^{18}F-FDG PET in sarcoidosis

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voor mijn ouders

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Chapter 1

General Introduction

1. Sarcoidosis

1.1 Epidemiology

Sarcoidosis was first described in 1877 by Jonathan Hutchinson, an English dermatologist. He observed purplish cutaneous lesions and initially, sarcoidosis was therefore thought to be limited to the skin. At the end of the 19th century the dermatologist Caesar Boeck described skin nodules, histologically characterized by epitheloid cells and giant cells.[1] In the following years it became clear that sarcoidosis can affect any organ, although the skin, lungs and eyes are most frequently involved.[2]
Sarcoidosis usually develops before the age of 40, with a peak incidence in the third decade.[3] There is a slightly higher incidence in women, but the incidence varies throughout the world from one to 40 cases per 100.000. The disease is common in the USA, northern European regions and Japan.[4]
The lifetime risk of sarcoidosis is three times higher in African-Americans than in Caucasians and it affects them more acutely and more severely.[5, 6] The overall mortality of sarcoidosis is 1-5%, merely caused by respiratory failure.[7] However, in the Japanese population, cardiac involvement is the main cause of death.

Sarcoidosis is known for its high spontaneous remission, particularly in patients with Löfgren's syndrome. This variant of sarcoidosis is characterized by the presence of bilateral hilar lymphadenopathy, erythema nodosum and arthritis.[8] Although the majority of patients with sarcoidosis will recover spontaneously within a few years, one third demonstrates at least a mild impairment of organ function.[2] A poorer prognosis is associated with some clinical variables, like splenomegaly, involvement of more than 3 organ systems, stage III pulmonary disease, the absence of erythema nodosum, black race, disease onset after the age of 40 and symptoms lasting for more than 6 months.[9, 10]
The use of corticosteroids is indicated in severe pulmonary, ocular, cardiac or neurologic involvement as well as hypercalcemia.[11] The necessity of systemic therapy in patients with mild to moderate pulmonary and/or other extra pulmonary disease is less clear, although most physicians suggest treatment when the symptoms become progressive. The choice of other immunosuppressive drugs,

such as methotrexate or infliximab, depends on the severity of the disease as well as effectiveness and adverse effects of corticosteroids.[12]

1.2 Immunopathogenesis

The cause of sarcoidosis remains unknown although the current general statement is that a genetically susceptible individual is exposed to a specific environmental factor.[13] The granuloma in sarcoidosis is very similar to granulomas caused by other diseases, like tuberculosis, hypersensitivity pneumonitis or berylliosis. Thus, it is postulated that granuloma formation in sarcoidosis is caused by an excessive immunological response to an infectious agent, organic particles or inorganic agents.
Research on immunological processes has contributed to the understanding of the pathogenesis of sarcoidosis and demonstrated that the development of the characteristic epitheloid cell granuloma is based on three phases.[14]

I. Granuloma formation is initiated by the incorporation of the unknown antigen by antigen presenting cells (APC). The antigen is attached to the major histocompatibility complex (MHC) class II molecule and presented to the naïve CD4+ T cells (Th0). Under the influence of interleukin (IL)-12 and IL-18, the CD4+ T cells will be activated and differentiate into T helper 1 cells (Th1).

II. Macrophages and their derivatives, epitheloid cells and giant multinucleated cells, are present in the centre of the granuloma and surrounded by a rim of lymphocytes and fibroblasts.[15] The continuous presentation of the antigen by alveolar macrophages to Th1 cells results in the formation of these granulomas. The alveolar macrophage releases IL-1, IL-6, IL-12, macrophage inflammatory protein-1 (MIP-1), monocyte chemotactic protein-1 (MCP-1) and RANTES, but above all tumor necrosis factor alpha (TNF-α). This specific cytokine has a crucial role in granuloma development.[14]
The Th1 response is mainly induced by IL-12. Activated Th1 cells predominantly secrete IL-2 and interferon-γ (IFN-γ) which also has a pivotal role in the granulomatous process. Additionally, the released chemokines boost the formation of the granuloma by the recruitment and proliferation of monocytes/macrophages and T cells.

III. The granulomatous inflammation may disappear without residual scarring, remain stable or lead to fibrosis with permanent damage to the affected tissue. Different immune responses are thought to clarify the varying disease course. Down regulation of the immune response might result in the spontaneous resolution of the granuloma, preceded by elimination of the eliciting factor.

In case of an inadequate Th1 response, Th2 cells are activated representing a more effective elimination of the antigen.[16] However, the switch from a Th1 to Th2 response is present in patients developing chronic sarcoidosis, potentially followed by fibrosis.[17]

Th2 cells are a second subset of CD4+ T cells capable of producing cytokines.[18] The Th2 cells release IL-4, IL-5, IL-10, IL-13 and stimulate B lymphocytes. The alternative activation of alveolar macrophages by the Th2 cytokines, results in the release of CC chemokine ligand 18 (CCL18) and fibronectin.[19] CCL18 up-regulates the production of collagen by pulmonary fibroblasts. This process increases the release of CCL18 by macrophages, again stimulating the fibroblasts, ultimately leading to fibrosis.[19]

1.3 Genetics

Sarcoidosis demonstrates familial clustering. Compared with control subjects, patients with sarcoidosis report five times more often to have siblings or parents with sarcoidosis.[20] Combined with ethnic differences in incidence, these findings suggest a genetic risk factor for sarcoidosis.

Human leukocyte antigens (HLA) are responsible for the antigen presentation and release of inflammation related molecules. HLA genes were therefore thought to be involved in sarcoidosis. Gene mapping demonstrates three HLA class regions comprising the human MHC.[21]

The search for HLA associations with sarcoidosis began with the evaluation of the HLA class I genes. HLA-B8 has been associated with acute onset of the disease and a favorable outcome.[22-25] HLA class II genes were subsequently analyzed and the allelic variation of HLA-DRB1 appeared be a major contributor to the genetic predisposition of sarcoidosis. In general, HLA-DRB1 is associated with a good prognosis. HLA-DRB1*1101 demonstrates a race dependant, increased susceptibility for sarcoidosis,[26] and HLA-DQB1*0201 has repetitively proven to correlate with a mild course of the disease.[27, 28] Conversely, HLA-DQB1*1501 and HLA-DQB1*0602

have been associated with chronic and severe sarcoidosis.[28, 29]

HLA class II alleles may clarify the different phenotypes of sarcoidosis. HLA-DRB1*0401 is associated with eye involvement in blacks and whites, and parotid and salivary gland involvement in blacks.[26] HLA-DRB3 is associated with bone marrow disease in blacks and HLA-DPB1*0101 with hypercalcemia in whites.[26]

The causative role of HLA genes in sarcoidosis has been extensively studied, but in approximately half of the patients these genes seem not to influence the disease risk. Therefore, several non-HLA genes have been assessed as well. Although correlations were found with a favorable or more dramatic course of the disease,[30-32] the results were inconsistent or remained unconfirmed.[33]

2. Sarcoidosis activity

A gold standard to assess the presence of active sarcoidosis is currently missing. Therefore, a combination of clinical parameters is frequently used for the assessment of disease activity. These parameters include symptoms, objective findings of activity, increased biomarkers of disease activity and the evolution of radiological and functional abnormalities over time. However, the majority of these parameters are unable to reflect the activity state accurately or demonstrate conflicting results when correlated. For example, serum markers can be increased while symptoms are absent and functional impairment does not occur. Conversely, normal levels of serum markers can be found in patients with severe, disabling symptoms as well as in progressive disease.

Worsening pulmonary function tests, however, almost invariably indicate active disease and is frequently accompanied by progressive dyspnoea and cough. However, the clinical condition of the patient needs to deteriorate first, before the decrease in pulmonary tests becomes evident. Furthermore, persistent dyspnoea and cough may occur in active as well as end-stage disease, *i.e.* fibrosis. Fibrosis is irreversible and these patients will therefore not benefit from immunosuppressive therapy.

Fatigue is another common symptom, present in both active and inactive disease. In patients with longstanding sarcoidosis, fatigue is reported by 57%, causing a significant impairment in quality of life.[34] When symptoms like dyspnoea and fatigue are based on active sarcoidosis, it may be reduced by the use of immunosuppressive therapy.

In symptomatic, newly diagnosed patients with histological proof of sarcoidosis, the presence of active disease is most obvious. However, in patients with long-standing disease, clinicians are frequently impeded by the lack of a gold standard for disease activity. Therefore, a new test, accurately determining the presence of active disease, would be a major step forward and contribute to a more adequate sarcoidosis management.

2.1 Consensus on sarcoidosis activity

In 1994, the American Thoracic Society, European Respiratory Society and World

Association of Sarcoidosis and Other Granulomatous disorders have published a consensus report on the definition of sarcoidosis activity.[35] Three elements of the pathogenesis of sarcoidosis were taken into account for the definition of active disease. Active sarcoidosis implies "an ongoing T-lymphocyte and macrophage inflammation, an evolving process of granuloma formation and progression towards fibrosis." Based on these different phases, active disease is present when patients have clinical signs of activity with/without active granuloma formation, with/without biological or immunological markers of alveolitis and/or active progression towards fibrosis. Inactive disease suggests regression or stable disease with biological markers within the normal range.

The consensus report recommends a combination of clinical investigation, chest radiography and lung function tests to evaluate the presence of active disease. Serum angiotensin converting enzyme (ACE), ^{67}Ga scintigraphy, high resolution CT (HRCT) and bronchoalveolar lavage (BAL) cell populations with $CD4^+/CD8^+$ ratio are optional.

2.2 Symptoms and clinical findings

Given that any organ can be involved, patients can present with varying symptoms and clinical findings.[36] The lung is involved in the majority of patients and progressive pulmonary symptoms like dyspnoea and cough, usually implies active disease. Patients with eye involvement often present with uveitis, optic neuritis or lacrimal gland swelling. Interestingly, when erythema nodosum and polyarthritis are present, the prognosis is usually good, whereas skin lesions other than erythema nodosum as well as splenomegaly suggest severe and chronic disease.[37, 38] Parotid enlargement, Bell's palsy and enlarged lymph nodes represent other extra pulmonary lesions, indicating disease activity. Furthermore, non specific symptoms like fatigue, weight loss and fever may be indicative for active sarcoidosis.

2.3 Pulmonary function tests

Restrictive or obstructive lung function is often found in sarcoidosis and is reflected by a reduced vital capacity (VC) and forced expiratory volume in one second (FEV_1), respectively.[2] Furthermore, the diffusion capacity of the lung for carbon monoxide (DLCO) can be decreased. Obstructive lung function can either

be caused by endobronchial involvement or peribronchovascular fibrosis.[39]

A recent study in 830 sarcoidosis patients, revealed that the initial pulmonary function tests (PFT) demonstrate an impaired DLCO in 26% of the patients. A decreased FEV_1 and VC was found in 15% and 5%, respectively.[40] Airflow obstruction expressed by a reduced FEV_1/VC was present in 12% of the patients.

Remarkably, impairment of PFT does not correlate with the radiographic findings. At the time of diagnosis, patients without parenchymal infiltrates on chest radiography show a decreased VC and DLCO in approximately 20% and 30%, respectively.[41-44] In the radiographically affected lung parenchyma, VC and DLCO are reduced in 65%.[41, 45, 46]

Baseline VC and DLCO do not predict disease outcome, but serial PFT can be used to monitor disease progression and are very informative in this respect.[47] In 80% of the patients with abnormal PFT at baseline, normalization occurs within 2 years.[48]

2.4 Serological markers

Several serum markers have been investigated with regard to sarcoidosis activity. These markers can be divided into 3 groups: macrophage and granuloma associated, lymphocyte associated and extracellular matrix associated.[35]

Angiotensin converting enzyme (ACE), lysosyme, chitotriosidase and calcium are macrophage/granuloma associated markers. ACE, perhaps the most widely used serological parameter, is produced by the epitheloid cells and macrophages and is thought to represent the total granuloma load.[49] Several studies have assessed the sensitivity of ACE with variable results. The average sensitivity in sarcoidosis was 55%, but the discovery of the insertion/deletion polymorphism of the ACE gene improved the sensitivity significantly.[50-55] ACE cannot predict disease outcome, however, it is suggested to be useful for treatment monitoring.[56, 57]

Like ACE, lysozyme is produced by macrophages and epitheloid cells in the granuloma. Remarkably, it can only be found in patients with freshly formed granulomas.[58] Although the sensitivity of lysozyme seems somewhat higher than the sensitivity of ACE, its specificity is even lower.[59, 60] Therefore, it is not regularly used in clinical practice.

Only recently, serum chitotriosidase was evaluated in sarcoidosis. Chitotriosidase is secreted by activated macrophages and can therefore be found in patients with active sarcoidosis and not in inactive disease.[61] It correlates with the radiographic stage and appears a promising marker for sarcoidosis activity.

Hypercalcaemia is present in 10% of the patients, while hypercalciuria is observed in 30%. Hypercalcaemia is caused by an overproduction of calcitriol, the active form of vitamin D_3. Calcitriol derives from the activated macrophages in the granuloma and leads to an increased absorption of calcium and phosphate from the gastro-intestinal tract.[62] This results in hypercalcaemia and hypercalciuria. A mild hypercalcaemia is often transient. Persistent hypercalcaemia however, is associated with nephrocalcinosis and is therefore an indication to start corticosteroids.[12]

The soluble interleukin-2 receptor (sIL-2R) is a lymphocyte associated marker. IL-2 receptors are found on the surface of T lymphocytes, B lymphocytes, monocytes and macrophages.[63-65] Its soluble form is associated with cellular immune reactions and might therefore be increased in sarcoidosis. The sensitivity of sIL-2R is approximately 70% and is higher in patients with parenchymal involvement.[66, 67] However, sIL-2R does not correlate with the radiographic changes and conflicting results have been found regarding the correlation between sIL-2R and lung functional outcome.[67, 68]

The aforementioned markers are not specific for sarcoidosis and are frequently elevated in several other diseases. Hyperthyroidism is associated with elevated ACE, as is silicosis, beryllium, leprosy and primary biliary cirrosis.[53, 69-71]
Since the origin of lysozyme is alike, increased levels can be found in the similar diseases.[72, 73]
Besides sarcoidosis, elevated chitotriosidase levels can be observed in patients with lysosomal lipid storage disorders, thalassemia, visceral Leishmaniasis and multiple sclerosis.[74-76] Increased sIL-2R is present in malignant lymphoma, inflammatory bowel disease, rheumatoid arthritis, systemic lupus erythematosus, HIV and tuberculosis.[77-81]

2.5 Bronchoalveolar lavage

The bronchoalveolar lavage procedure (BAL) is performed after local anesthesia of the larynx and lower airways combined with oxygen supply, if necessary.[82] In most patients, a fiberoptic bronchoscopy is used, providing biopsy possibilities. Sedatives can be administered to reduce patient discomfort and facilitate the procedure.[83]
BAL has a central role in the diagnostic process of diffuse lung diseases. BAL enables the analysis of different alveolar cell types and although a definite diagnosis may remain absent, it is helpful in excluding other causes of diffuse lung diseases such as infections.[84]
In 90% of the sarcoidosis patients, BAL reveals an increased number of T lymphocytes at the time of diagnosis.[85] Although this finding is not specific, the shift towards Th1 cells is. The Th1 cells express CD4 on the cell surface and are characterized by the excretion of IFN-γ and IL-2. Compared to the cytotoxic T cells, expressing CD8, the number of $CD4^+$ T cells is much higher. Therefore, an increased $CD4^+/CD8^+$ ratio is a typical finding in sarcoidosis.[86, 87]
Besides the lymphocytic alveolitis and increased $CD4^+/CD8^+$ ratio, the number of neutrophils can be increased. Increased neutrophils in BAL are particularly seen in patients with advanced sarcoidosis and seem to correlate with a more severe course of the disease.[88, 89] Despite their diagnostic value, the number of lymphocytes and the $CD4^+/CD8^+$ ratio do not have a predictive value.

3. Imaging in sarcoidosis

3.1 Conventional chest radiography

Sarcoidosis can be classified into 5 stages based on conventional radiography of the chest. Stage 0 correlates with a normal chest radiography and stage I is defined as bilateral hilar lymphadenopathy. Stage II represents bilateral hilar lymphadenopathy combined with parenchymal infiltrates while stage III correlates with parenchymal infiltrates without lymph node enlargement. In stage IV, signs of fibrosis are present with retraction of the hila, cystic changes and bullae.[90] At presentation, stage 0 can be found in approximately 10% of the patients; stage I in 45%, stage II in 30%, stage III in 10% and stage IV in 5%.[91]

In more than 50% of the patients, a spontaneous remission is seen within 3 years, seldomly followed by disease recurrence.[2] The remission rate is related to the radiographic stage, but may vary due to the different ethnic backgrounds.[5, 91-94] Approximately 45-80% of the patients with stage I will recover from sarcoidosis, which is 30-70% in stage II, 10-20% in stage III and 0% in stage IV.

3.2 High resolution CT

Due to the spatial resolution of CT, this modality is superior to conventional radiography in detecting interstitial abnormalities, early fibrosis and parenchymal distortion.[95] Typical CT features in sarcoidosis are thoracic lymphadenopathy, nodules, thickened interlobular septa, ground glass, fibrosis and scarring. However, CT findings vary greatly and may be present in other lung diseases as well.[96]

Enlarged lymph nodes are predominantly located on the right side and calcifications occur in prolonged disease. Nodules can be found subpleurally and around the bronchovascular bundles. At the onset of sarcoidosis, ground glass may be present, representing the granulomatous inflammation. This CT feature can be found in fibrosis as well, although often accompanied by architectural distortion.

Conflicting results have been found about the predictive role of HRCT features and disease outcome.[97, 98] In studies describing a predictive role, conventional chest imaging equaled HRCT with regard to the decrease in PFT. The extent of the disease depicted by HRCT is unable to predict functional impairment and inconsistent results have been found regarding the correlation of HRCT with BAL parameters, ACE and sIL-2R.[98-101]

HRCT is not recommended in routine analysis, but is indicated in case of an atypical clinical presentation and/or chest radiography, to detect the complications of sarcoidosis, like fibrosis or bronchiectasis, and in the presence of a normal chest radiography but a clinical suspicion of the disease.[11] The radiation burden of HRCT as compared to conventional chest radiography is higher as the effective radiation dose is approximately 2.3 mSv.[102]

3.3 ^{67}Gallium scintigraphy

In vivo, Gallium-67 (^{67}Ga) acts like an iron analogue and binds to transferrin. In inflammatory lesions, ^{67}Ga will subsequently bind to lactoferrin which is an important binding protein in polymorphonuclear leukocytes.[103]

During the first 24 hours after administration, ^{67}Ga citrate is excreted by the kidneys. Subsequently, the hepatobiliary system will excrete the radiopharmacon. Due to the slow plasma clearance, a substantial amount of ^{67}Ga citrate remains in the body and will be distributed to 'lactoferrin rich' tissues. This explains the activity in bone marrow, spleen, liver and lacrimal and salivary glands.

Several authors have described the sensitivity of ^{67}Ga scintigraphy to diagnose sarcoidosis activity. Sensitivity ranges between 60-90%, with a low specificity of approximately 50%.[104-109] However, negative ^{67}Ga scintigraphy combined with normal ACE has a high negative predictive value.[51, 110]

^{67}Ga scintigraphy correlates with ACE values and clinically effective steroid therapy is associated with an improvement of ^{67}Ga scintigraphy and decrease in ACE.[51,107,111,112] Rizzato et al. assessed the predictive value of ^{67}Ga scintigraphy, chest radiography and ACE in 382 patients.[113] ^{67}Ga scintigraphy appeared to be more sensitive than chest radiography in detecting disease progression and improvement. Furthermore, uptake of ^{67}Ga citrate was suppressed by the use of corticosteroids but to a lesser extent than ACE.

