COVID-19 imaging trial.

Imaging

$^{18}$F-$\alpha_v\beta_6$-BP PET/CT images were acquired during recovery 66 days post initial chest CT scan. The patient was injected with $^{18}$F-$\alpha_v\beta_6$-BP (340 MBq) as a rapid intravenous bolus. Immediately before and after the injection the patients vital signs (blood pressure, heart rate, pulse oximetry value and body temperature) were measured. The patient rested for 1 hour prior to the PET/CT scan. The PET scan was
performed on a GE Discovery 690 PET/CT scanner at 2 minutes per bed position. A PET/CT acquisition of the thorax with arms up was performed with a typical low dose level CT scan (140kV ‘smart mA’ [50-350mA], noise index 20) of the thorax for attenuation correction. Immediately following, a second non-attenuation corrected PET scan was acquired from the skull vertex to the proximal thighs with arms up.

Data analysis

Reconstructed PET/CT images were displayed using GE Advantage Workstation Client (GE AW Server 3.2 ext3 (VolumeViewer 14 ext4), reoriented into maximum intensity projection (MIP) transaxial, coronal and sagittal images. PET, fused PET/CT and CT images were reviewed. The PET images were interpreted qualitatively and semi-quantitatively. Semi-quantitative analysis included regions of interest (ROIs) placed around tracer avid foci suspicious for lung damage in order to obtain standard uptake values (SUV), including \( \text{SUV}_{\text{max}} \) and \( \text{SUV}_{\text{mean}} \).

RESULTS

No changes in vital signs were noted during the study and the patient’s SpO₂ was 100%. The transaxial CT images (FIGURE 2 left panels) through both the upper and lower lungs showed improved areas of bilateral patchy opacities as compared to the initial chest CT (FIGURE 1 middle and right). Transaxial co-registered attenuation corrected \(^{18}\text{F-}\alpha_6\beta_0\text{-BP}\) PET images through the upper and lower lungs (scale \( \text{SUV}_{\text{max}} \) 5.0, FIGURE 2, middle panels) demonstrated elevated uptake of \(^{18}\text{F-}\alpha_6\beta_0\text{-BP}\) (\( \text{SUV}_{\text{max}} \) of 3.0) in areas corresponding to areas of opacities noted on the CT. Concurrently, regions of normal lung parenchyma by CT demonstrated low levels of \(^{18}\text{F-}\alpha_6\beta_0\text{-BP}\) uptake by PET with \( \text{SUV}_{\text{max}} \) of 0.8-1.0.

DISCUSSION

The long-term systemic health impact of COVID-19 in both symptomatic as well as asymptomatic subjects is yet to be determined. In the future it will be critically important to non-invasively evaluate the persistence and potential progression of abnormalities of the lung and other organs. As was recently
described by George et al. long-term follow up studies to establish the true prevalence of post COVID-19 fibrosis are essential and these preliminary data suggest $^{18}$F-$\alpha_v\beta_6$-BP PET/CT is a promising non-invasive strategy to address this (1).

Although anatomical imaging with CT of patients infected with SARS-CoV-2 often shows a mix of consolidation and ground glass opacities in the lung, early identification is often confounded by delayed radiographic presentations (9). In addition, lung damage may be missed for the large fraction of asymptomatic patients. Long et al. recently reported the clinical and immunological assessment of asymptomatic patients where in radiological and laboratory findings they noted that 11/37 patients showed focal ground-glass opacities on CT, and 14/21 patients had abnormal radiological findings in at least one lung (10). Several incidental findings of COVID-19 have also been noted in patients undergoing $^{18}$F-FDG PET/CT studies for routine oncological indications (11).

Considering that the anatomical observations made by CT are caused by major changes of the tissue, molecularly targeted non-invasive imaging strategies, such as $^{18}$F-$\alpha_v\beta_6$-BP PET, can provide essential complementary clinical information to further understand the nature of the underlying tissue remodeling resulting in these changes noted by CT. The quantitative information from the $^{18}$F-$\alpha_v\beta_6$-BP PET scans could help identify potential damage sooner (prior to clinical/symptom manifestation) and ascertain if the damage is transient or progressive. Molecular imaging can thus also contribute to improved clinical detection and the longitudinal study of recovery from damage to lungs as well as other organs following infection. Other radiopharmaceuticals such as $^{18}$F-FDG and $^{18}$F-FAPI (12) could also provide complimentary information about SARS-CoV-2 infection.

The integrin $\alpha_v\beta_6$ has previously been described as a potential biomarker of fibrotic lung disease including idiopathic pulmonary fibrosis (IPF), non-specific interstitial pneumonitis (NSIP), and chronic hypersensitivity pneumonitis (HP) (6,8) and it has been recognized as important activator of TGF-$\beta$1 during tissue remodeling (2,3). Semi-quantitative analysis of the integrin $\alpha_v\beta_6$ expression in lung biopsy
specimens from individuals with IPF has been shown to have potential prognostic significance, with higher levels predicting more rapid progression and mortality (8).

