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Perspective on Fibroblast Activation Protein Specific PET/CT Imaging in Fibrotic Interstitial Lung Diseases: Imaging Fibrosis - a New Paradigm for Molecular Imaging?

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Running Title: FAPI PET/CT for imaging of fibrosis

Interstitial lung diseases (ILDs) are a heterogeneous group of parenchymal pulmonary disorders. Their hallmark is pulmonary infiltration by immune-competent cells followed by lung fibrosis [1]. Depending on the subtype of ILD, their prognosis varies. However, several of these disease entities present with a chronic, irreversible, and progressive clinical course and are associated with worsening lung function, impaired quality of life, and limited life expectancy. Despite the possibility to detect ILDs with the current standard imaging technique high resolution CT (HRCT) and pulmonary function tests, monitoring of disease activity remains challenging, since the course of disease e.g. in systemic-sclerosis associated ILD (SSC-ILD) is highly variable [2].

To date, HRCT is the mainstay to establish the diagnosis of ILDs since certain morphologic disease patterns indicate specific disease entities. However, morphologic imaging using HRCT is not capable of determining tissue remodeling. Furthermore, the measurement of functional decline by pulmonary function tests requires long-term follow-up. Molecular imaging of ILDs for the assessment of disease activity is so far based on the use of ^{18}F-fluor-desoxy-glucose (^{18}F-FDG) positron emission tomography/ computed tomography (PET/CT). However, ^{18}F-FDG PET/CT can generally only assess the level of inflammation, but cannot evaluate the degree of fibrotic activity in ILDs and other fibroinflammatory diseases [3]. Hence, novel non-invasive diagnostic approaches to evaluate disease activity and for monitoring treatment of ILDs are of considerable interest. Persistent activation and local accumulation of myofibroblasts play a key role in the development of fibrotic diseases of the lung such as in SSc-ILD or idiopathic pulmonary fibrosis [4]. Fibroblast activation protein alpha (FAP) is a type II transmembrane protease with dipeptidyl peptidase and endopeptidase activity which is induced in fibroblasts upon activation and is negligible or absent in resting fibroblasts or other cell types [5] . The recently developed radiolabelled quinoline-based PET tracers binding to FAP demonstrate tracer uptake in various tumor entities as well as in fibrotic diseases and are a major advance for molecular imaging [6].

In a recently published translational exploratory study, Röhrich et al. evaluated the static and dynamic imaging properties of ^{68}Ga-FAPI-46 PET/CT in fifteen patients suffering from fibrotic interstitial lung diseases (fILD) and suspected lung cancer (LC) [7]. They performed static PET/CT scans in twelve patients and dynamic scans in another three patients. Standardized uptake values (SUV) were measured in a total of 55 CT-morphologically typical fibrotic lesions and three LC lesions. Furthermore, FAP immunohistochemistry of four human fILD biopsy samples and of fibrotic lungs of Nedd4-2$^{-/-}$ mice was carried out. fILD and LC lesions had considerably elevated uptake at each of the static imaging time points. SUVmax and SUVmean values of both fILD and LC lesions decreased over time with a more pronounced decrease in fILD lesions compared to LC lesions. In contrast, due to the decreasing background activity over time, fILD manifestations demonstrated relatively stable target to background ratios, while target to

background ratios of LC manifestations showed a tendency to increase during the sequential PET examinations. These findings highlight the potential use of quantitative PET imaging at sequential time points to differentiate between malignant and fibrotic lesions. Analogous results were obtained in the dynamic PET acquisitions: While fILD lesions showed an early peak in tracer accumulation with a slowly decreasing signal intensity over time, LC manifestations presented an increasing time activity curve with a delayed peak and gradual washout. Interestingly, the peak of the tracer uptake in LC lesions was between 10 and 30 minutes with a corresponding fast blood clearance resulting in high target to background ratios. These findings suggest that, for ^{68}Ga-FAPI-46, the optimal imaging time point might be earlier than the current standard 60 min p.i. for LC and fibrosis imaging in use for ^{18}F-FDG. Immunohistochemistry of both human fILD biopsies and whole lung sections of Nedd4-2$^{-/-}$ mice serving as a gold standard were evaluated for FAP expression. FAP-positive areas were localized in the transition zone between healthy lung tissue and fibrotic areas in human fILD sections. In Nedd4-2$^{-/-}$ mice, healthy lung parenchyma demonstrated only low FAP expression, but fibrotic lesions exhibited FAP upregulation. These impressive results suggest a promising role of fibroblast activation protein imaging in fibrotic lung diseases for evaluation of disease activity. The early peaks in tracer uptake of LC and fibrotic lesions between 10 to 30 minutes allow for early image acquisition, which is an important logistical advantage in times of an increasing number of PET/CT examinations.