The lambda and panda signs in ^{67}Ga scintigraphy are suggested to be a characteristic feature of sarcoidosis. Bilateral hilar activity combined with predominantly right sided active lymph nodes in the mediastinum represent the lambda sign. The panda sign is based on the symmetrical activity in the lacrimal and parotid glands. However, these signs have demonstrated a poor diagnostic sensitivity.[114-116] Furthermore, the panda sign can be found in patients with HIV, malignant lymphomas and Sjögren's syndrome as well.

In general, ^{67}Ga scintigraphy is performed with 185 MBq ^{67}Ga citrate, resulting in an effective radiation dose of 18.5 mSv.[117, 118]

3.4 ^{18}F-FDG PET

Fluorine-18 fluorodeoxyglucose positron emission tomography (^{18}F-FDG PET) is widely used in the imaging of malignant tumors. An increased glucose metabolism of malignant cells causes the accumulation of ^{18}F-FDG. Once ^{18}F-FDG is transported through the cell membrane into the cytosol, it is phosphorylated by hexokinase. Here, the ^{18}F-FDG is metabolically trapped as ^{18}F-FDG-6-phosphate. Glucose transporters (GLUT) across the cell membrane account for the transport of ^{18}F-FDG into the cell. In malignant cells, the expression of mainly GLUT-1 is responsible for ^{18}F-FDG accumulation.[119-122] Activated leukocytes express the GLUT-1 transporter as well.[122] Consequently, ^{18}F-FDG PET can be used in leukocyte mediated processes, like inflammatory lesions.

In 1994, the use of ^{18}F-FDG PET in sarcoidosis was first reported by Lewis and Salama.[123] Although autoradiographic studies of sarcoid lesions are missing, the accumulation of ^{18}F-FDG in macrophages and CD4^{+} T lymphocytes may explain the in vivo imaging of this granulomatous process.[124, 125]

Nishiyama et al. compared ^{18}F-FDG PET and ^{67}Ga scintigraphy retrospectively in 18 patients.[108] Sensitivity for the detection of thoracic disease was 100% for ^{18}F-FDG PET and 81% for ^{67}Ga scintigraphy while extra thoracic lesions were adequately observed in 90% and 48%, respectively. In a similar study, Prager et al. reported a significantly higher, overall detection rate for ^{18}F-FDG PET.[109] Thoracic disease was found in 96% of the patients. ^{67}Ga scintigraphy revealed thoracic abnormalities in 88%. Nineteen extra thoracic locations were found in ^{18}F-FDG PET and 12 in ^{67}Ga scintigraphy.

It might be suggested that a combined imaging modality of ^{18}F-FDG PET and CT is even more sensitive than PET alone. Braun et al. retrospectively evaluated ^{18}F-FDG PET/CT in 20 patients, both new and previously diagnosed sarcoidosis.[104] This technique correctly demonstrated 78% of the biopsy proven locations, with a 100% sensitivity for thoracic and sinonasal disease. Skin lesions were the main reason for the decreased overall sensitivity, probably explained by the skin thickness, causing difficulties in granuloma detection. In 12 patients, ^{67}Ga scintigraphy was previously

performed, with a correct detection of biopsy proven lesions in 58%. Sensitivity of thoracic and sinonasal disease was 71% and 75%, respectively.

These data suggest that ^{18}F-FDG PET is a sensitive technique for the detection of sarcoidosis. Sensitivity is higher than in ^{67}Ga scintigraphy, although these retrospective studies are performed with a small number of patients.

As described previously, ^{18}F-FDG presumably accumulates in different cell types involved in the inflammatory response of sarcoidosis. Methionine accumulates in cells with an increased amino acid metabolism.[126] Both tracers are increased in granulomatous lesions, although the degree of uptake varies. To predict outcome, uptake of ^{18}F-FDG was compared with ^{11}C-Methionine in 31 patients with stage I and stage II thoracic sarcoidosis.[127] Outcome was based on changes in chest radiography and clinical findings. The authors found that a predominant ^{18}F-FDG uptake was correlated with a favorable outcome, while a higher ^{11}C-Methionine uptake had a poorer prognosis.

Teirstein et al. retrospectively analyzed 188 ^{18}F-FDG PET/CT scans in 137 patients and demonstrated that this technique exhibits adequate sites for diagnostic biopsy. In 51 patients, ^{18}F-FDG PET/CT was repeated to evaluate the effect of corticosteroids. Overall, the improvement seen by ^{18}F-FDG PET/CT correlated well with changes in symptoms and clinical findings, although limited data have been provided.[128]

Radiation dose of ^{18}F-FDG PET is approximately 5.8 mSv for the first generation, standalone PET scanners. In the current PET/CT systems, the administered ^{18}F-FDG activity is reduced to an average of 150-200 MBq, resulting in an effective radiation dose of 2.9-3.8 mSv.[117] More accurate disease location can be achieved by the concurrently obtained CT, also performed for attenuation correction. This low dose CT adds approximately 2.9 mSv to the radiation dose.[129]

3.5 Somatostatin receptor scintigraphy

Somatostatin receptors are present in several cell types, for example activated macrophages.[130] There are 5 somatostatin receptor subtypes and in vitro autoradiography of histological biopsies of sarcoidosis revealed that the somatostatin receptor subtype 2 (sst_2) is expressed in epitheloid cells en giant cells.[131] Somatostatin receptor scintigraphy (SRS) is most frequently performed with Indium-111 (^{111}In) pentetreotide. Pentetreotide shows high affinity for the sst_2

receptor and might therefore be used in the imaging of sarcoidosis. In general, SRS is performed with 200 MBq ^{111}In-pentetreotide resulting in an effective radiation dose of 10.8 mSv.[132] Planar whole body images and SPECT of the chest are performed 24 hours after injection.

Kwekkeboom et al. performed SRS in 46 sarcoidosis patients and demonstrated active lesions in 97% of the disease locations imaged by conventional chest radiography.[133] Additional thoracic and extra thoracic lesions were found but several other extra thoracic lesions were not properly demonstrated. Lebtahi et al. compared SRS with ^{67}Ga imaging in 18 patients and found that SRS revealed significantly more sarcoidosis lesions at the clinically involved sites than ^{67}Ga imaging (82% and 64%, respectively).[134] Although SRS demonstrated additional extra thoracic sites that were not suspected clinically, still 40% of the known extra thoracic lesions were not diagnosed. SRS seems therefore sensitive in the assessment of active thoracic sarcoidosis but the presence of extra thoracic disease can be missed.

4. Outline of the thesis

In clinical practice, determining sarcoidosis activity is based on a combination of symptoms, clinical findings, chest radiography, serological and immunological markers as well as pulmonary function tests. These activity parameters are derivatives of the actual inflammatory process.
^{18}F-FDG PET is an in-vivo, non invasive imaging technique. It has obtained a crucial role in oncology and its application is still evolving. The aim of this thesis is to evaluate the utility of ^{18}F-FDG PET in the assessment of sarcoidosis activity. Therefore, ^{18}F-FDG PET is compared with the currently used markers of disease activity.

Chapter 2 describes the prospective analysis of ^{18}F-FDG PET and ^{67}Ga imaging. Sensitivity for thoracic and extra thoracic lesions is assessed and inter observer agreement is determined in newly diagnosed and histologically proven sarcoidosis patients.

In **Chapter 3**, ^{18}F-FDG PET is compared with genotype corrected ACE and sIL-2R, two serological markers that are widely used to assess disease activity and to monitor treatment efficacy. The degree of metabolic activity as reflected by SUV_{max} and SUV_{avg} is correlated with ACE and sIL-2R values, representatives of the granuloma load.

The invasive BAL procedure is often performed at the initial diagnosis. When disease recurrence is suspected, BAL is generally not repeated. To assess whether ^{18}F-FDG PET represents the different BAL cell profiles, metabolic activity is correlated with the different cell types. These results are presented in **Chapter 4**.

Chapter 5 describes the results of specific ^{18}F-FDG PET patterns in the pulmonary tract with regard to clinical outcome. The degree of metabolic activity in the lung parenchyma is correlated with the change in pulmonary function tests after one year follow-up.

In **Chapter 6**, the results of infliximab treatment monitored by ^{18}F-FDG PET are presented. Change in metabolic activity after 6 cycles of inflximab is compared with

changes in conventional markers like symptoms, ACE, sIL-2R, chest radiography and PFT.
Several phenotypic presentations are known in sarcoidosis, all correlating with a certain clinical outcome. ^{18}F-FDG PET might contribute to the accurate assessment of phenotypes. Categorizing ^{18}F-FDG PET into 4 major phenotypic presentations, based on the type and extent of organ involvement, is set out in **Chapter 7**.

Chapter 8 summarizes our results and provides concluding remarks.

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Chapter 2

Imaging the inflammatory activity of sarcoidosis: sensitivity and inter observer agreement of ^{67}Ga imaging and ^{18}F-FDG PET

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Abstract

Background

The aim of this study was to investigate the sensitivity of ^{67}Ga imaging and ^{18}F-FDG PET for sarcoidosis activity and their inter observer variability.

Methods

Thirty-four newly diagnosed, histologically proven sarcoidosis patients were analyzed prospectively. ^{67}Ga imaging and ^{18}F-FDG PET were performed, the presence of pulmonary and extra pulmonary lesions was evaluated and inter observer variability of both techniques was assessed.

Results

Overall sensitivity to detect active sarcoidosis was 88% for ^{67}Ga imaging and 97% for ^{18}F-FDG PET. Although these results were not significantly different, ^{18}F-FDG PET detected more lesions in the mediastinum ($p < 0.05$), hila ($p < 0.05$), lymph nodes ($p < 0.001$) and extra pulmonary regions in general ($p < 0.001$). Inter observer agreement was poor to moderate for ^{67}Ga imaging (kappa 0.19-0.59) and good to very good for ^{18}F-FDG PET (kappa 0.65-1.00).

Conclusion

^{18}F-FDG PET is more sensitive than ^{67}Ga imaging in the assessment of sarcoidosis activity with regard to the mediastinum, hila, lymph nodes and extra pulmonary lesions in general. Furthermore, ^{18}F-FDG PET demonstrates a very good inter observer agreement in contrast with ^{67}Ga imaging and ^{18}F-FDG PET is therefore the nuclear imaging technique of choice in sarcoidosis assessment.

Introduction

Sarcoidosis is a systemic granulomatous disease of unknown origin that primarily affects the lung. Diagnosis is based on the presence of noncaseating epitheloid cell granulomas in histological biopsy. In the pulmonary tract, sarcoidosis is particularly located in the parenchyma and in lymph nodes in the mediastinum and hila. Sarcoidosis of the lung parenchyma may lead to abnormal pulmonary function tests (PFT) and commonly causes symptoms like dyspnoea and coughing. However, the presence of pulmonary symptoms and an abnormal PFT do not always implicate active disease. Actually, defining and determining active sarcoidosis appears to be difficult and leads to extensive debate. In 1993, a consensus was reached and experts concluded that active disease should primarily be based on clinical findings, chest radiography and change of PFT.[1]

There is increasing interest in the role of molecular imaging techniques in assessing disease processes in terms of activity. Gallium-67 (^{67}Ga) imaging has been used for many years to demonstrate active sarcoidosis and shows good sensitivity, but due to its low specificity it is only of limited value in clinical practice.[2, 3] In the last few years, several case reports and studies have been published about the use of Fluor-18 deoxyglucose (^{18}F-FDG) PET in the assessment of sarcoidosis activity.[4-7] An excellent sensitivity was found, varying between 97% and 100%. Like other authors, we found remarkable differences in the uptake of ^{67}Ga citrate and ^{18}F-FDG in some sarcoidosis patients.[8] The aim of the current study was to measure sensitivity of both ^{67}Ga imaging and ^{18}F-FDG PET in the assessment of sarcoidosis activity and to evaluate the inter observer agreement for both techniques.

Given the absence of a single ‘gold standard’ for sarcoidosis activity, only newly diagnosed patients with histologically proven sarcoidosis were included, representing truly active disease.

Materials and Methods

Patients

In this prospective study, 34 consecutive patients with newly diagnosed sarcoidosis were included. Patients were recruited at the outpatient clinic of the St. Antonius Hospital between May 2003 and May 2006. The diagnosis of sarcoidosis was based on clinical findings, supported by histological evidence and after the exclusion of other known causes of granulomatosis in accordance with the consensus of the American Thoracic Society (ATS) / European Respiratory Society (ERS) / World Association of Sarcoidosis and other Granulomatous Disorders (WASOG) statement on sarcoidosis.[9] All patients were symptomatic and in all patients histology was obtained which confirmed the diagnosis sarcoidosis. The maximum time interval between histological confirmation and ^{67}Ga imaging and ^{18}F-FDG PET was less than 3 months. None of these patients used immunosuppressive drugs.

This study protocol was approved by the St. Antonius Hospital medical ethical committee.

^{67}Ga imaging

The day ^{18}F-FDG PET was performed, 185 MBq ^{67}Ga citrate was administered intravenously (Covidien, Petten, the Netherlands). Forty-eight hours later, whole body images were obtained, including single photon emission computed tomography (SPECT) of the chest. Acquisition was performed using an ADAC double-headed gamma camera with a 64 x 64 matrix and medium energy general purpose collimators (Philips Medical Systems, Eindhoven, the Netherlands). Three photopeaks were used; 93 keV, 185 keV and 300 keV with a window of 20%, 15% and 10%, respectively. Whole body imaging was carried out with a collimator speed of 8 cm per minute. In SPECT, we used 64 angles and 60 seconds per angle. SPECT was reconstructed with OSEM using 4 iterations and 8 subsets.

^{18}F-FDG PET

The patient fasted for at least six hours and before the intravenous injection of ^{18}F-FDG, 5 milligram of diazepam was administered to reduce muscle activity and accumulation of ^{18}F-FDG in brown fat. In order to reduce radiation exposure and accelerate ^{18}F-FDG excretion by the kidneys, 20 milligrams of furosemide was injected intravenously. Subsequently, 295-400 MBq ^{18}F-FDG (Covidien, Petten,

the Netherlands) was administered intravenously. 295 MBq ^{18}F-FDG was given to patients with a body weight less than 80 kilograms. When the body weight exceeded 80 kilograms a calculated dose was used (body weight /10 * 37 MBq) with a maximum of 400 MBq ^{18}F-FDG. PET was performed using the Philips Allegro PET system with external Cesium-137 source for transmission scanning (Philips Medical Systems, Eindhoven, the Netherlands). Sixty minutes after administration of ^{18}F-FDG, transmission scan was started. Transmission time was 23 seconds per bed position. Emission scan was performed from the subinguinal region to the head with an acquisition time of three minutes per bed position. Reconstruction was performed in accordance with the 3D-RAMLA protocol applying 4 iterations with a 144 x 144 matrix.[10]

Interpretation of ^{67}Ga imaging and ^{18}F-FDG PET

Two experienced nuclear medicine physicians independently interpreted both imaging modalities. First ^{67}Ga imaging was interpreted, two weeks later followed by ^{18}F-FDG PET with the patient list assessed in the opposite direction. In case of a disagreement between the observers, a third nuclear medicine physician interpreted the images leading to the final conclusion by majority of votes.

Disease activity was determined in the mediastinum, hila, lung parenchyma, extra pulmonary lymph nodes, spleen, liver, skeletal, parotid glands and muscles. These sites were scored either positive or negative (positive = increased metabolic activity, negative = no increased metabolic activity).

^{67}Ga imaging was analysed semi-quantitatively. Homogenous activity in the mediastinum was defined as normal. Hila and lung parenchyma were scored positive when the activity exceeded the mediastinal activity. When the mediastinal activity was focally increased, the mediastinum was scored positive. In case the activity in hila and lung parenchyma exceeded the normal background activity in the mediastinum, these organs were scored positive as well. Lymph nodes were involved when the activity in the lymphatic regions was focally increased. Parotid glands were scored positive when the activity was higher than the surrounding background.

^{18}F-FDG uptake was increased when the activity was higher than the background activity in the affected organ. In case of diffuse activity in the spleen, this was defined positive when it exceeded the activity of the liver.

Statistics

The statistical evaluation was performed using SPSS 16 (SPSS Inc, Chicago, IL, USA). Continuous data were expressed as the mean ± SD. Cohen's kappa was used to determine the inter observer agreement in ^{67}Ga imaging and ^{18}F-FDG PET at all different anatomical sites. The strength of agreement, expressed as kappa value, was subdivided into 5 categories: < 0.20 (poor), 0.21-40 (fair), 0.41-0.60 (moderate), 0.61-0.80 (good) and 0.81-1.0 (very good).[11] Chi-Square test and Fisher's Exact Test were used to determine statistical significance. For both tests, $p \leq 0.05$ was considered significant.

Results

Patients

Patient characteristics are summarized in Table 1. The time interval between the imaging techniques and the histological confirmation of sarcoidosis was 3.4 weeks (± 4.5 weeks).

Table 1 Patient characteristics

	n = 34
Age (years)	42.0 (± 9.9)
Sex	
Female	10
Male	24
Smoker	
Never	20
Ex-smoker	14
Chest radiographic stage	
0	3
I	9
II	18
III	2
IV	2
Serum markers	
ACE	61.7 (± 26.4)
sIL-2R	925 (± 671)

ACE = angiotensin converting enzyme (reference value 12-68 U/l), sIL-2R = soluble interleukin-2 receptor (reference value < 700 U/ml)

Sensitivity of ^{67}Ga imaging and ^{18}F-FDG PET

^{18}F-FDG PET was found positive in 33 out of 34 patients while ^{67}Ga imaging was positive in 33 out of 34 patients. This resulted in a sensitivity of 97% (95% CI 0.91-1.00) for ^{18}F-FDG PET and 88% (95% CI 0.77-0.99) for ^{67}Ga imaging (p = 0.61).

In ^{67}Ga imaging, 8 patients (24%) showed extra pulmonary lesions at 9 different sites, compared to 22 (65%) in ^{18}F-FDG PET in 27 different organs.^{18}F-FDG PET revealed significantly more lesions in the mediastinum (p = 0.02), hila (p = 0.01),

lymph nodes ($p < 0.001$) and extra pulmonary regions in general ($p < 0.001$). Table 2 illustrates the affected organs per imaging technique.

Table 2 ^{67}Ga imaging and ^{18}F-FDG PET results, *n* = 34

	^{67}Ga imaging	^{18}F-FDG PET
Pulmonary activity	30 (88%)	30 (88%)
Mediastinum	18 (53%)	27 (79%) *
Hila	21 (62%)	30 (88%) *
Lung parenchyma	22 (65%)	21 (62%)
Extra pulmonary activity	8 (24%)	22 (65%) **
Lymph nodes	4 (12%)	19 (56%) **
Spleen	0 (0%)	4 (12%)
Liver	0 (0%)	0(0%)
Skeletal	2 (6%)	1 (3%)
Parotid glands	2 (6%)	2 (6%)
Muscles	1 (3%)	1 (3%)

* $p < 0.05$; ** $p < 0.001$

Planar ^{67}Ga images detected hilar involvement in 18 patients and SPECT found increased activity in 21 patients ($p = 0.46$), while parenchymal activity was found in 20 and 19 patients, respectively ($p = 0.81$).

An example of ^{67}Ga imaging and ^{18}F-FDG PET is shown in Figure 1, illustrating the differences seen in these two techniques.

Of the 4 patients with a negative ^{67}Ga imaging, ^{18}F-FDG PET showed intra thoracic involvement in a variable extent. This pulmonary involvement was histologically confirmed. Two of these patients also showed extra pulmonary lymph nodes.

The one patient with a negative ^{18}F-FDG PET showed uptake of Ga^{67} citrate only in the lung parenchyma while histology was obtained from a peripheral lymph node.