We and others have developed radiolabeled peptides to image integrin α₆β₅ expression (6-8). A recent study by Lukey et al. evaluated ¹⁸F-FB-A20FMDV2 in healthy and fibrotic lung and concluded that lung uptake of ¹⁸F-FB-A20FMDV2 was markedly increased in subjects with pulmonary fibrosis as compared with healthy volunteers (SUVmean 1.03 and 0.54, respectively) (8). In our study using ¹⁸F-α₆β₅-BP (NCT03164486) in patients with cancer, PET images showed significant uptake of ¹⁸F-α₆β₅-BP in both the primary lesion and metastases, including metastasis to brain, bone, liver and lung; immunohistochemical analysis of biopsy specimen confirmed high expression of integrin α₆β₅ for tissues showing high uptake by ¹⁸F-α₆β₅-BP PET (7). SUVmax values in the primary tumors and metastases were as high as 25.0, while low levels of uptake were noted in normal lung parenchyma with SUVmax ≤1.0 (range 0.3 – 1.0).

This preliminary study has shown correlation of integrin α₆β₅-targeted ¹⁸F-α₆β₅-BP PET with lung damage identified by CT. For areas of lung that corresponded to SARS-CoV-2 related ground glass and consolidation by CT, the SUVmax observed by ¹⁸F-α₆β₅-BP PET were approximately 3.0. These values are three times those reported by Lukey et al. for ¹⁸F-FB-A20FMDV2 in fibrotic lung, and represent an almost 4-fold increase in uptake of ¹⁸F-α₆β₅-BP in abnormal vs normal lung tissue and clear visualization of damage. These observations suggest ¹⁸F-α₆β₅-BP PET/CT as a promising strategy to detect and monitor the development and progression of lung fibrosis post SARS-CoV-2 infection, and to further understand the nature of the tissue remodeling and progression in recovering patients over time. The main limitation of this study is the single imaging time point, and follow-up ¹⁸F-α₆β₅-BP PET/CT scans will be critically important to evaluate the persistence and potential progression of abnormalities of the lung and other organs. ¹⁸F-α₆β₅-BP PET/CT scans are currently scheduled for 3 and 6 months and a total of 10 patients will be enrolled in the study. These longitudinal studies are needed to determine the ability of this imaging test to predict/monitor post COVID lung fibrosis.
CONCLUSION

As COVID-19 continues to spread through the global population, the burden of diseases such as fibrotic lung post-acute COVID-19 are yet to be determined and close follow up of patients is critical. This study has shown correlation of integrin \(\alpha_v\beta_6\)-targeted \(^{18}\text{F}-\alpha_v\beta_6\)-BP with lung damage identified by CT; we therefore will further investigate the role \(^{18}\text{F}-\alpha_v\beta_6\)-BP as a PET imaging agent for early detection of lung damage and monitoring of disease progression.

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Disclosure

Drs Sutcliffe and Hausner are named inventors on WO2015160770. Dr Sutcliffe is the founder and stock holder of Luminance Biosciences, Inc. Luminance Biosciences, Inc has licensed WO2015160770. No other potential conflicts of interest relevant to this article exist.

Key points

Question

Can \(^{18}\text{F}-\alpha_v\beta_6\)-BP PET detect damage to the lungs following SARS-CoV-2 infection?

Pertinent findings

\(^{18}\text{F}-\alpha_v\beta_6\)-BP PET is a non-invasive approach to identify the presence and potentially monitor the persistence/ progression of lung damage.

Implications for patient care
This approach has the potential to not only detect organ damage but also to guide and monitor response to novel molecularly targeted treatments.
REFERENCES


FIGURE 1: Initial chest X-ray at hospital admission showing diffuse pulmonary opacities in the mid and peripheral lungs bilaterally (left panel), and CT scan of the thorax on day 4 after admission: Transaxial view of the upper lung (middle panel) and lower lung (right panel) showing areas of ground glass and consolidative changes.
FIGURE 2: Transaxial CT images through the upper lungs (top left) and lower lungs (bottom left) showing areas of bilateral patchy opacities at time of the $^{18}$F-$\alpha_v\beta_6$-BP PET/CT scan. Transaxial co-registered attenuation corrected $^{18}$F-$\alpha_v\beta_6$-BP PET images (scale SUV$_{max}$ 5) through the upper lungs (top middle) and lower lungs (bottom middle), showing increased uptake of $^{18}$F-$\alpha_v\beta_6$-BP. Fused $^{18}$F-$\alpha_v\beta_6$-BP PET/CT images through the upper lungs (top right) and lower lungs (bottom right).