While the current imaging standard HRCT is not capable of determining disease activity, ^{18}F-FDG PET/CT is of limited use for the assessment of response to antifibrotic drugs [8]. This is not surprising since ^{18}F-FDG PET/CT visualizes increases in glucose metabolism caused by inflammatory processes, but not the activity of activated fibroblasts that play a key role in the development of lung fibrosis. Recently, Bergmann et al. reported the use of ^{68}Ga-FAPI-04 PET/CT in 21 patients suffering from SSc-ILD and showed that fibroblast activation protein imaging directly visualizes activated fibroblasts in vivo [9]. Furthermore, ^{68}Ga-FAPI-04 uptake was higher in patients with extensive disease, with previous ILD progression, or high European Sclerodermia Trials and Research Group activity scores than in those with limited disease, previously stable ILD, or low European Sclerodermia Trials and Research Group activity scores. Increased ^{68}Ga-FAPI-04 uptake at baseline was associated with progression of ILD independently of extent of involvement on HRCT scan and of the forced vital capacity at baseline. Moreover, changes in ^{68}Ga-FAPI-04 uptake were concordant with the observed response to the fibroblast targeting antifibrotic drug nintedanib [9].

To date, FAP- PET/CT is the only clinically available imaging approach that can directly visualize and quantify the activity of activated fibroblasts in fibrotic and tumor diseases. In contrast to other techniques, e.g. pulmonary function tests or HRCT that measure the cumulative result of tissue damage ^{68}Ga-FAPI PET/CT can directly assess the dynamic of this process. ^{68}Ga-FAPI PET/CT might improve risk assessment

of patients suffering from fibrotic diseases and allow earlier and more accurate treatment as well as dynamic monitoring of the molecular response to fibroblast-targeting therapies.

FAP imaging might open a completely new perspective for the nuclear medicine community. This was also demonstrated in a publication on FAPI PET/CT in IgG_4-related disease (IgG_4-RD) [10]. IgG_4-RD is a paradigm of the inflammation versus fibrosis dichotomy and is characterized by autoimmune inflammation associated with tumefactive tissue fibrosis. The disorder has a predilection for the pancreas, the biliary tree, the salivary glands, the kidney, and the aorta among others. Histopathology studies and clinical correlations have suggested progression of IgG4-RD from a proliferative to a fibrotic phase. While in the proliferative IgG_4-RD phase dense lympho-plasmacytic infiltrates occur, the fibrotic phase has relatively sparse cellular infiltrates and a greater degree of tissue fibrosis Fibrotic lesions responded far less pronounced to anti-inflammatory treatment with rituximab than inflammatory lesions. The conclusion of this study was that FAP-specific PET/CT permits the discrimination between inflammatory and fibrotic activity in IgG4-related disease. This finding may profoundly change the management of certain forms of immune-mediated disease, such as IgG4-related disease, as subtypes dominated by fibrosis may require different approaches to control disease progression than those that are predominantly inflammatory. For example, in the former, specific antifibrotic agents rather than broad-spectrum anti-inflammatory treatments might be useful.

The above-reviewed evidence has demonstrated that imaging of active fibrotic processes has become feasible. Future clinical research will reveal whether the new paradigm of imaging activated fibroblasts by PET is of clinical utility in ILDs and other rheumatic disorders.

Conflict of interest statement: No potential conflicts of interest relevant to this article exist.

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