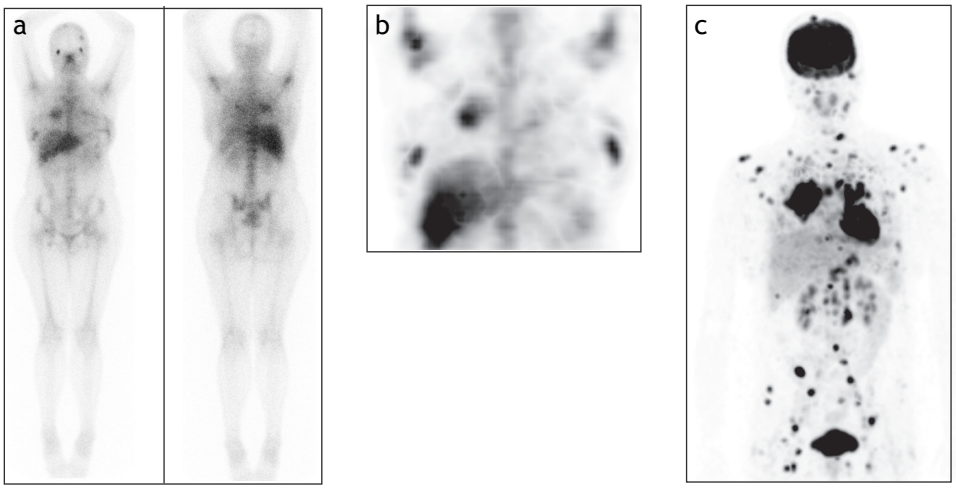

Figure 1 ^{67}Ga planar images (a) show uptake of ^{67}Ga citrate in the hila, lung parenchyma, skeletal and right inguinal region. SPECT of the thorax (b) only demonstrates uptake in the hila. The increased uptake in the mammae is considered to be physiologic. In the same patient, ^{18}F-FDG PET (c) shows lesions in the mediastinum, hila, lung parenchyma, skeletal and lymph nodes in cervical, axillary, para-aortic and inguinal regions. Histological confirmation of sarcoidosis was obtained from the right upper and middle lobe by video-assisted thoracoscopic surgery.

Inter observer agreement

The inter observer agreement, expressed as kappa values, is illustrated in Table 3. In ^{67}Ga imaging, kappa was 0.22 for overall disease, 0.59 for planar imaging of the hila, 0.34 for planar imaging of the lung parenchyma, 0.50 for SPECT of the mediastinum, 0.34 for SPECT of the hila, 0.19 for SPECT of the lung parenchyma and 0.43 for extra pulmonary disease activity.

In ^{18}F-FDG PET, kappa was 1.00 for overall disease, 0.65 for the assessment of the mediastinum, 0.84 for the hila, 0.69 for the lung parenchyma and 0.71 for extra pulmonary lesions.

Table 3 Positive imaging results per observer and the inter observer agreement

	Observer 1	Observer 2	Kappa [95% CI]
^{67}Ga imaging			
Overall disease	29 (85%)	32 (94%)	0.22 [0.00-0.63]
Planar hila	17 (50%)	24 (71%)	0.59 [0.24-0.78]
Planar lung parenchyma	18 (53%)	21 (62%)	0.34 [0.00-0.61]
SPECT mediastinum	14 (41%)	23 (68%)	0.50 [0.13-0.71]
SPECT hila	20 (59%)	28 (82%)	0.34 [0.00-0.59]
SPECT lung parenchyma	20 (59%)	23 (68%)	0.19 [0.00-0.48]
Extra pulmonary activity	6 (18%)	15 (44%)	0.43 [0.04-0.65]
^{18}F-FDG PET			
Overall disease	33 (97%)	33 (97%)	1.00 [0.22-1.00]
Mediastinum	25 (74%)	29 (85%)	0.65 [0.26-0.86]
Hila	30 (88%)	31 (91%)	0.84 [0.36-0.97]
Lung parenchyma	20 (59%)	21 (62%)	0.69 [0.38-0.87]
Extra pulmonary activity	17 (50%)	22 (65%)	0.71 [0.39-0.87]

CI = Confidence Interval

Discussion

In this prospective study, ^{67}Ga imaging and ^{18}F-FDG PET were compared and high sensitivity for disease activity was found for both techniques (88% and 97%, respectively). Due to the absence of a reliable test to determine the sarcoidosis activity state, we only included symptomatic, newly diagnosed and histologically proven sarcoidosis patients, representing truly active disease.
Previous studies comparing both methods found a sensitivity of 67-81% for ^{67}Ga imaging and 86-100% for ^{18}F-FDG PET.[4, 8] In both studies, small patient populations were retrospectively analysed and histological biopsy was used as the gold standard. Their studies were designed to illustrate the ability of imaging granulomas with ^{18}F-FDG PET.

In the current study, the sensitivity of ^{18}F-FDG PET to detect the overall presence of active sarcoidosis was higher than in ^{67}Ga imaging, although the difference was not statistically significant. However, ^{18}F-FDG PET revealed significantly more lesions in the mediastinum and hila when scored per anatomical region. In addition, significantly more extra pulmonary lesions were detected by ^{18}F-FDG PET (65%) than by ^{67}Ga imaging (24%), predominantly explained by the increased detection of lymph node involvement.
The results for ^{18}F-FDG PET found in our study correspond with previous reports showing the high value of this technique in the assessment of sarcoidosis.[4, 8] An increased detection of granulomas in thoracic lymph nodes and extra pulmonary regions by ^{18}F-FDG PET was previously reported, but a significant difference between ^{67}Ga imaging and ^{18}F-FDG PET in the detection of pulmonary or extra pulmonary involvement could not be confirmed.[8]
We hypothesize that the superiority of ^{18}F-FDG PET in detecting extra pulmonary lesions might be explained by the complete tomographic aspect, while in ^{67}Ga imaging, only SPECT of the chest is performed. Therefore, additional ^{67}Ga imaging studies are required including whole body SPECT, to compare the presence of extra pulmonary sarcoidosis lesions in ^{67}Ga imaging and ^{18}F-FDG PET.

Inter observer agreement was compared for both ^{67}Ga imaging and ^{18}F-FDG PET. In ^{67}Ga imaging, kappa ranged between 0.19 and 0.59 with the lowest values for

SPECT. Although SPECT images are considered to increase the diagnostic value of ^{67}Ga imaging, our results could not confirm this premise.
In ^{18}F-FDG PET, kappa ranged between 0.65 and 1.00. Our kappa values are similar with earlier studies comparing ^{67}Ga imaging and ^{18}F-FDG PET in non-Hodgkin's lymphoma patients. Zijlstra et al found kappa values of 0.53 for ^{67}Ga imaging and 0.98 for ^{18}F-FDG PET.[12] The highly variable inter observer results in ^{67}Ga imaging make its use limited in clinical practice and suggest the preference of ^{18}F-FDG PET in both sarcoidosis and non-Hodgkin's lymphoma.

Besides the abovementioned advantages of ^{18}F-FDG PET, a few other aspects need to be discussed. In ^{67}Ga imaging, acquisition starts only two days after the administration of the radiopharmaceutical, which entails an additional hospital visit. ^{18}F-FDG PET on the other hand, is performed within two hours.
The radiation dose is significantly higher for ^{67}Ga imaging than for ^{18}F-FDG PET. In ^{67}Ga imaging, the radiation dose is 18.5 mSv compared with 5.6-7.6 mSv for ^{18}F-FDG PET,[13] depending on the patients body weight. In the new generation PET cameras based on 'time of flight', the dosage of ^{18}F-FDG can be reduced, resulting in an even lower radiation dose.
In this study, a standalone ^{18}F-FDG PET was used although combined ^{18}F-FDG PET/CT has become available. This hybrid imaging technique offers functional as well as anatomical information. Furthermore, ^{18}F-FDG PET is able to quantify its abnormalities, in contrast with ^{67}Ga imaging. Quantifying sarcoidosis lesions might be of great importance since it reflects disease extent. Furthermore, quantification might even be useful in therapy monitoring.
However, ^{18}F-FDG PET is more expensive than ^{67}Ga imaging. Although the above mentioned aspects and the findings of the current study are in favour of ^{18}F-FDG PET, cost/benefit analysis are necessary to justify the use of ^{18}F-FDG PET in future sarcoidosis assessment.

As mentioned before, the assessment of sarcoidosis activity is hampered by the absence of a reliable gold standard. Nowadays, the presence of active disease is based on a combination of clinical, serological, functional and radiological parameters. However, each parameter has its limitation.[1]
Our initial study protocol included an evaluation of the disease activity parameters in a larger patient population. The population consisted of patients with both new

and previously diagnosed sarcoidosis assuming that patients with inactive disease were represented as well. In that manner, both sensitivity and specificity could be determined for ^{67}Ga imaging and ^{18}F-FDG PET.
Disease activity of each patient was assessed by blinded sarcoidosis experts, including activity parameters during follow-up when available. However, in only 29% of the patients an agreement was reached about the state of disease activity, resulting in a fair inter observer agreement. Consequently, the initial gold standard could not be used in the present study. For that reason, only patients with recent onset of sarcoidosis were included representing active disease.
The disagreement between the pulmonologists reflects the difficulties in determining sarcoidosis activity but may also raise doubts about the present 'guideline' regarding sarcoidosis assessment. It seems clear that a reliable gold standard is required to determine sarcoidosis activity in which we believe, ^{18}F-FDG PET might play an important role.

In conclusion, ^{18}F-FDG PET is more sensitive in the assessment of sarcoidosis activity than ^{67}Ga imaging with regard to the mediastinum, hila, lymph node involvement and extra pulmonary lesions in general. In contrast with ^{67}Ga imaging, the inter observer agreement of ^{18}F-FDG PET is very good. These results advocate the use of ^{18}F-FDG PET in future assessment of sarcoidosis activity.

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Chapter 3

^{18}F-FDG PET, genotype-corrected ACE and sIL-2R in newly diagnosed sarcoidosis

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Abstract

Introduction

Angiotensin-converting enzyme (ACE) and soluble interleukin-2 receptor (sIL-2R) are serological markers, widely used for determining sarcoidosis activity. ^{18}F-FDG PET has proven to be a sensitive technique in the imaging of sarcoidosis. The aim of this study was to determine sensitivity of ^{18}F-FDG PET, genotype corrected ACE and sIL-2R in active sarcoidosis as well as their correlation.

Methods

This retrospective study included 36 newly diagnosed, symptomatic sarcoidosis patients. ACE and sIL-2R levels were simultaneously obtained within 4 weeks of ^{18}F-FDG PET. ACE was corrected for genotype and expressed as Z-score. ^{18}F-FDG PET was visually evaluated and scored as positive or negative. Maximum and average Standardized Uptake Value (SUV_{max} and SUV_{avg}) were compared with ACE and sIL-2R.

Results

^{18}F-FDG PET was found positive in 34 of 36 patients (94%). Thirteen patients (36%) showed an increased ACE with the highest sensitivity found in patients with the I/I genotype (67%). Seventeen patients (47%) showed an increased sIL-2R. No correlation was found between SUV and ACE or sIL-2R. Increased ACE and sIL-2R correlated with a positive ^{18}F-FDG PET in 12 patients (92%) and 16 patients (94%), respectively.

Conclusion

^{18}F-FDG PET is a very sensitive technique to assess active sarcoidosis, in contrast with ACE and sIL-2R, suggesting a pivotal role for ^{18}F-FDG PET in future sarcoidosis assessment.

Introduction

Sarcoidosis remains an intriguing disease despite decades of research. First, its etiology is still unknown. The current hypothesis is that environmental factors trigger an immunological response which results in granuloma formation.[1] Although several agents have been thought to elicit this immunological response, there is no indisputable evidence for a specific environmental factor or gene.[2] Second, sarcoidosis is indefinite in terms of 'disease activity'. Symptoms and objective parameters may differ in reflecting the level of disease activity. Conventional serum markers for sarcoidosis activity like angiotensin converting enzyme (ACE) and soluble interleukin-2 receptor (sIL-2R) may be increased while the patient does not have any symptom or functional impairment. Other patients may have severe and disabling symptoms while the increase in ACE or sIL-2R remains absent. ACE is produced by the granuloma itself,[3] while sIL-2R is mainly released by activated T cells.[4] Both parameters are considered to reflect the granulomatous inflammation. ACE has been studied extensively over the last 30 years and sensitivity varied widely between 34-77%.[5-8] Discovering the insertion/deletion polymorphism of the ACE gene in the early 1990s,[9] was thought to increase sensitivity of ACE in sarcoidosis.[10] Sensitivity indeed improved for the diagnosis of sarcoidosis [11, 12] and in newly diagnosed and therefore active sarcoidosis, sensitivity was even 83%.[13] Although this was at the expense of the already low specificity, ACE is widely used in the assessment and follow-up of sarcoidosis.[14]

sIL-2R has been shown to be an accurate parameter in the assessment of pulmonary sarcoidosis [15] and correlates with active disease,[16] although it is still not recommended as an activity marker.[17] Actually, there is still no gold standard to define disease activity in sarcoidosis which hampers clinicians involved in sarcoidosis management. Without a gold standard, scientific research will be impeded to develop new, reliable activity markers. Molecular imaging by ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET might offer such a promising technique in determining sarcoidosis activity. We hypothesized that ^{18}F-FDG PET is currently the best available technique to assess sarcoidosis activity. Therefore, the aim of this study was to determine the sensitivity of ^{18}F-FDG PET, genotype-corrected ACE and sIL-2R together with their correlation in patients with active sarcoidosis.

Materials and methods

Patients

This retrospective study included 36 consecutive patients with newly diagnosed sarcoidosis. Patients were recruited at the outpatient clinic of the St. Antonius Hospital between February 2004 and August 2007. The diagnosis of sarcoidosis was based on clinical findings, supported by histological evidence and after the exclusion of other known causes of granulomatosis in accordance with the consensus statement on sarcoidosis of the American Thoracic Society (ATS) / European Respiratory Society (ERS) / World Association of Sarcoidosis and other Granulomatous Disorders (WASOG).[17] All patients were symptomatic and in all patients histology results were obtained which confirmed the diagnosis sarcoidosis. The maximum time interval between histological confirmation and ^{18}F-FDG PET was less than 3 months. None of these patients used immunosuppressive drugs or ACE inhibitors.
This study was approved by the local Medical Ethics Committee.

^{18}F-FDG PET

The patient fasted for at least 6 h and before the intravenous injection of ^{18}F-FDG, 5 mg of diazepam was administered to reduce muscle activity and accumulation of ^{18}F-FDG in brown fat. In order to reduce radiation exposure and accelerate ^{18}F-FDG excretion by the kidneys, 20 mg of furosemide was injected intravenously. Subsequently, 295-400 MBq ^{18}F-FDG (Covidien, Petten, the Netherlands) was administered intravenously; 295 MBq ^{18}F-FDG was given to patients with a body weight less than 80 kilograms. When the body weight exceeded 80 kilograms a calculated dose was used (body weight /10 * 37 MBq) with a maximum of 400 MBq ^{18}F-FDG. PET was performed using the Philips Allegro PET system with external ^{137}Cs source for transmission scanning (Philips Medical Systems, Eindhoven, the Netherlands). The transmission scan was started 60 minutes after administration of ^{18}F-FDG. Transmission time was 23 seconds per bed position. The emission scan was performed from the subinguinal region to the head with an acquisition time of 3 minutes per bed position. Reconstruction was performed in accordance with the 3D-RAMLA protocol applying 4 iterations with a 144 x 144 matrix.[18]
Two experienced nuclear medicine physicians independently interpreted ^{18}F-FDG

PET. In the case of a disagreement, a third nuclear medicine physician scored the ^{18}F-FDG PET. Disease activity was determined in the mediastinum, hila, lung parenchyma, extra pulmonary lymph nodes, liver, spleen, skeleton, parotid glands and muscles. These sites were scored either as positive or negative (positive = increased ^{18}F-FDG uptake compared to the background in the affected organ, negative = no increased ^{18}F-FDG uptake). The final PET result was defined as 'positive' or 'negative'. Maximum and average standardized uptake value (SUV_{max} and SUV_{avg}) were measured in the organs scored 'positive' by drawing a region of interest (ROI). ROIs were drawn automatically by using the 'quick ROI' button in the visually affected part of the organ. The maximum SUV_{max} and SUV_{avg} were correlated to ACE and sIL-2R.

ACE and sIL-2R

ACE and sIL-2R were measured within 4 weeks of ^{18}F-FDG PET. Blood samples to determine ACE and sIL-2R level were simultaneously obtained. Quantification of ACE activity was measured in lithium heparin plasma using the Bühlmann ACE kinetic test, in accordance with previously described methods (Siemens Medical Solutions Diagnostics, Breda, the Netherlands).[19, 20]

Serum ACE is influenced by an insertion (I)/deletion (D) ACE gene polymorphism.[9] The deletion variant is associated with higher serum ACE values. As a result, ACE values will be higher in patients with the D/D genotype, followed by the I/D and I/I genotype, respectively.

ACE I/D polymorphisms were determined by real-time polymerase chain reaction (PCR) using fluorescent hybridization probes and a LightCycler (Roche Diagnostics, Almere, the Netherlands). ACE level was considered positive or negative in accordance with genotype-corrected reference values. The reference value for I/I was 9-43 U/l, for I/D 14-62 U/l and for D/D 24-82 U/l. A for genotype-corrected ACE was calculated, expressed as Z-score.[21]

Serum sIL-2R was quantitatively determined using enzyme linked immunosorbent assay (Emelca Bioscience, Breda, the Netherlands). Serum sIL-2R above 700 U/ml was considered increased.

Statistical analysis

The Z-score for ACE was calculated as (ACE patient - mean ACE reference group) / SD reference group, where mean ACE reference group and SD reference group were

calculated from the ACE values measured in either the I/I, I/D or D/D reference group. If Z was greater than +1.96 above the mean, the ACE value was considered positive.[21] Data were expressed as mean values (± SD). The analysis of variance (ANOVA) test was used to compare ACE Z-scores between the different genotypes. Kruskall Wallis test was used to compare SUV_{max} and SUV_{avg} between the different genotypes. Correlations between serum parameters and SUV_{max} and SUV_{avg} were determined using Spearman's rank. The statistical evaluation was performed using SPSS 15 (SPSS Inc., Chicago, IL, USA).

Results

Patients

Patients' characteristics are summarized in Table 1. The average time interval between ^{18}F-FDG PET and the histological confirmation of sarcoidosis was 3.8 weeks (± 3.8 weeks).
None of these patients were diagnosed with Löfgren's syndrome.

Table 1 Patient characteristics

	n = 36
Age (years)	39 (range: 23–71)
Sex	
Female	20
Male	16
Smoker	
Never	16
Ex-smoker	16
Current smoker	4
Chest radiographic stage	
0	1
I	18
II	11
III	5
IV	1

^{18}F-FDG PET

The median time interval between ^{18}F-FDG PET and ACE and sIL-2R measurements was 1.5 weeks (± 1.2 weeks). The average blood glucose level before the administration of ^{18}F-FDG was 4.9 (± 0.8). There were 34 patients (94%) with a positive ^{18}F-FDG PET and 32 of these patients had pulmonary involvement. Of the 32 patients with pulmonary involvement, extra pulmonary lesions were seen in 19 patients. Two patients had extra-pulmonary lesions without pulmonary involvement. Thus, a total of 21 patients (58%) had extra-pulmonary involvement. The average SUV_{max} and SUV_{avg} in patients with an abnormal ^{18}F-FDG PET was 9.0 (± 7.4) and 5.6 (± 4.0), respectively.

ACE and sIL-2R

Positive ACE was seen in 13 patients (36%) and positive sIL-2R in 17 patients (47%). ACE and sIL-2R results are illustrated in Figure 1.

The D/D genotype was seen in 11 patients, the I/D genotype in 16 patients and the I/I genotype in 9 patients. An increased ACE was found in 3 patients (27%) with the D/D genotype, 4 patients (25%) with the I/D genotype and 6 patients (67%) with the I/I genotype. The average ACE Z-score was 2.07 (± 3.04). The Z-score was 0.88 (± 1.33) for the D/D genotype, 2.01 (± 3.39) for the I/D genotype and 3.63 (± 3.49) for the I/I genotype. There was no statistically significant difference in the Z-scores between the different ACE genotypes (p = 0.13). The average sIL-2R level was 1170 (± 964) U/ml. Per ACE genotype, no significant difference in sIL-2R value (p = 0.28) was observed. There was a moderate but significant correlation between ACE and sIL-2R (r = 0.42, p = 0.01).

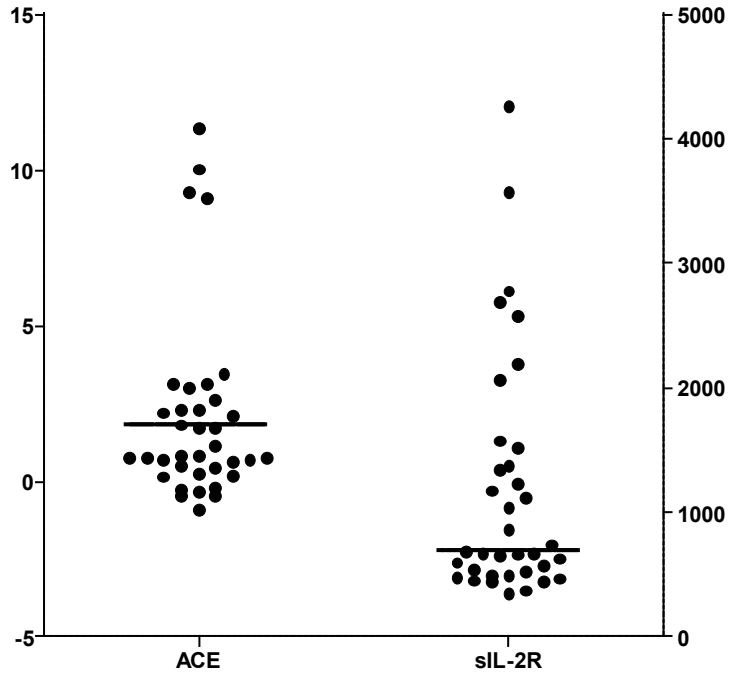

Figure 1 ACE and sIL-2R in 36 newly diagnosed sarcoidosis patients.
The lines indicate the upper reference value which is 1.96 for ACE, expressed as Z-score, and 700 U/ml for sIL-2R.

Correlation between ^{18}F-FDG PET, ACE and sIL-2R

Of the 34 patients with a positive ^{18}F-FDG PET result, 12 patients showed an increased ACE (35%) and 16 patients showed an increased sIL-2R (47%). Fourteen patients had a negative ACE as well as sIL-2R (41%).

There was a non-significant, negative correlation between SUV_{max} and ACE (r = -0.21, p = 0.2). The average SUV_{max} was higher in the D/D genotype group (9.9 ± 8.3) followed by the I/D genotype (8.9 ± 8.8) and the I/I genotype (8.1 ± 3.9), although not significantly different (p = 0.64). The correlation between SUV_{max} and sIL-2R was not significant either (r = 0.15, p = 0.4). SUV_{avg} did not correlate significantly with ACE (r = -0.26, p = 0.12) or sIL-2R (r = 0.16, p = 0.37). SUV_{avg} was higher in the D/D genotype group (6.4 ± 5.0) followed by the I/I genotype (5.3 ± 2.7) and the I/D genotype (5.2 ± 4.0), but not significantly different between groups (p = 0.67). The results of SUV_{max} and SUV_{avg} per ACE genotype are demonstrated in Figure 2.

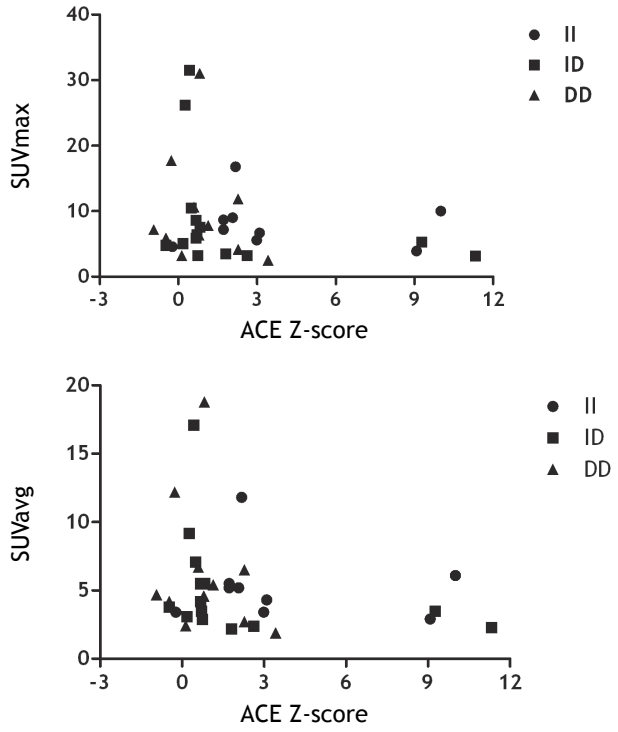

Figure 2 Scatter plot of SUV_{max} (a) and SUV_{avg} (b) per ACE genotype. There was a negative, non-significant correlation between SUV and ACE (r = -0.21, p = 0.2 for SUV_{max} and r = -0.26, p = 0.12 for SUV_{avg}).

The number of affected organs was evaluated in the 22 patients with positive ^{18}F-FDG PET and negative ACE. In 20 patients (91%), the mediastinum showed increased metabolic activity, in 21 (96%) the hila, in 12 (55%) the lung parenchyma, in 11 (50%) extra pulmonary lymphnodes, in 4 (18%) the spleen and in 2 (9%) the parotid glands.

In the 18 patients with positive ^{18}F-FDG PET and negative sIL-2R, this was evaluated as well. In 16 patients (89%), the mediastinum showed increased metabolic activity, in 17 (94%) the hila, in 12 (67%) the lung parenchyma, in 6 (33%) extra pulmonary lymphnodes, in 3 (17%) the spleen and in 1 (6%) the parotid glands. In both groups, the skeleton, muscles and liver were not affected. Figure 3 shows ^{18}F-FDG PET of two patients without increased serum parameters.

Of the 2 patients with a negative ^{18}F-FDG PET, one showed a positive ACE and sIL-2R and one a negative ACE and sIL-2R. CT scan of the chest of the patient with positive serological markers showed diffuse groundglass, bronchiectasies and a few, calcified lymphnodes in the mediastinum. The patient with negative serological markers showed multiple, intrapulmonary nodules on CT scan with large lymphnodes in the right hilum and mediastinum.

Of 13 patients with an increased ACE, 12 showed a positive ^{18}F-FDG PET (92%). Of 17 patients with an increased sIL-2R, 16 showed a positive ^{18}F-FDG PET (94%).

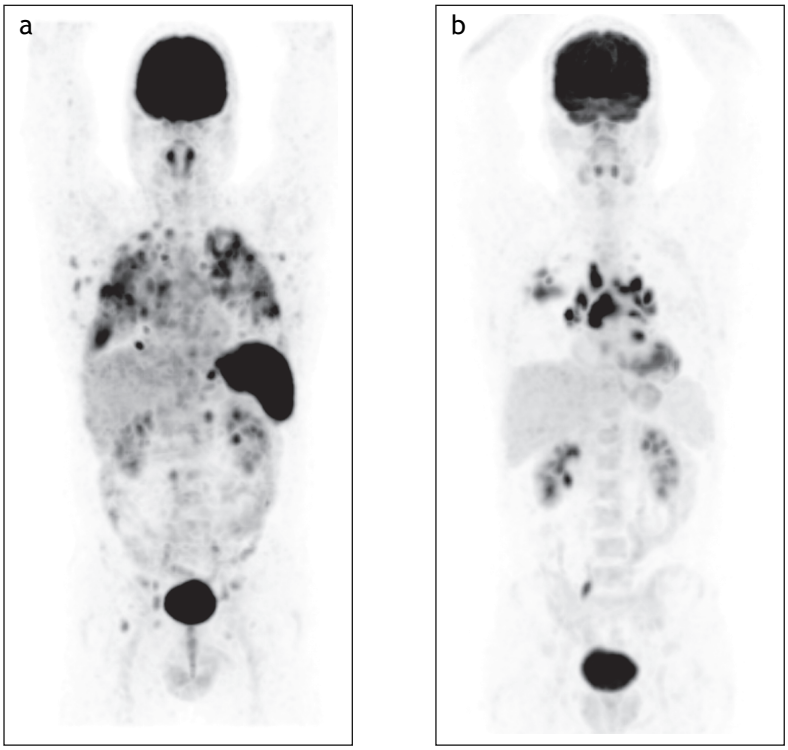

Figure 3 ^{18}F-FDG PET (a) shows increased metabolic activity in the hila, lung parenchyma, extra pulmonary lymph nodes and spleen. Seven weeks before ^{18}F-FDG PET was performed, histological proof of sarcoidosis was obtained from the right lung. ACE was 48 U/l with the I/D genotype (normal 14-62 U/l) and sIL-2R was 657 U/ml (normal < 700 U/ml).
A completely different ^{18}F-FDG PET pattern is seen in the right patient (b) with increased activity in the mediastinum, hila and focally in the right lung parenchyma. Three weeks after ^{18}F-FDG PET was performed, histological results were obtained from lymph node number 7 confirming sarcoidosis. ACE was 46 U/l with the I/D genotype and sIL-2R was 571 U/ml.

Discussion

The current study shows that ^{18}F-FDG PET is a very sensitive technique (94%) in demonstrating active disease, unlike genotype-corrected ACE (36%) and sIL-2R (47%). Due to the absence of an unfailing test to determine sarcoidosis activity state, we only included newly diagnosed, symptomatic and histologically proven sarcoidosis patients, representing truly active disease. Specificity of ^{18}F-FDG PET in sarcoidosis could therefore not be determined although it is known that ^{18}F-FDG is a non-specific marker. In fact, ^{18}F-FDG PET of sarcoidosis patients might suggest other inflammatory or even malignant processes. ACE and sIL-2R are non-specific parameters as well, since increased values can be found in several other diseases.[6, 22, 23]

The high sensitivity of ^{18}F-FDG PET in our study corresponds with previous reports showing a sensitivity ranging between 87-100%.[24-26] Like these studies, we determined sensitivity of ^{18}F-FDG PET in newly diagnosed sarcoidosis. In addition, we simultaneously measured sensitivity of the most widely used serological activity parameter together with sIL-2R. However, the found sensitivity of genotype-corrected ACE to diagnose active sarcoidosis could not confirm the sensitivity of 83% reported by Tomita et. al. although we did find the highest sensitivity to be in the I/I genotype group.[13] Furthermore, our results were not even close to the sensitivity of 69-70% reported in two studies evaluating the sensitivity of ACE in the diagnosis of this disease.[11, 12] In these two studies, patients were previously diagnosed with sarcoidosis and given the longer disease duration, part of these patients might perhaps have had inactive disease. We could not confirm the earlier reported sensitivity of sIL-2R of approximately 70% either.[15, 27]

The uptake of ^{18}F-FDG in sarcoidosis has not been elucidated yet. It is known that ^{18}F-FDG accumulates in activated macrophages in tumor processes.[28] Activated macrophages are an important element of the granuloma in sarcoidosis as well. Together with macrophages, these granulomas contain epitheloid cells.[29] In sarcoidosis, epitheloid cells secrete ACE and the serum ACE levels are therefore thought to represent the total granuloma load in the body.[30] Besides ACE, sIL-2R is important in determining the presence of active disease. sIL-2R derives from activated T cells although macrophages do have an IL-2R as well.[31] Both cell types

are important in the formation of granulomas. Consequently, sIL-2R can be used to estimate the total inflammatory activity. Considering macrophages to be the main target cell of ^{18}F-FDG in sarcoidosis, it is surprising that ^{18}F-FDG PET, ACE and sIL-2R, as representatives of active disease, show such different sensitivity. Furthermore, no correlation was found between the degree of metabolic activity, expressed by SUV_{max} or SUV_{avg}, and ACE or sIL-2R. Autoradiographic studies are necessary to support our hypothesis that ^{18}F-FDG is actually incorporated in macrophages or whether other cell types play a role.
There was a significant correlation between ACE and sIL-2R while sensitivity of both markers in determining active sarcoidosis was low. Our findings could be confirmed by previous reported results,[32] although others did not find a correlation between ACE and sIL-2R values.[16]

The overall assumption that ACE and sIL-2R represent the total granuloma load, *i.e.* the total load of active disease, suggests a correlation between these two parameters and the extent of metabolic activity. Metabolic activity depicted by ^{18}F-FDG PET can be quantified by calculating SUV_{max}. However, such correlation could not be found in the current study. This might be explained by the use of SUV_{max}, measuring the maximum activity in only one pixel. To overcome this problem, SUV_{avg} was determined, but no correlation was found between SUV_{avg} and the two serological parameters either.
Since ACE and sIL-2R represent the total mass of sarcoidosis, it would be of great interest to determine the total volume of sarcoidosis lesions imaged by ^{18}F-FDG PET. However, to date, reliable volumetric measurements with ^{18}F-FDG PET in sarcoidosis patients are not available. Combined ^{18}F-FDG PET/CT, on the other hand, might offer the possibility of volumetric measurements. Future studies are necessary to assess the role of this combined modality in functional and anatomical imaging of sarcoidosis.

The current study is of importance since ACE is still widely used in the assessment and follow-up of sarcoidosis. In 1999, the ATS and ERS adopted the joint statement on sarcoidosis in which ACE is suggested to be an appropriate marker to determine sarcoidosis activity.[17] Our study illustrates that, even after genotype-correction, ACE is not a reliable activity marker. ACE as well as sIL-2R should be interpreted with care, as a negative test does not exclude active disease. Unlike ACE and sIL-

2R, ^{18}F-FDG PET will contribute to an improved detection of disease activity. Given the low sensitivity of ACE and sIL-2R, an additional ^{18}F-FDG PET can be considered in those patients with normal serum markers, but still suspected of active disease. In conclusion, unlike ^{18}F-FDG PET, genotype-corrected ACE and sIL-2R have a low sensitivity in the detection of active sarcoidosis. Positive ACE and sIL-2R correlate well with ^{18}F-FDG PET, suggesting that ^{18}F-FDG PET might be omitted when these serum markers are elevated. However, given the low test performance of ACE and sIL-2R, the future role of ^{18}F-FDG PET in sarcoidosis assessment seems to be significant.

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Chapter 4

^{18}F-FDG PET patterns and BAL cell profiles in pulmonary sarcoidosis

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Abstract

Purpose

Bronchoalveolar lavage (BAL) and ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET can both demonstrate sarcoid activity. To assess whether metabolic activity imaged by ^{18}F-FDG PET represents signs of disease activity as reflected by BAL, ^{18}F-FDG PET patterns were compared with BAL cell profiles.

Methods

In this retrospective analysis, 77 newly diagnosed, pulmonary sarcoidosis patients underwent BAL and ^{18}F-FDG PET. Based on ^{18}F-FDG PET, patients were diagnosed with exclusively mediastinal/hilar activity (group A) and activity in the lung parenchyma (group B). Per group, BAL lymphocytes (%), $CD4^+/CD8^+$ ratio, $CD103^+CD4^+/CD4^+$ ratio and neutrophils (%) were compared with the extent of metabolic activity expressed as the maximum standardized uptake value (SUV_{max}). Additionally, SUV_{max} and BAL parameters per radiographic stage were analyzed.

Results

Overall, the SUV_{max} in the lung parenchyma correlated with neutrophils and SUV_{max} of the mediastinum/hila correlated with the $CD4^+/CD8^+$ ratio. In both groups, a significant, negative correlation between the SUV_{max} of the mediastinum/hila and $CD103^+CD4^+/CD4^+$ ratio was found. In group B, the SUV_{max} of the mediastinum/hila correlated with the $CD4^+/CD8^+$ ratio, while SUV_{max} in the lung parenchyma correlated with the $CD103^+CD4^+/CD4^+$ ratio and neutrophils. Significant differences were found in the SUV_{max}, $CD4^+/CD8^+$ ratio, $CD103^+CD4^+/CD4^+$ ratio and neutrophils between the radiographic stages. The SUV_{max} of the lung parenchyma was positively related to the radiographic stage, while SUV_{max} of the mediastinum/hila and $CD4^+/CD8^+$ ratio were inversed related.

Conclusion

^{18}F-FDG PET correlates with the $CD4^+/CD8^+$ ratio and neutrophils, suggesting that ^{18}F-FDG PET represents this specific cell profile in BAL. High SUV_{max} values of the lung parenchyma may therefore correlate with more severe parenchymal involvement, particularly when accompanied by a low SUV_{max} of the mediastinum/hila.

Introduction

Sarcoidosis is an inflammatory disorder that can affect several organs although the lung is most frequently involved.[1] The characteristic epitheloid cell granulomas can be found in the mediastinum and hila, with or without involvement of the lung parenchyma. These different forms of sarcoidosis in the lung are reflected in the chest radiographic stages according to Scadding.[2] Stage 0 is defined as normal, stage I disease as bihilar lymphadenopathy, stage II as bihilar lymphadenopathy combined with parenchymal involvement, stage III as exclusive parenchymal involvement and stage IV as fibrosis.

In the last decade, several studies have shown that ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET is a useful molecular imaging technique in sarcoidosis to depict disease activity.[3-5] In accordance with the chest radiographic stages, ^{18}F-FDG PET might demonstrate increased metabolic activity at different intra-thoracic sites. This increased metabolic activity is considered to reflect active sarcoidosis lesions.

Bronchoalveolar lavage (BAL) is frequently performed in sarcoidosis and specific cell types reflect active disease as well. Lymphocytosis in BAL fluid represents an alveolitis in which the $CD4^+/CD8^+$ ratio might be increased. This lymphocytosis is sensitive for sarcoidosis while an increased $CD4^+/CD8^+$ ratio is not sensitive but highly specific.[6] Although a high lymphocyte count in BAL and an increased $CD4^+/CD8^+$ ratio cannot predict the clinical outcome,[7,8] increased neutrophils are related to future pulmonary deterioration in terms of fibrosis.[8, 9] In patients with stage IV disease, characterized by fibrosis, it is known that the number of neutrophils is higher than in patients with lower stages.[9]

Since $CD4^+$ T cells are not sensitive to diagnose sarcoidosis, CD103, a subset of $CD4^+$ T cells, was thought to be more discriminative. CD103 ($\alpha^E\beta7$ integrin) can be found on $CD4^+$ T lymphocytes in the epithelium of bronchi and bronchial glands. In contrast with other interstitial lung diseases, a low ratio of $CD4^+$ T cells expressing CD103 ($CD103^+CD4^+/CD4^+$) is found in sarcoidosis.[10, 11] This low $CD103^+CD4^+/CD4^+$ ratio might be explained by a recruitment of $CD4^+$ T cells from the blood instead of the epithelium, explaining the relatively low $CD4^+/CD8^+$ ratio in the blood.

Since BAL and ^{18}F-FDG PET both have the potential to reflect sarcoidosis activity, ^{18}F-FDG PET patterns might be able to represent the different BAL cell profiles. We hypothesized that the number of neutrophils in BAL correlates with the extent of

metabolic activity in the lung parenchyma and that the number of lymphocytes and $CD4^+/CD8^+$ ratio correlate with the metabolic activity in lymph nodes localized in the mediastinum/hila. In addition, the $CD103^+$ subset of $CD4^+$cells T cells, defined as $CD103^+CD4^+/CD4^+$ ratio, might be low in the current population, consisting of patients with newly diagnosed, histologically proven, pulmonary sarcoidosis.

If BAL and ^{18}F-FDG PET are both capable of demonstrating sarcoidosis activity, the non-invasive ^{18}F-FDG PET might be a valuable alternative for the invasive BAL procedure in the future. Therefore, the aim of this study was to correlate BAL cell profiles with the different intra-thoracic patterns of ^{18}F-FDG PET. In addition, the extent of pulmonary metabolic activity and BAL cell types per radiographic stage were analyzed.

Materials and methods

Patients

This retrospective study included 77 consecutive patients with newly diagnosed and histologically proven pulmonary sarcoidosis. The patients were seen at the Department of Pulmonology of the St. Antonius Hospital between February 2004 and January 2009. The patients underwent BAL as well as ^{18}F-FDG PET as part of their routine analysis, while none of them used immunosuppressive treatment. The smoking status of all patients was evaluated. The diagnosis of sarcoidosis was based on clinical findings, supported by histological evidence and after the exclusion of other known causes of granulomatosis in accordance with the consensus statement on sarcoidosis of the American Thoracic Society (ATS) / European Respiratory Society (ERS) / World Association of Sarcoidosis and other Granulomatous Disorders (WASOG).[12] Radiographic stage was determined based upon conventional chest radiography according to Scadding.[2] Stage 0 was defined as normal, stage I as bihilar lymphadenopathy, stage II as bihilar lymphadenopathy combined with parenchymal infiltrates, stage III as exclusive parenchymal infiltrates and stage IV as fibrosis. BAL parameters and the maximum standardized uptake value (SUV_{max}) were analyzed per radiographic stage. Serum angiotensin/ converting enzyme (ACE) was determined and evaluated per radiographic stage and per ^{18}F-FDG PET group. The ACE level was considered positive or negative in accordance with genotype-corrected reference values. A for genotype corrected ACE was calculated, expressed as the Z-score.[13]
This study was approved by the local Medical Ethics Committee.

Bronchoalveolar lavage

BAL was performed during flexible fiberoptic bronchoscopy at the time of the diagnosis according to a standardized and validated procedure.[14] The procedure involved detailed explanation to the patient and local anaesthesia of the larynx and lower airways (0.5 % tetracaine in the oropharynx, 8 cc 0.5% tetracaine in lower airways). If necessary, 100% oxygen was delivered via a nasal cannula (2 l/min) during the procedure, whilst transcutaneous oxygen saturation was continuously monitored by an oximeter with a finger probe. BAL was performed, preferably in the right middle lobe, with four 50-ml aliquots of sterile isotonic saline solution

(37 ºC). The aspirated lavage fluid from the first 50-ml aliquot was kept apart and excluded from further analysis. The BAL fluid recovered from the three subsequent aliquots was collected in a siliconized specimen trap and kept on ice. BAL fluid was filtered through nylon gauze and centrifuged (10 min at 400 *g* at 4 ºC).

The cell pellet was washed twice, counted and resuspended in minimal essential medium/RPMI 1640 (Gibco, Grand Island, NY, USA), supplemented with 0.5 % bovine serum albumin (Organon Teknika, Boxtel, the Netherlands). Cells were counted in a Bürker chamber. Cell viability was determined by Trypan blue exclusion. Smears for cell differentiation were prepared by cytocentrifugation (Shandon, Runcorn, UK). Cell differentiation was performed on a cytospin slide after staining with May-Grünwald-Giemsa (Merck, Darmstadt, Germany), and at least 2 x 500 cells were counted.

The percentage of BAL fluid recovery needed to be at least 30%. Lymphocytosis was defined as lymphocytes > 15%.[15] $CD4^+/CD8^+$ ratio > 3.5, $CD103^+CD4^+/CD4^+$ ratio > 0.2 and neutrophils > 1.5% were considered to be increased.[10, 16]

^{18}F-FDG PET

The patient fasted for at least 6 h and before the intravenous injection of ^{18}F-FDG, 5 mg of diazepam was administered to reduce muscle activity. In order to reduce radiation exposure and accelerate ^{18}F-FDG excretion by the kidneys, 20 mg of furosemide was injected intravenously. This was followed by 295-400 MBq ^{18}F-FDG (Covidien, Petten, the Netherlands). In patients with a body weight less than 80 kilograms, 295 MBq ^{18}F-FDG was administered. When the body weight exceeded 80 kilograms a calculated dose was used (body weight /10 * 37 MBq) with a maximum of 400 MBq. PET was performed using the Philips Allegro PET system with external ^{137}Cs source for transmission scanning (Philips Medical Systems, Eindhoven, the Netherlands). Sixty minutes after administration of ^{18}F-FDG, the transmission scan was started followed by emission scans from the head to the subinguinal region. Acquisition time per bed position was 3 min.

Two experienced, independent nuclear medicine physicians interpreted the ^{18}F-FDG PET. Disease activity was determined in the mediastinum/hila and lung parenchyma. All sites were scored as either positive or negative. Positive ^{18}F-FDG PET in the mediastinum and hila was defined as increased metabolic activity compared to the mediastinal background, i.e. the blood pool. Any increased metabolic activity in

the lung parenchyma was considered to be abnormal. In cases of disagreement, a third nuclear medicine physician scored the ^{18}F-FDG PET.

The SUV_{max} was calculated in the mediastinum/hilar region as well as in the lung parenchyma. Regions of interest (ROI) were drawn over the visually affected part of the organ to measure SUV_{max}. This was performed by using the automatic ROI drawing program provided by Hermes Diagnostics. As the region definition was operator-independent, only one reader performed the SUV_{max} measurements.

Patients were grouped based on the results of ^{18}F-FDG PET with regard to the pulmonary tract. Patients without increased intra-thoracic activity were therefore not included in this analysis. Group A consisted of patients with exclusively mediastinal/hilar abnormalities, while patients in group B showed increased metabolic activity in the lung parenchyma. The absolute number of lymphocytes in BAL, percentage of lymphocytes, $CD4^+/CD8^+$ ratio, $CD103^+CD4^+/CD4^+$ ratio, percentage of neutrophils and SUV_{max} per group were compared.

In addition, BAL parameters were correlated with the SUV_{max} of the mediastinum/hila as well as the lung parenchyma in all 77 patients.

Statistical analysis

Data are expressed as mean values (± SD). The time interval between ^{18}F-FDG PET and histological confirmation as well as ^{18}F-FDG PET and BAL is expressed as median (range). The Kolmogorov-Smirnov test was used to assess the distribution of the different parameters. The Mann-Whitney test was used to compare BAL parameters between the smoking and non-smoking group and BAL parameters between groups A and B. BAL parameters, SUV_{max} and ACE Z-score per radiographic stage were analyzed by the Kruskal-Wallis test. Correlations between SUV_{max} and BAL parameters were determined using Spearman's rank. The statistical evaluation was performed using SPSS 15 (SPSS Inc., Chicago, IL, USA).

Results

Patients

The characteristics of the 77 sarcoidosis patients are summarized in Table 1. The median time interval between BAL and ^{18}F-FDG PET was 6 days (range: 0-79 days). The time interval between histological confirmation of sarcoidosis and ^{18}F-FDG PET was 15 days (range: 0-84 days). None of these patients had Löfgren's syndrome.

Table 1 Patient characteristics

	n = 77
Age (years)	39 (± 12.4)
Sex	
Female	32
Male	45
Smoker	
Never	38
Ex-smoker	30
Current smoker	9
Chest radiographic stage	
0	4
I	31
II	30
III	9
IV	3

According to the chest radiographic stages, there were four patients with stage 0 disease. In 31 patients, sarcoidosis was limited to hilar lymph nodes (stage I) while 39 patients did have parenchymal abnormalities (stage II/III). Three patients already had signs of fibrosis (stage IV). Per radiographic stage, there was a significant difference between SUV_{max} of the mediastinum/hila ($p < 0.05$) and SUV_{max} of the lung parenchyma ($p < 0.001$). In BAL, significant differences were found for the $CD4^+/CD8^+$ ratio, $CD103^+CD4^+/CD4^+$ ratio and percentage of neutrophils ($p < 0.05$ for all). No differences were found between the lymphocytes (%) per radiographic stage. SUV_{max} and BAL parameters in the various chest radiographic stages are de-

scribed in Table 2. The ACE Z-score did not differ significantly per radiographic stage (p = 0.3). The ACE Z-score was 3.9 (± 5.1) in stage 0, 2.5 (± 2.8) in stage I, 3.2 (± 2.9) in stage II, 1.2 (± 2.8) in stage III and 1.9 (± 1.5) in stage IV.

Bronchoalveolar lavage

There were no statistical differences in BAL parameters between smokers and non-smokers (data not shown). Therefore, smokers and non-smokers were considered as one group.

Overall, there were 59 patients (77%) with lymphocytosis and 39 of these patients showed an increased $CD4^+/CD8^+$ ratio. Eight patients with an increased $CD4^+/CD8^+$ ratio did not have lymphocytosis. Consequently, 47 patients (61%) had an increased $CD4^+/CD8^+$ ratio. The $CD103^+CD4^+/CD4^+$ ratio was decreased in 55 patients (71%). Increased neutrophils were seen in 21 patients (27%).

Table 2 ^{18}F-FDG PET per chest radiographic stage

Stage	*n*	Increased uptake mediastinum/hila	Increased uptake Lung parenchyma	SUV_{max} mediastinum/hila*	SUV_{max} lung parenchyma**	Lymphocytes (%)	$CD4^+/CD8^+$ ratio*	$CD103^+$ $CD4^+$/ CD4+ ratio*	Neutrophils(%)*
0	4	4 (100%)	1 (25%)	6.3 (± 3.5)	1.2 (± 0.2)	42.2 ± 29.5	11.2 ± 2.0	0.17 ± 0.25	0.78 ± 0.34
I	31	31 (100%)	16 (52%)	9.1 (± 4.9)	2.4 (± 1.8)	31.2 ± 18.3	7.3 ± 7.3	0.11 ± 0.09	1.2 ± 1.0
II	30	27 (90%)	23 (77%)	9.2 (± 8.1)	4.4 (± 3.1)	30.4 ± 19.8	5.5 ± 4.2	0.11 ± 0.13	2.9 ± 4.6
III	9	8 (89%)	7 (78%)	5.4 (± 2.4)	4.3 (± 2.5)	27.8 ± 12.1	4.1 ± 4.6	0.24 ± 0.15	1.6 ± 0.6
IV	3	3 (100%)	3 (100%)	3.1 (± 0.6)	5.9 (± 2.6)	29.4 ± 15.1	2.0 ± 0.6	0.30 ± 0.06	11.3 ± 16.4

SUV_{max} and BAL parameters are presented as mean ± SD. Stage 0 = no abnormalities, stage I = bihilar lymphadenopathy, stage II = bihilar lymphadenopathy and parenchymal infiltrates, stage III = parenchymal infiltrates, stage IV = signs of fibrosis

* $p < 0.05$

** $p < 0.001$

^{18}F-FDG PET

There were 75 patients (97%) with a positive ^{18}F-FDG PET and 73 of them demonstrated thoracic involvement (95%). Mediastinal/hilar activity was present in all 73 patients, which was accompanied by parenchymal activity in 50 patients. There were no patients with increased activity in the lung parenchyma without mediastinal/hilar involvement. Group A (exclusively mediastinal/hilar activity) consisted of 23 patients. Group B (increased metabolic activity in the lung parenchyma) consisted of 50 patients. Therefore, four patients could not be evaluated in this analysis. In Figure 1, ^{18}F-FDG PET of a patient from group A and from group B are shown.

In group A, the average SUV_{max} in the mediastinum/hila was 8.6 (± 6.7) and in the lung parenchyma 1.2 (± 0.5), in Group B, 8.6 (± 6.1) and 4.7 (± 2.6), respectively. The SUV_{max} in the lung parenchyma was significantly higher in group B as compared to group A ($p < 0.0001$). The ACE Z-score was 2.3 (± 3.6) in group A and 2.9 (± 2.8) in group B ($p = 0.2$).

Correlation of ^{18}F-FDG PET and cell profiles in BAL

In group A, there were 21 patients (91%) with an increased percentage of lymphocytes, 15 patients (65%) with an increased $CD4^+/CD8^+$ ratio, 17 patients (74%) with a decreased $CD103^+CD4^+/CD4^+$ ratio and 3 (13%) with an increased number of neutrophils. In group B, there were 36 patients (72%) with an increased percentage of lymphocytes, 30 patients (60%) with an increased $CD4^+/CD8^+$ ratio, 35 patients (70%) with a decreased $CD103^+CD4^+/CD4^+$ ratio and 17 (34%) with an increased number of neutrophils.

The total lymphocyte count, the percentage of lymphocytes, $CD4^+/CD8^+$ ratio, $CD103^+CD4^+/CD4^+$ ratio and the percentage of neutrophils in groups A and B are presented in Table 3. The number of neutrophils was significantly higher in group B, but no other differences in cellular BAL parameters between groups A and B were found.

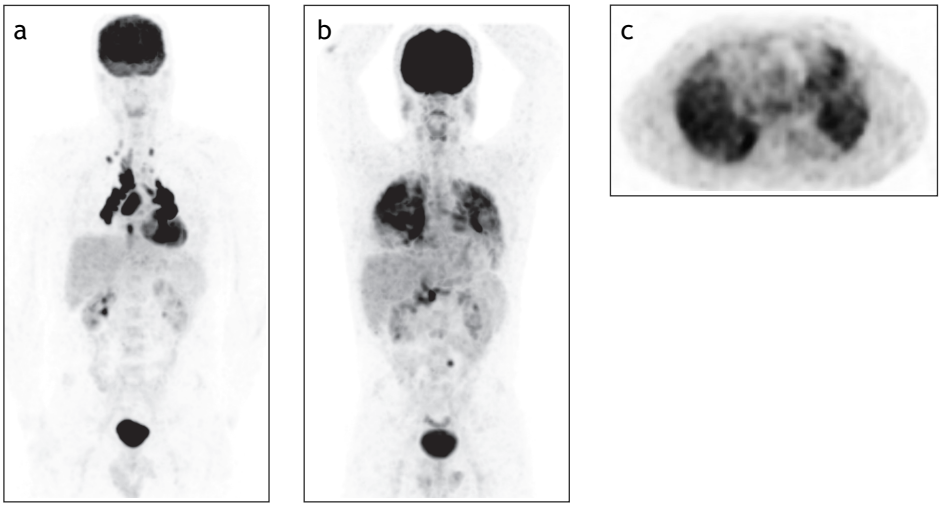

Figure 1 ^{18}F-FDG PET (a) of a patient from group A with increased metabolic activity in the mediastinum and hila without parenchymal or extra pulmonary activity. BAL fluid showed lymphocytes of 45.9% with a $CD4^+/CD8^+$ ratio of 30.3 and 0.5% neutrophils. ^{18}F-FDG PET (b) of a patient from group B with increased metabolic activity in the mediastinum, hila, lung parenchyma and abdominal lymph nodes. The transversal image of the chest (c) illustrates the affected lung parenchyma. BAL fluid showed 7.8% lymphocytes, normal $CD4^+/CD8^+$ ratio and 1.1% neutrophils.

Table 3 BAL parameters and ^{18}F-FDG PET activity in newly diagnosed pulmonary sarcoidosis

	Mediastinal/hilar activity only *n* = 23	Parenchymal activity *n* = 50
Total lymphocyte count	9.1 (± 6.6)	7.7 (± 8.5)
Lymphocytes (%)	35.5 (± 17.6)	29.9 (± 19.2)
$CD4^+/CD8^+$ ratio	6.2 (± 6.2)	6.2 (± 5.8)
$CD103^+CD4^+/CD4^+$ ratio	0.13 (± 0.11)	0.14 (± 0.14)
Neutrophils (%)	1.3 (± 1.7)	2.5 (± 5.1) *

* $p < 0.05$

Overall, a significant correlation was found between SUV_{max} of the mediastinum/hila and the $CD4^+/CD8^+$ ratio ($r = 0.39$, $p < 0.01$) with a negative correlation between SUV_{max} of the mediastinum/hila and $CD103^+CD4^+/CD4^+$ ratio ($r = -0.39$, $p < 0.01$). The SUV_{max} in the lung parenchyma correlated significantly with the percentage of neutrophils ($r = 0.38$, $p < 0.01$). A scatterplot demonstrating this correlation is presented in Figure 2.

In group A, a negative correlation between SUV_{max} of the mediastinum/hila and $CD103^+CD4^+/CD4^+$ ratio was found ($r = -0.52$, $p < 0.05$). No other correlations could be found in this group between SUV_{max} and BAL parameters.

In group B, a positive correlation between the SUV_{max} of the mediastinum/hila and the $CD4^+/CD8^+$ ratio was found ($r = 0.45$, $p < 0.01$). A negative correlation between SUV_{max} of the mediastinum/hila and the number of $CD103^+CD4^+/CD4^+$ ratio was found ($r = -0.38$, $p < 0.01$), while the SUV_{max} in the lung parenchyma showed a positive correlation ($r = 0.40$, $p < 0.01$).

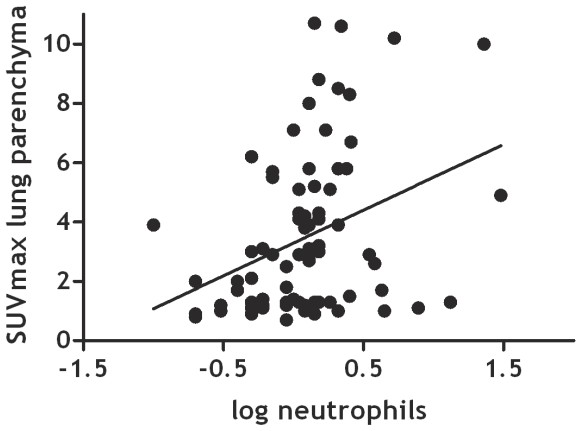

Figure 2 Scatterplot of SUV_{max} in the lung parenchyma and the percentage of neutrophils in BAL in 77 newly diagnosed sarcoidosis patients ($r = 0.39$, $p < 0.01$).

The SUV_{max} in the lung parenchyma correlated significantly with the number of neutrophils ($r = 0.36$, $p = 0.01$). Scatterplots of SUV_{max} in the mediastinum/hila and

$CD103^+CD4^+/CD4^+$ ratio for groups A and B are shown in Figure 3. A scatterplot of the SUV_{max} in the lung parenchyma and $CD103^+CD4^+/CD4^+$ ratio for group B is shown in Figure 4.

Two of the four patients without increased metabolic activity in the chest showed increased lymphocytes and one of these had an increased $CD4^+/CD8^+$ ratio. In one patient, a normal lymphocyte count was found with an increased $CD4^+/CD8^+$ ratio, combined with increased neutrophils. The fourth patient had no increased lymphocytes or $CD4^+/CD8^+$ ratio.

Seven of the eight patients with an increased $CD4^+/CD8^+$ ratio but without lymphocytosis showed increased metabolic activity in mediastinum/hila and lung parenchyma. The eighth patient had a normal ^{18}F-FDG PET.

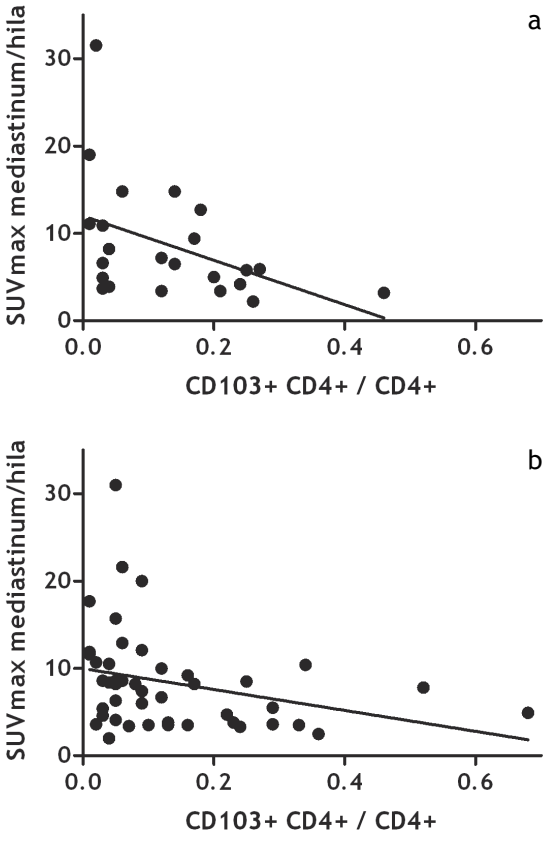

Figure 3 Scatterplot of SUV_{max} in the mediastinum/hila and $CD103^+CD4^+/CD4^+$ ratio in patients with metabolic activity based on ^{18}F-FDG PET in the mediastinum/hila only (a) and lung parenchyma and mediastinum/hila (b). There was a negative correlation between SUV_{max} in the mediastinum/hila and $CD103^+CD4^+/CD4^+$ ratio in both groups ($r = -0.52$, $p < 0.05$ and $r = -0.38$, $p < 0.01$, respectively).

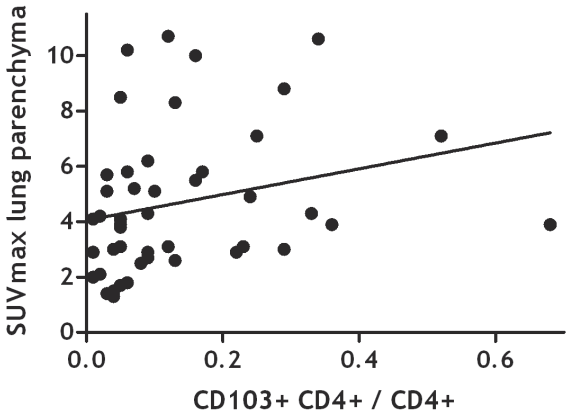

Figure 4 Scatterplot of SUV_{max} in the lung parenchyma and $CD103^{+}CD4^{+}/CD4^{+}$ ratio in patients with metabolic activity based on ^{18}F-FDG PET in the lung parenchyma and mediastinum/hila. There was a positive correlation between SUV_{max} and $CD103^{+}CD4^{+}/CD4^{+}$ ratio ($r = 0.40$, $p < 0.01$).

Discussion

In the current study, different intra-thoracic ^{18}F-FDG PET patterns and SUV_{max} were compared with BAL cell profiles in newly diagnosed, histologically proven, pulmonary sarcoidosis patients. Based on ^{18}F-FDG PET, patients were divided in group A (mediastinal/hilar activity only) and group B (mediastinal/hilar and parenchymal activity). In group B, the number of neutrophils was significantly higher but no other differences in BAL parameters between groups A and B were found. Overall, there was a significant correlation between the SUV_{max} of the mediastinum/hila and the $CD4^+/CD8^+$ ratio. In both groups, a significant, negative correlation was found between the SUV_{max} of the mediastinum/hila and the $CD103^+CD4^+/CD4^+$ ratio. In group B, the SUV_{max} in the lung parenchyma showed a positive, significant correlation with the $CD103^+CD4^+/CD4^+$ ratio and the number of neutrophils. Additionally, SUV_{max} and BAL parameters were analyzed per radiographic stages according to Scadding. Significant differences were found for the SUV_{max} of the mediastinum/hila and the SUV_{max} of the lung parenchyma as well as $CD4^+/CD8^+$ ratio, $CD103^+CD4^+/CD4^+$ ratio and neutrophils (%).

Yamada et al. correlated ^{18}F-FDG uptake in the mediastinum/hilar region with BAL parameters in 21 patients with stage I and II disease.[5] They found a significant correlation between ^{18}F-FDG uptake and the absolute lymphocyte count, but not with the lymphocyte percentage or $CD4^+/CD8^+$ ratio. In the present study, an overall, significant correlation between ^{18}F-FDG uptake in the mediastinum/hila and $CD4^+/CD8^+$ ratio was found. The differences between the results of Yamada et al. and our findings might be explained by the number of patients as well as the method used to quantify metabolic activity. In the current study, the generally accepted SUV_{max} was used while Yamada et al. used a derivative, the 'differential absorption rate'.

The $CD103^+CD4^+/CD4^+$ ratio in relation to intra-thoracic patterns based upon ^{18}F-FDG PET has never been evaluated. Since an increased $CD4^+/CD8^+$ ratio in BAL is not a sensitive parameter for sarcoidosis, it was thought that lymphocyte subsets like $CD4^+$ T cells expressing $CD103^+$ might be more discriminative. Indeed, a low $CD103^+CD4^+/CD4^+$ ratio can be observed in contrast to other interstitial

lung diseases. The lower $CD103^+CD4^+/CD4^+$ ratio in sarcoidosis might be due to a recruitment of $CD4^+$ T cells from the blood rather than from the epithelium. This explains the relative low $CD4^+/CD8^+$ ratio in the blood. In the current study, a negative correlation was found between the degree of metabolic activity in the mediastinum/hila, expressed as SUV_{max}, and the $CD103^+CD4^+/CD4^+$ ratio. Remarkably, a positive correlation was found between the extent of metabolic activity in the lung parenchyma and the $CD103^+CD4^+/CD4^+$ ratio. This finding seems in line with previous results, describing a significantly higher $CD103^+CD4^+/CD4^+$ ratio in patients with stage II-IV disease.[10] From these data, it might be hypothesized that $CD4^+$ T cells involved in the parenchymal activity of sarcoidosis originate from the lung itself, given their CD103 expression. This might imply that formation of granulomas in the lung parenchyma is based on a different immunological pathway than the formation of granulomas in the mediastinum/hila, although additional immunohistochemical studies of granulomas from the different intra-thoracic sites are necessary to investigate this theory.

Since ^{18}F-FDG PET is able to depict active sarcoidosis,[1, 4] the parenchymal activity can be regarded as active disease. Active pulmonary disease might lead to a decline in pulmonary function and it is known that increased neutrophils in BAL are associated with future deterioration.[8, 9] Therefore, we hypothesized that the amount of neutrophils in patients with parenchymal activity would correlate with the extent of parenchymal metabolic activity expressed by the SUV_{max}. This hypothesis could be confirmed, but whether parenchymal activity imaged by ^{18}F-FDG PET does have an actual predictive value for the clinical course of the disease and final outcome needs to be elucidated by follow-up studies.
Instead of using SUV_{max}, it would be of great interest to quantify the total amount of ^{18}F-FDG in the lung parenchyma of sarcoidosis patients, representing the extent of active parenchymal disease. The extent of affected lung parenchyma might correlate with future pulmonary function. Quantifying the volume of active granulomas might therefore be of clinical significance, for example therapy monitoring and adjustment. However, reliable volumetric measurements are not available as in the current study a stand-alone PET scan was used. Integrated ^{18}F-FDG PET/CT might offer the possibility of volumetric measurements. Future studies are necessary to determine the role of this combined technique in functional and anatomical imaging of sarcoidosis.

In the current study, conventional chest radiography was used to determine the different stages of thoracic sarcoidosis. Per radiological stage, the metabolic activity expressed as the SUV_{max} was determined and significant differences were found for the SUV_{max} of the mediastinum/hila as well as the lung parenchyma. Remarkably, the SUV_{max} of the mediastinum/hila was inversely related to the radiographic stages. On the other hand, the SUV_{max} of the lung parenchyma showed higher values when the radiographic stage increased.

In line with previous results, the $CD4^+/CD8^+$ ratio was inversely related to the radiographic stages,[17] while the percentage of neutrophils and $CD103^+CD4^+/CD4^+$ ratio showed a positive correlation.[9, 10] Parenchymal involvement of sarcoidosis can lead to pulmonary decline and immunosuppressive treatment is frequently required. The $CD4^+/CD8^+$ ratio does not have a predictive value, but the percentage of neutrophils correlates with a more severe course of the disease.[9] Therefore, the current findings may have clinical consequences. High SUV_{max} values of the lung parenchyma may imply severe parenchymal involvement, especially when combined with a low SUV_{max} of the mediastinum/hila.

In addition, it seems that ^{18}F-FDG PET is able to demonstrate more sarcoidosis lesions than conventional radiography. In stage 0 and IV, defined as inactive pulmonary disease and end-stage sarcoidosis, respectively, ^{18}F-FDG PET revealed active sarcoidosis lesions in the mediastinum/hila in all patients and parenchymal activity in 57%. Even in stage I disease, defined as bilateral hilar lymphadenopathy without parenchymal involvement, increased metabolic activity in the lung parenchyma was seen in 52% of the patients. These findings are important since chest radiography is still used in the diagnosis of sarcoidosis as well as to assess the presence of active disease. Adequately diagnosing active sarcoidosis helps clinicians in making the decision on whether immunosuppressive treatment needs to be started or (dis)continued. Our data show that conventional chest radiography appears not as reliable as currently thought in determining disease activity, in contrast to ^{18}F-FDG PET.

Conclusion

The SUV_{max} of the lung parenchyma is positively related to the radiographic stage, while the SUV_{max} of the mediastinum/hila is inversely related. This implies that high SUV_{max} values of the lung parenchyma may correlate with more severe parenchymal involvement, especially when combined with a low SUV_{max} of the mediastinum/hila.

The extent of metabolic activity in the mediastinum/hila, expressed as the SUV_{max}, correlates with the $CD4^+/CD8^+$ ratio in BAL, while the degree of metabolic activity in the lung parenchyma correlates with the number of neutrophils. ^{18}F-FDG uptake in the lung parenchyma correlates with the $CD103^+CD4^+/CD4^+$ ratio which might imply an association between these specific T lymphocytes located in epithelium of the bronchi and bronchial glands and the granulomatous inflammation of the lung parenchyma, while granulomas in the mediastinum/hila seem to lack CD103.

These data suggest that ^{18}F-FDG PET might be a helpful substitute for the invasive BAL procedure in sarcoidosis patients, although prospective studies are required to determine its contribution as a predictor of pulmonary decline.

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Chapter 5

^{18}F-FDG PET as a predictor of pulmonary function in sarcoidosis

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Submitted

Abstract

Introduction

Fluor-18 fluorodeoxyglucose (^{18}F-FDG) PET is able to demonstrate sarcoidosis activity. Ongoing pulmonary sarcoidosis activity can be reflected by a decline in pulmonary function tests (PFT). To assess whether diffuse metabolic activity of the lung parenchyma imaged by ^{18}F-FDG PET predicts future pulmonary deterioration, ^{18}F-FDG PET was compared with PFT.

Methods

In this retrospective cohort study, 43 newly diagnosed, sarcoidosis patients were analyzed. Based on ^{18}F-FDG PET, patients were diagnosed with diffuse parenchymal disease activity, without or with immunosuppressive treatment, started after ^{18}F-FDG PET was performed. As a control, sarcoidosis patients with mediastinal/hilar disease activity but without metabolic activity in the lung parenchyma were analyzed, all without treatment. Vital capacity (VC), forced expiratory volume (FEV_1) and diffusion capacity of the lung for carbon monoxide (DLCO) were analyzed per group at baseline, *i.e.* at the time ^{18}F-FDG PET was performed, and after one year follow-up.

Results

At follow-up, a significant decrease in DLCO was found in untreated patients with diffuse parenchymal activity. No change in VC or FEV_1 could be observed. Treated patients with parenchymal activity showed a significant increase in VC, FEV_1 and DLCO, while patients without parenchymal activity did not show any change in PFT.

Conclusions

In sarcoidosis, diffuse parenchymal disease imaged by ^{18}F-FDG PET, predicts a future deterioration of DLCO when untreated. Treatment however, improves VC, FEV_1 and DLCO significantly. The absence of metabolic activity in the lung parenchyma justifies a wait-and-see policy.

Introduction

The lung is the most frequently affected organ in sarcoidosis.[1, 2] The characteristic non caseating epitheloid cell granuloma can be found in intra thoracic lymph nodes as well as the lung parenchyma. Thoracic involvement of sarcoidosis is reflected by conventional chest radiography and can be classified into five stages. Stage 0 is defined as normal, stage I disease as bihilar lymphadenopathy, stage II as bihilar lymphadenopathy combined with parenchymal involvement, stage III as exclusive parenchymal involvement and stage IV as fibrosis.[3] Each radiological stage correlates with a spontaneous remission rate of sarcoidosis. The higher the stage, the lower the spontaneous recovery rate.[4]

Pulmonary function tests (PFT) may be abnormal in sarcoidosis, particularly the diffusion capacity of the lung for carbon monoxide.[5] Remarkably, PFT does not correlate with the radiographic abnormalities. Abnormal PFT can be found in patients without parenchymal involvement and vice versa.[6] However, serial PFT is suggested to be used in monitoring disease progression.[6] PFT is a derivative of the actual involvement of pulmonary tissue. Therefore, the disease needs to progress first before it becomes evident. A tool, adequately assessing the actual presence of active pulmonary disease, might be desirable since active sarcoidosis may require therapeutic intervention.

Fluor-18 fluorodeoxyglucose (^{18}F-FDG) PET is an in vivo imaging technique and has proven to be useful in depicting sarcoidosis activity.[7-9] However, ^{18}F-FDG PET with regard to clinical outcome has not been evaluated yet. This study was performed to evaluate the significance of parenchymal activity with regard to future pulmonary function.

Patients and methods

Patients

In this retrospective cohort study, 49 consecutive patients with newly diagnosed and histologically proven sarcoidosis were evaluated. The patients were seen at the department of pulmonology of the St. Antonius Hospital between January 2004 and December 2009. The diagnosis of sarcoidosis was based on clinical findings, supported by histological evidence and after the exclusion of other known causes of granulomatosis in accordance with the consensus statement on sarcoidosis of the American Thoracic Society (ATS) / European Respiratory Society (ERS) / World Association of Sarcoidosis and other Granulomatous Disorders (WASOG).[1]

Patients underwent PFT and ^{18}F-FDG PET as part of their routine analysis, and none of them used immunosuppressive treatment. Patients were grouped based on the results of ^{18}F-FDG PET with regard to lung parenchymal activity. Group A consisted of patients with diffusely increased metabolic activity in the lung parenchyma without the use of corticosteroids or immunosuppressive drugs during follow-up. Patients in group B showed diffuse parenchymal activity and received corticosteroids or immunosuppressive drugs after ^{18}F-FDG PET was performed. The pulmonologist decided whether treatment was indicated, based on a combination of symptoms, clinical findings, PFT and serological markers and ^{18}F-FDG PET.

Group C was the control group, consisting of patients with increased metabolic activity in the mediastinum and hila but without activity in the lung parenchyma. These patients did not receive corticosteroids or immunosuppressive drugs during follow-up.

Chest radiographic stage according to Scadding was evaluated.[3]

This study was approved by the local medical ethical committee.

Serum markers

ACE and sIL-2R were analyzed and compared between the groups. ACE level was considered positive or negative in accordance with genotype corrected reference values. Reference value for I/I was 9-43 U/l, for I/D 14-62 U/l and for D/D 24-82 U/l. A for genotype corrected ACE was calculated and expressed as Z-score.[10] Serum sIL-2R above 700 U/ml was considered increased.

Pulmonary Function Tests

VC, FEV_1 and DLCO were evaluated at baseline, *i.e.* at the time ^{18}F-FDG PET was performed, and after one year follow-up. For follow-up, a time interval of 10-14 months was allowed. In patients requiring prednisone or immunosuppressive drugs before follow-up was completed, PFT performed at that time was used as follow-up. PFT was interpolated when performed outside the suggested range of 10-14 months after ^{18}F-FDG PET.

Absolute change in percentage of predicted VC, FEV_1 and DLCO was calculated per group and corrected for the time of inclusion. Spirometry was performed in accordance with the guidelines provided by the ERS.[11-13] DLCO was corrected for the hemoglobin concentration. VC, FEV_1 and DLCO were determined by using a MS-PFT analyzer unit (Jaeger, Würzburg, Germany).

^{18}F-FDG PET

The patient fasted for at least six hours and before the intravenous injection of ^{18}F-FDG, 5 milligram of diazepam was administered to reduce muscle activity and accumulation of ^{18}F-FDG in brown fat. In order to reduce radiation exposure and accelerate ^{18}F-FDG excretion by the kidneys, 20 milligrams of furosemide was injected intravenously. Subsequently, 295-400 MBq ^{18}F-FDG (Covidien, Petten, the Netherlands) was administered intravenously. 295 MBq ^{18}F-FDG was given to patients with a body weight less than 80 kilograms. When the body weight exceeded 80 kilograms a calculated dose was used (body weight /10 * 37 MBq) with a maximum of 400 MBq ^{18}F-FDG. PET was performed using the Philips Allegro PET system with external Cesium-137 source for transmission scanning (Philips Medical Systems, Eindhoven, the Netherlands). Sixty minutes after administration of ^{18}F-FDG, transmission scan was started. Transmission time was 23 seconds per bed position. Emission scan was performed from the subinguinal region to the head

with an acquisition time of three minutes per bed position. Reconstruction was performed in accordance with the 3D-RAMLA protocol applying 4 iterations with a 144 x 144 matrix.[14]

Two experienced, independent nuclear medicine physicians interpreted ^{18}F-FDG PET. Diffusely affected lung parenchyma was present when at least two third of the lung parenchyma showed an increased metabolic activity. The degree of increased metabolic activity may vary but needed to be higher than the mediastinal background, *i.e.* the blood pool. In case of disagreement, a third nuclear medicine physician evaluated ^{18}F-FDG PET.

Maximum Standardized Uptake Value (SUV_{max}) was calculated in the mediastinum/ hilar region as well as in the lung parenchyma. Regions of interest (ROI) were drawn over the visually affected part of the organ to measure SUV_{max}. This was performed by using the automatic ROI drawing program provided by Hermes Diagnostics. As the region definition was operator-independent, only one reader performed the SUV_{max} measurements.

Statistical analysis

Continuous data were expressed as mean values ± SD or median (interquartile range) where appropriate. Categorical data were analyzed by chi-square and continuous data by analysis of variance (ANOVA) with a Tukey HSD post hoc test were appropriate. Changes in VC, FEV_1 and DLCO were normalized per time unit (months) and expressed as percentage predicted. The Kruskal-Wallis test was used to assess ACE values between the groups with an additional Mann-Whitney test. To assess the predictive value of the PET stage, SUV_{max} , ACE and sIL-2R, ANOVA with a LSD post hoc test was performed.

The statistical evaluation was performed using SPSS 16.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Patients

Of the 49 analyzed patients, 5 patients with exclusive mediastinal/hilar activity were lost to follow-up. Control PFT was performed in these patients but the time interval was less than 12 months. One patient with diffuse parenchymal activity on ^{18}F-FDG PET and prednison was excluded because of a severe pneumonia with prolonged pulmonary deterioration. Therefore, 43 patients were included in this analysis. Group A consisted of 11 patients and group B and C of 16 patients each. In Table 1, baseline characteristics of the 43 sarcoidosis patients are summarized. In all patients, histological confirmation of sarcoidosis was obtained within 2.7 weeks (range 0-11 weeks) of ^{18}F-FDG PET. In group A, 4 patients required immunosuppressive drugs before the follow-up of 12 months was completed. In the control group, no immunosuppressive drugs were started.

According to the chest radiographic stages, there was one patient with stage 0 disease. In 10 patients, sarcoidosis was limited to hilar lymph nodes (stage I) and 30 patients did have parenchymal abnormalities (stage II/III). Two patients had signs of fibrosis (stage IV). All patients in group A and B showed a radiographic stage of ≥ 2, except one. Consequently, 6 patients in Group C showed a radiographic stage of ≥ 2.

Overall, ACE was increased in 23 patients (54%) and sIL-2R was increased in 27 patients (63%). In group A, both ACE and sIL-2R were increased in 5 patients (45%). In group B, 14 patients (88%) showed an increased ACE while sIL-2R was increased in 13 patients (81%). In group C, increased ACE was present in 4 patients (25%) and increased sIL-2R in 9 (56%) patients. ACE and sIL-2R differed between the groups ($p < 0.001$ and $p < 0.05$, respectively) with a significant difference for both parameters between groups A and B (both $p < 0.05$).

Table 1 Patient characteristics at baseline

	Group A Parenchymal activity, untreated	Group B Parenchymal activity, treated	Group C No parenchymal activity
Number of patients	*n* = 11	*n* = 16	*n* = 16
Age	43.2 ± 9.5	40.8 ± 11.0	46.1 ± 11.6
Female sex (%)	5 (46%)	7 (44%)	4 (25%)
Chest radiographic stage	2.1 ± 0.3	2.4 ± 0.8	1.5 ± 0.9
VC	89.0 ± 17.9	80.4 ± 18.4	102.1 ± 12.0
FEV_1	86.9 ± 15.7	72.1 ± 21.5	94.0 ± 11.2
DLCO	80.7 ± 13.8	64.6 ± 9.5 *	87.0 ± 14.4
ACE Z-score	1.6 (0.8-3.0)	3.4 (2.4-9.6) **	1.1 (0.2-1.7)
sIL-2R	810 ± 278	1603 ± 893 **	1001 ± 657
SUV_{max} mediastinum/hila	5.7 ± 2.1	5.7 ± 2.8	10.2 ± 7.6
SUV_{max} lung parenchyma	6.9 ± 3.3	7.2 ± 3.2	1.2 ± 0.3

Data are presented as the mean ± SD or median (interquartile range). Pulmonary function tests are presented as the percentage predicted. VC = vital capacity, FEV_1 = forced expiratory volume in 1 second, DLCO = diffusion capacity of the lung for carbon monoxide, ACE Z-score = angiotension converting enzyme corrected for genotype, sIL-2R = soluble interleukin-2 receptor.
Group A vs. B: * = $p < 0.01$; ** = $p < 0.05$

Patients in group B received therapy, based on progressive (pulmonary) symptoms combined with severe impaired baseline PFT in 10 patients. Extra pulmonary disease requiring therapy was present in three patients and hypercalcaemia in one. One patient demonstrated severe endobronchial involvement. Chest radiography revealed signs of fibrosis in two patients. In these patients, therapy was started because of the symptoms combined with increased serum markers.

^{18}F-FDG PET

Group A consisted of 11 patients. In 8 patients (73%), extra pulmonary lesions were found. SUV_{max} was 5.7 (± 2.1) in the mediastinum/hilar region and 6.9 (± 3.3) in the lung parenchyma. Group B consisted of 16 patients. Extra pulmonary lesions were found in 15 patients (94%). The difference in extra pulmonary findings between groups A and B was not significant ($p = 0.13$). SUV_{max} was 5.7 (± 2.8) in the mediastinum/hilar region and 7.2 (± 3.2) in the lung parenchyma.

Group C consisted of 16 patients and extra pulmonary lesions were found in 8 (50%). SUV_{max} was 10.2 (± 7.6) in the mediastinum/hilar region and 1.2 (± 0.3) in the lung parenchyma.

SUV_{max} in the lung parenchyma differed between the groups ($p < 0.01$), but not between Group A and B ($p = 0.87$). No differences were found in SUV_{max} of the mediastinum/hila between the groups ($p = 0.14$). In Figure 1, ^{18}F-FDG PET of a patient in group A is shown.

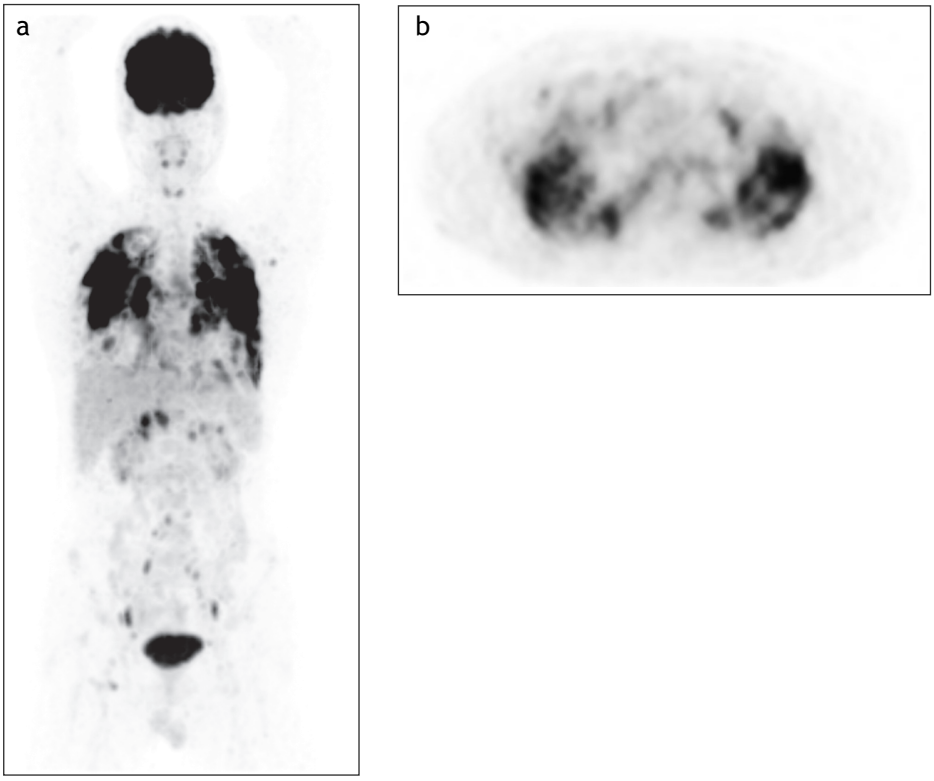

Figure 1 ^{18}F-FDG PET demonstrating diffuse metabolic activity in the lung parenchyma. Furthermore, increased activity is seen in lymph nodes in the hila, mediastinum and extra pulmonary regions. Eight months after ^{18}F-FDG PET was performed, VC, FEV_1 and DLCO showed a decrease of 10%, 8% and 15%, respectively.

Pulmonary function tests

Baseline PFT was performed within 2 weeks of ^{18}F-FDG PET (range -10 weeks-5 weeks).The median time interval between follow-up PFT and ^{18}F-FDG PET was 11.7 months (range 7-14 months). Interpolation of PFT was performed in 1 patient in group B and in 2 patients in group C. In all other patients, PFT was performed in the suggested time interval.

At baseline, there was a difference in VC, FEV_1 and DLCO between the groups ($p < 0.01$ for all). Group A and B did not differ significantly in VC and FEV_1, but they differed in DLCO ($p < 0.01$).

PFT results at baseline and follow-up are presented in Table 2. In untreated patients with parenchymal activity (group A), no change in VC or FEV_1 was found after one year, but there was a significant decrease in DLCO of 7.8% (± 7.1%). Prednisone was started in 4 patients before follow-up was completed (range 7-11 months). Three patients showed non-significant decreasing PFT, progressive dyspnoea, worsening chest radiography and increasing serological markers. One patient had stable PFT and no pulmonary symptoms. Because of progressive arthralgia and worsening chest radiography, prednisone was started.

In group B, all patients were treated after ^{18}F-FDG PET was performed. One patient received methotrexate given the presence of severe osteoporosis. All other patients received prednisone. In these patients, improvement of VC, FEV_1 and DLCO was seen after one year. The increase in VC was 11.6% (± 11.2%), in FEV_1 9.4% (± 10.0%) and in DLCO 8.7% (± 8.0%). The improvement was significant for all lung functional parameters ($p < 0.01$).

In patients without parenchymal activity (group C), no changes in PFT could be found.

Corrected for the time of inclusion, PFT of all groups was compared. Box-and-whisker diagrams of the change in VC, FEV_1 and DLCO per time unit are shown in Figure 2.

Comparing groups A and C, there was a significant decrease in DLCO in patients with diffuse parenchymal activity ($p < 0.01$), but no change in VC or FEV_1 could be observed.

Table 2 PFT at baseline and one year follow-up in patients with and without metabolic activity in the lung parenchyma based on ^{18}F-FDG PET

	VC baseline	VC follow-up	*p*	FEV_1 baseline	FEV_1 follow-up	*p*	DLCO baseline	DLCO follow-up	*p*
Parenchymal activity, untreated	89% (± 18)	88% (± 20)	ns	87% (± 16)	87% (± 20)	ns	81% (± 14)	73% (± 13)	< 0.05
Parenchymal activity, treated	80% (± 18)	92% (± 12)	< 0.01	72% (± 22)	82% (± 19)	< 0.01	65% (± 9)	72% (± 11)	< 0.01
No parenchymal activity	102% (± 12)	102% (± 11)	ns	94% (± 11)	94% (± 10)	ns	87 (± 14)	89% (± 14)	ns

Data are presented as the mean ± SD. Pulmonary function tests are presented as the percentage predicted.
VC = vital capacity, FEV_1 = forced expiratory volume in 1 second, DLCO = diffusion capacity of the lung for carbon monoxide, ns = not significant

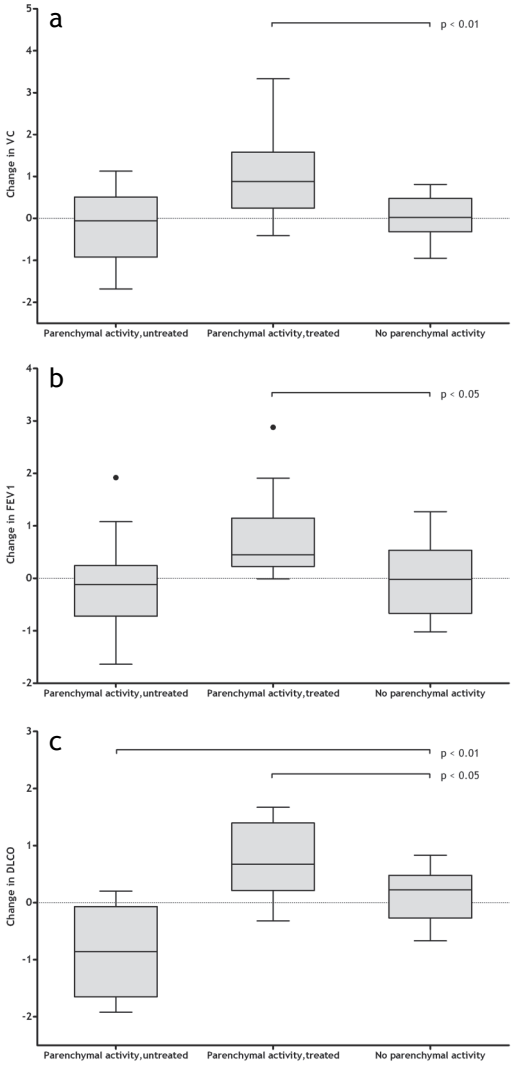

Figure 2 Box and whisker diagram of the change in VC (a), FEV_1 (b) and DLCO (c), expressed as % predicted per month.

Patients in group B showed a significant increase of VC, FEV_1 and DLCO compared to group C.

Baseline ACE, sIL-2R, PET pattern and SUV_{max} in the lung parenchyma and mediastinum/hila were assessed with regard to their predictive value in long functional outcome. The PET pattern showed to be a predictive factor for the change in VC and DLCO ($p < 0.05$ and $p < 0.01$, respectively). ACE predicted the change in DLCO ($p < 0.05$).

Discussion

In the current study, intra-thoracic metabolic activity imaged by ^{18}F-FDG PET was correlated with PFT in newly diagnosed, pulmonary sarcoidosis patients. The presence of diffusely increased metabolic activity in the lung parenchyma correlated with a future decrease of DLCO when untreated. Such a correlation could not be found for VC or FEV_1. Patients with diffuse active disease in the lung parenchyma receiving corticosteroids or immunosuppressive drugs showed a significant increase in VC, FEV_1 and DLCO. Furthermore, patients without increased metabolic activity in the lung parenchyma showed stable lung function tests during follow-up.

This is the first study, assessing the presence of ^{18}F-FDG activity in sarcoidosis patients with regard to clinical outcome. The current data support the hypothesis that increased metabolic activity in the lung parenchyma represents active disease since a decline in DLCO was observed. Intervention was required in 4 patients (36%) with diffuse parenchymal activity. Conversely, patients without metabolic activity in the lung parenchyma showed stable PFT after one year. A wait-and-see policy appears to be justified in the absence of parenchymal activity while a regular PFT during follow-up is required in patients with active parenchymal disease. In patients with pulmonary involvement receiving corticosteroids or other immunosuppressive drugs, a significant improvement of PFT was found. All patients exhibited ongoing inflammatory activity in the lung parenchyma as shown by ^{18}F-FDG PET, implying that metabolic active disease correlates with reversible abnormalities.
Serial PFT is advised to monitor disease progression since a decrease in PFT suggests pulmonary activity. However, the use of serial PFT might have limitations since a pulmonary decline only becomes evident when sarcoidosis has progressed and the patient has deteriorated. This clinical worsening may consist of disabling symptoms and impair the quality of life. Impaired quality of life frequently includes a reduced working capacity, all in a predominantly young population.[15]

In our study, a selected patient population was observed. Patients with diffusely affected lung parenchyma were included. The inclusion criterion was restricted to this specific pulmonary pattern, since quantitative analysis of the affected

lung parenchyma is not possible yet. When the extent of affected tissue could be quantified, the degree of metabolic activity can be taken into account. The currently available PET/CT systems might offer a solution for disease quantification. However, when quantification models become accessible, additional research is required to determine the significance of partly increased metabolic activity in the lung parenchyma.

There was a significant difference in DLCO, serum ACE and sIL-2R between groups A and B at baseline. Increased ACE and sIL-2R, biomarkers for active sarcoidosis, combined with lower PFT might explain the urge for treatment in group B. Based on ^{18}F-FDG PET, patients in groups A and B demonstrated a similar pulmonary involvement. Apparently, ^{18}F-FDG PET is unable to distinguish patients with regard to baseline PFT. Disease duration might clarify these differences since patients delay can already have caused a decrease in PFT.

Serum ACE and sIL-2R was significantly higher in patients receiving treatment after ^{18}F-FDG PET. The amount of ACE and sIL-2R is suggested to be related with the total granuloma load and total T cell activity, respectively.[16, 17] Indeed, besides pulmonary involvement, ^{18}F-FDG PET demonstrated active extra pulmonary lesions in 94% of the patients receiving treatment, while this was 73% in untreated patients. Although this might suggest that the total granuloma load is larger in the treated group, the difference in extra pulmonary findings was not significant. In addition, the extra pulmonary findings were not quantified.

A previous study has shown that normal ACE and sIL-2R does not rule out active disease.[18] Indeed, the majority of untreated patients with diffuse parenchymal disease did not have increased serum levels while a significant decrease in DLCO after one year was found. Since diffuse lung parenchymal activity was demonstrated by ^{18}F-FDG PET, it appears that this nuclear imaging technique more adequately reflects the actual sarcoidosis activity state than serum markers.

Based on ^{18}F-FDG PET patterns, patients were assigned to different groups. The control group consisted of patients with mediastinal and hilar lymph nodes without parenchymal activity, implying stage I disease on chest radiography. However, radiography revealed stage II or III disease in 6 patients (38%) while PFT did not change during follow-up. This finding is in line with previous studies describing that PFT is not related with pulmonary abnormalities on radiography,[6] and might suggest that ^{18}F-FDG PET more adequately reflects the presence of clinical relevant active disease than chest radiography.

Conclusion

Diffuse parenchymal activity in sarcoidosis patients, imaged by ^{18}F-FDG PET, predicts a future deterioration of DLCO when medical treatment is withheld. Treatment however, improves VC, FEV_1 and DLCO significantly. In sarcoidosis patients without metabolic activity in the lung parenchyma, surveillance seems allowed even in patients with radiographic signs of parenchymal disease.

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Chapter 6

^{18}F-FDG PET in sarcoidosis: an observational study in 12 patients treated with infliximab

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Abstract

Background

^{18}F-FDG PET is a promising technique in sarcoidosis imaging, although it is not incorporated in routine activity assessment. The purpose of this study was to correlate ^{18}F-FDG PET with standard sarcoidosis activity parameters during infliximab treatment.

Methods

Twelve patients with refractory sarcoidosis were treated with 6 cycles of infliximab. Pre- and post-therapy ^{18}F-FDG PET was visually evaluated and SUV_{max} was measured. In addition, the effect of infliximab was evaluated by changes in symptoms, angiotensin converting enzyme (ACE), soluble interleukin-2 receptor (sIL-2R), vital capacity (VC), diffusion capacity of the lung for carbon monoxide (DLCO) and chest radiography. SUV_{max} and conventional parameters were correlated.

Results

Clinical improvement as judged by conventional parameters was seen in all patients, though with a minor response in one. Symptoms improved in 11/12 patients while chest radiographic stages did not change. The decrease in ACE was 39% and in sIL-2R 47% ($p < 0.01$). Improvement of VC and DLCO was 5.4% and 3.3% ($p < 0.05$), respectively. ^{18}F-FDG PET revealed either improvement or normalization in 11/12 (92%) clinically responding patients. The overall decrease in SUV_{max} was 55% ($p < 0.01$); the patient with a limited response showed a 34% increase. A decrease in SUV_{max} of the lung parenchyma correlated with an improvement of VC ($r=-0.75$, $p < 0.01$). No significant correlation between SUV_{max} and other parameters was found.

Conclusion

Changes imaged by ^{18}F-FDG PET during infliximab treatment in sarcoidosis patients correlate with signs of clinical improvement to a considerate extent, which supports the hypothesis that ^{18}F-FDG uptake represents disease activity.

Introduction

Sarcoidosis is a multi organ disease, characterized by the presence of epitheloid cell granulomas. The formation of these granulomas is, among others, regulated by tumor necrosis factor (TNF)-α.[1] Anti-TNF-α has already shown to be an effective therapy in other TNF-α mediated conditions e.g., rheumatoid arthritis, inflammatory bowel disease and ankylosing spondylitis.[2-4] Several studies indicate that TNF-α therapy might also be useful in the treatment of severe pulmonary and extra pulmonary sarcoidosis.[5-7]

To determine the effect of anti-TNF-α in sarcoidosis, several parameters can be used such as the evaluation of symptoms, serum concentrations of angiotensin-converting enzyme (ACE), soluble interleukin-2 receptor (sIL-2R), pulmonary function tests (PFT) and chest radiography. However, each activity parameter has its limitation. Symptoms might be difficult to interpret; especially fatigue is a very common and persisting symptom in sarcoidosis patients.[8] ACE is an unattractive tool due to its moderate sensitivity [9] and cannot be used as a reliable follow-up tool.[10] sIL-2R appears somewhat more reliable but is not widely available.[11, 12] An improvement of PFT is likely to reflect a positive therapeutic effect. However, persistent abnormal PFT might represent an ineffective drug, an inadequate dosage or even irreversible fibrotic changes. Despite these shortcomings, decisions regarding treatment and treatment monitoring are currently based on a combination of the aforementioned parameters.

Fluor-18 deoxyglucose (^{18}F-FDG) PET is thought to represent the active granuloma in sarcoidosis and it has already shown to be a sensitive tool in the assessment of this disease.[13-15] The use of ^{18}F-FDG PET as an imaging tool to evaluate infliximab treatment has not been described previously. The aim of this study is to correlate changes in ^{18}F-FDG PET with conventional activity parameters in sarcoidosis patients during anti-TNF-α therapy in order to illustrate that ^{18}F-FDG uptake indeed represents active disease and might be used to monitor therapeutic effects.

Materials and Methods

Patients

Nineteen biopsy proven sarcoidosis patients who had received infliximab in the St. Antonius Hospital Nieuwegein were evaluated. Three patients had a Gallium-67 scan prior to therapy, one patient had a common variable immunodeficiency and one patient received only 3 cycles of infliximab due to psychiatric problems. One patient had cardiac sarcoidosis and one patient neurosarcoidosis, both without other signs of sarcoidosis based on ^{18}F-FDG PET. Since the brain and heart show a high physiologic uptake of ^{18}F-FDG and the role of ^{18}F-FDG PET in neuro- and cardiac sarcoidosis has not been elucidated yet, these patients were not included. In total, 12 sarcoidosis patients treated with infliximab between March 2005 and September 2007 were included in this retrospective study. All patients had therapy resistant sarcoidosis or did not tolerate conventional therapy. Active or latent tuberculosis was excluded in all patients. Infliximab was administered in a dose of 5 mg/kg bodyweight in week 0, 2, 6, 12, 18 and 24.[5] During infliximab treatment, no additional therapy was started and dosages of currently used drugs were maintained at the same level.
This study was approved by the local medical ethical committee.

Evaluation of the infliximab effect by conventional parameters

At the time of inclusion, all symptoms were described in the patients medical record as well as their smoking status and prior use of immunosuppressive drugs. Serum ACE and sIL-2R were simultaneously obtained, vital capacity (VC) and diffusion capacity of the lung for carbon monoxide (DLCO) were performed and chest radiography was made. Symptoms were evaluated at the time of the last infliximab treatment and all previously mentioned parameters of disease activity were repeated.
ACE was corrected for genotype.[16] ACE was considered normal or increased in accordance with genotype corrected reference values. Reference value for I/I was 9-43 U/l, for I/D 14-62 U/l and for D/D 24-82 U/l. Serum sIL-2R above 700 U/ml was considered increased. PFT was defined as the percentage predicted and the absolute change in percentage predicted VC and DLCO was used to evaluate the effect.

The pulmonologist determined the effect of infliximab based on the changes of the above mentioned parameters.

^{18}F-FDG PET

The patient fasted for at least six hours and before the intravenous injection of ^{18}F-FDG, 5 milligram of diazepam was administered orally to reduce muscle activity and appearance of brown fat. In order to reduce radiation exposure and accelerate ^{18}F-FDG excretion by the kidneys, 20 milligrams of furosemide was injected intravenously. This was followed by 295-400 MBq ^{18}F-FDG (Covideon, Petten, the Netherlands). 295 MBq was given to patients with a body weight less than 80 kilograms. When the body weight exceeded 80 kilograms a calculated dose was used (body weight /10 * 37 MBq) with a maximum of 400 MBq. PET was performed using the Philips Allegro PET system with external Cesium-137 source for transmission scanning (Philips Medical Systems, Eindhoven, the Netherlands). Sixty minutes after administration of ^{18}F-FDG, transmission scan was started followed by emission scans from the subinguinal region to the head. Acquisition time per bed position was three minutes.

Two independent nuclear medicine physicians, blinded for all clinical data, interpreted ^{18}F-FDG PET. In case of disagreement between the observers, a third nuclear medicine physician interpreted the images and final conclusion was based on majority of votes.

^{18}F-FDG PET was visually evaluated. Disease activity was assessed separately in the mediastinum, hila, lung parenchyma, extra pulmonary lymph nodes, liver, spleen, bone marrow, skeletal and muscles. These sites were scored either positive or negative (positive = increased ^{18}F-FDG uptake, negative = no increased ^{18}F-FDG uptake). In addition, quantitative assessment was performed, based on maximum standardized uptake value (SUV_{max}) corrected for body weight. To correlate ACE and sIL-2R with SUV_{max}, the maximum change of SUV_{max} in any of the affected organs was used because these serum parameters are thought to represent the total granuloma load.[17] VC and DLCO were correlated with changes in SUV_{max} measured in the lung parenchyma.

Statistical analysis

Paired samples t-test was used to compare pre and post therapy values. A two-sided

p-value < 0.05 was considered to be statistically significant. Correlations between ACE, sIL-2R, VC, DLCO and SUV_{max} were determined using Spearman's rank. Data are presented as mean values ± standard deviation. The statistical evaluation was performed using SPSS 15 (SPSS Inc., Chicago, IL, USA). There has been no external funding source for this study.

Results

Patients

Patients' characteristics are summarized in Table 1. Chest radiographic stage I disease was seen in 3 patients. Besides pulmonary involvement, these patients had severe extra pulmonary sarcoidosis for which infliximab was indicated. Two patients suffered from a visual loss due to uveitis and optic neuritis, respectively. The third patient had extensive muscle and skin involvement.

Table 1 Patient characteristics at baseline

	n = 12
Age (years)	43.6 (± 9.3)
Female/Male	6/6
Smoker	
Never	8
Ex-smoker	4
Disease duration (years)	3.9 (± 3.1)
Pulmonary function tests (% predicted)	
VC	85% (± 19)
DLCO	61% (± 18)
Chest radiographic stage	
I	3
II	6
III	2
IV	1
Prior therapy	
Prednisone	1
Methotrexate with prior use of prednisone	3
Prednisone and methotrexate	7
Prednisone and plaquenil	1

Evaluation of the infliximab effect by conventional parameters

Changes in symptoms, serum ACE and sIL-2R, VC and DLCO after completion of the final infliximab treatment are shown in Table 2.

Although not validated by a standardized dyspnoea score or a 6-minute walk-distance test, 11 patients noticed an improvement of prior symptoms. One patient did not notice any improvement and PFT remained about the same level while the serological markers showed a dramatic decrease.

Prior to therapy, there were 6 patients with a normal ACE and only one patient with a normal sIL-2R. An average decrease of 40 U/l (± 43) was found for ACE ($p < 0.01$), representing a decrease of 39% (± 27). One patient showed an increase of ACE, however, still within the normal range. Eleven patients did show a decrease and 5 of these patients already had a normal ACE prior to infliximab treatment. Despite a decrease during treatment, 2 of the 11 patients still had an elevated ACE after the final infliximab treatment.

An average decrease of 902 U/ml (± 753) was found for sIL-2R ($p < 0.01$), representing a decrease of 47% (± 37). Two patients showed an increase of sIL-2R; one within the normal range. Ten patients showed a decrease of sIL-2R, all with an elevated sIL-2R level prior to therapy. Three of these 10 patients still had an abnormal sIL-2R after the final infliximab treatment.

Overall, an increase in VC of 5.4% (± 5.8) was found ($p < 0.01$). DLCO showed an improvement of 3.3% (± 5.1), which was also statistically significant ($p < 0.05$). Ten patients showed an increase of VC with an average increase of 7.5% in this subgroup (range 4%-15%). Two patients showed a decrease of VC of 5.0%. Eight patients showed an improvement of DLCO with an average increase of 6.0%. Four patients showed a decrease of DLCO ranging between 1%-3%.

Table 2 Changes in chest radiography, serological markers, pulmonary function tests and symptoms during infliximab treatment

Patient	Symptoms prior to therapy	Chest radiographic stage	Chest radiographic changes during treatment	ACE	sIL-2R	VC	DLCO	Symptoms
Overall				- 39%*	- 47%*	+ 5.4%*	+ 3.3%*	
1	Fatigue, coughing, weight loss	II	Improvement of interstitial abnormalities	- 46%**	- 73%	+ 6%	+ 12%	Improved
2	Dyspnea, coughing	IV	Decrease of lymphadenopathy	- 47%**	- 62%	- 5%	+ 3%	No change
3	Fatigue, visual loss due to neuritis optica	I	No changes	- 33%**	- 69%	+ 7%	- 3%	Improved
4	Dyspnea d'effort, thoracic pain	II	Decrease of lymphadenopathy and improvement of interstitial abnormalities	- 47%	- 72%	+ 11%	- 3%	Improved
5	Fatigue, dyspnea d'effort	III	No changes	- 12%**	- 40%	+ 15%	+ 4%	Improved
6	Fatigue, dyspnea, muscle pain in arms and legs	I	Decrease of lymphadenopathy	- 53%**	- 52%***	+ 5%	- 1%	Improved
7	Fatigue, dyspnea d'effort, thoracic pain	II	No changes	- 49%	- 11%***	+ 4%	+ 3%	Improved
8	Fatigue, visual loss, due to uveitis	I	Decrease of lymphadenopathy	- 57%	- 80%	- 5%	+ 3%	Improved
9	Dyspea d'effort, stridor, coughing	II	No changes	+ 18%**	+ 34%**	+ 10%	+ 4%	Improved
10	Fatigue, dyspnea, coughing, skin lesions	III	No changes	- 72%***	- 76%	+ 5%	+ 11%	Improved
11	Fatigue, dyspnea, arthralgia	II	No changes	- 66%	- 72%***	+ 7%	+ 8%	Improved
12	Fatigue, coughing, night sweating, abdominal pain, arthralgia	II	Improvement of interstitial abnormalities	- 3%***	+ 6%***	+ 5%	- 2%	Less fatigue, other symptoms did not improve

ACE = angiotensin/converting enzyme, sIL-2R = soluble interleukin-2 receptor, VC = vital capacity, DLCO = diffusion capacity of the lung for carbon monoxide

* = statistically significant, ** = normal pre infliximab level, *** = abnormal post infliximab level

Chest radiographic stages did not change during infliximab treatment. However, 6 patients did show an improvement; in 3 patients there was a decrease of lymphadenopathy, in 2 there was an improvement of interstitial abnormalities and one patient showed both signs of improvement.

Based on the above mentioned conventional activity parameters, 11 patients were considered to benefit from the infliximab treatment. In one patient (patient 12), infliximab appeared to have a minor clinical effect.

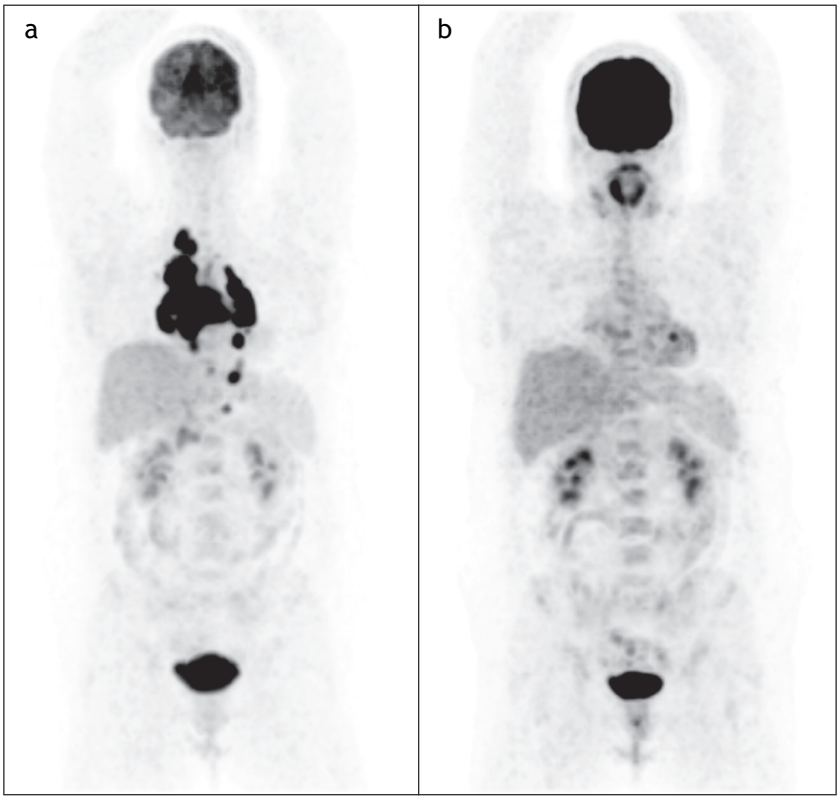

Figure 1 Baseline ^{18}F-FDG PET (a) of patient number 3 shows increased metabolic activity in the mediastinum, hila and para-aortic lymph nodes. Post infliximab ^{18}F-FDG PET (b) shows a normalization together with a decrease of serum ACE and sIL-2R, improvement of symptoms and VC.

^{18}F-FDG PET

Pre and post infliximab ^{18}F-FDG PET results are illustrated in Table 3. Pre infliximab ^{18}F-FDG PET was abnormal in all patients. Involvement of the pulmonary tract was seen in all 12 patients, while 8 patients showed extra pulmonary involvement.

Table 3 Pre and post therapy localizations of increased ^{18}F-FDG uptake and change in SUV_{max}

Patient	Pre therapy ^{18}F-FDG PET	Post therapy ^{18}F-FDG PET	Maximum change in SUV_{max}
1	med-hila-par-bone marrow*	Normalized	-83%
2	med-hila-par	Normalized	-12%
3	med-hila-lnn	Normalized	-83%
4	med-hila-par	Normalized	-82%
5	med-hila-par	par	-78%
6	med-hila-par-muscles*	med-hila	-47%
7	med-hila-par-lnn	med-hila-par-lnn	-45%
8	med-hila-par-lnn-parotids	med-hila-par-parotids	-61%
9	med-hila-par	med-hila	-82%
10	med-hila-par-lnn-spleen-parotids	hila-par-spleen-parotids	-55%
11	med-hila-par-lnn	med-par	-66%
12	med-hila-lnn-liver*-muscles-aortic wall	med-hila-lnn-liver muscles-aortic wall	+34%

med = mediastinum, par = lung parenchyma, lnn = lymph nodes, SUV_{max} = maximum standardized uptake value
* = histologically proven

The average interval between ^{18}F-FDG PET and the final infliximab treatment was 2.8 weeks (± 2.2). Four patients showed a normalized post infliximab ^{18}F-FDG PET (Figure 1). Seven patients showed an improvement, while one patient did not show any change.

The overall decrease in SUV_{max} was 5.4 (± 5.0) corresponding with an average decrease of 55% (± 35) ($p < 0.01$). In all patients with a normalization or improvement, the average decrease in SUV_{max} was 63% (± 22). The one patient without any visual change on ^{18}F-FDG PET showed an increase in SUV_{max} of 34% (Figure 2).

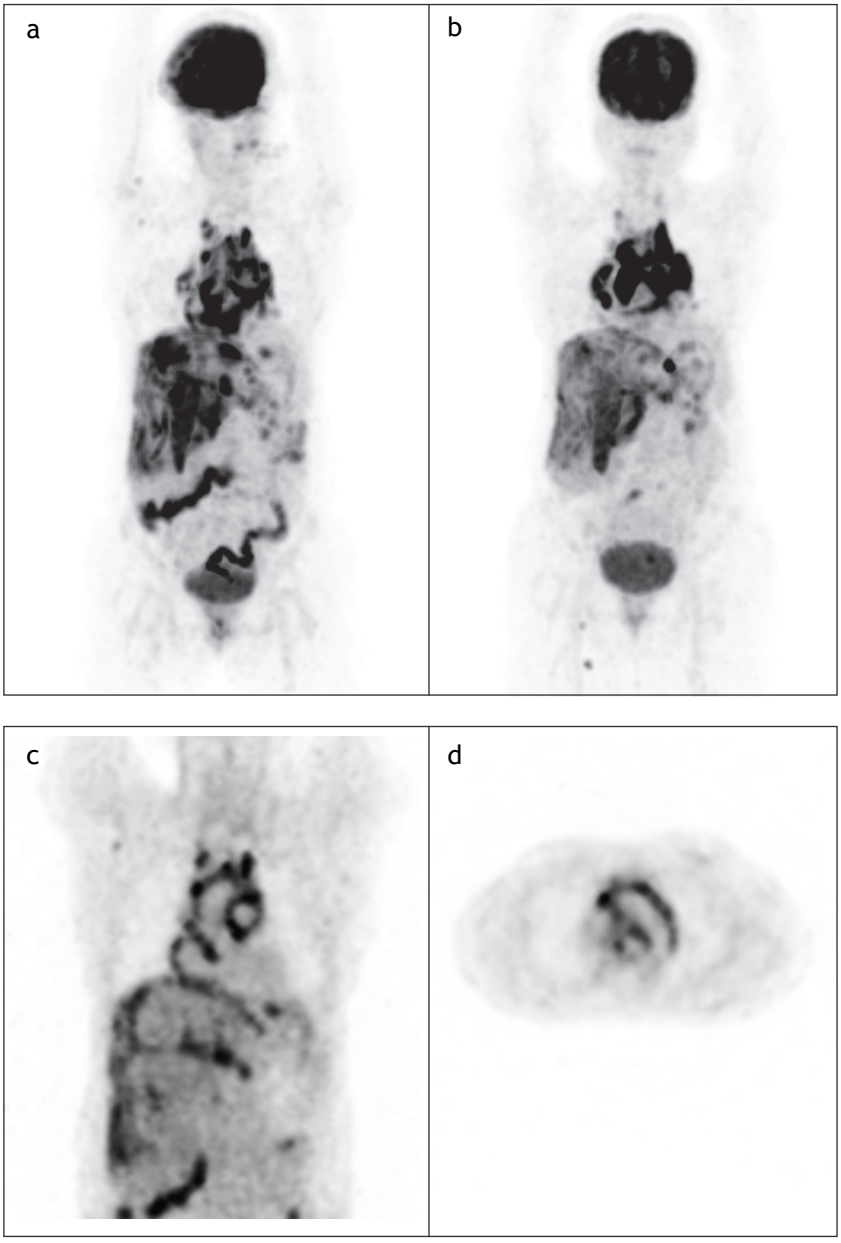

Figure 2 ^{18}F-FDG PET of patient number 12 before (a) and after (b) infliximab treatment. There is increased metabolic activity in the mediastinum, hila, lymph nodes, liver, ascending aorta (c) and arcus aortae (d). The increased uptake in the aorta might indicate an aortitis. SUV_{max} of the thoracic abnormalities showed an increase of 34% after infliximab treatment. Clinically, this patient showed a minor, pulmonary improvement.

The average decrease in SUV_{max} of the lung parenchyma was 2.5 (± 3.7) which correlated with an improvement of VC (r = -0.75, p < 0.01) (Figure 3). There was no significant correlation between SUV_{max} and DLCO, ACE or sIL-2R.

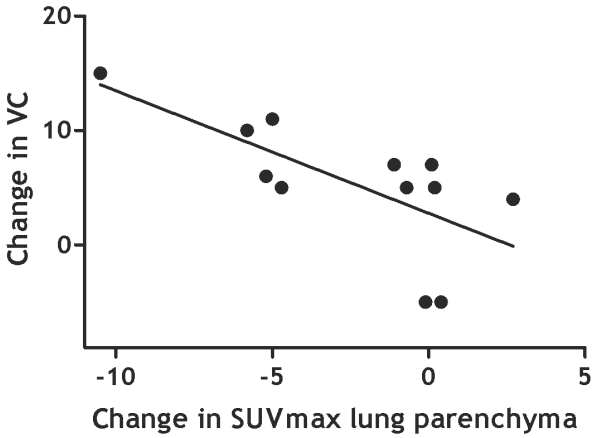

Figure 3 Correlation between change in SUV_{max} in the lung parenchyma and VC. Spearman's rho was –0.75 (p < 0.01). The lines represent the mean with 95% confidence interval.

Discussion

In this case series of sarcoidosis patients treated with infliximab, visually evaluated ^{18}F-FDG PET activity correlated with signs of clinical improvement in 11 out of 12 patients. All patients with a clear clinical improvement based on a combination of conventional activity parameters demonstrated an improvement or even normalization of ^{18}F-FDG PET. The patient with a minor clinical response showed no visual change on ^{18}F-FDG PET, while SUV_{max} showed an increase. The current study shows an overall decrease in SUV_{max} of 55% with a significant correlation between the decrease in SUV_{max} measured in the lung parenchyma and an improvement of VC.

The use of corticosteroids and its effect imaged by ^{18}F-FDG PET were previously described in a small patient population.[15] To our knowledge, this is the first observational study that illustrates a simultaneous decrease in metabolic activity and an improvement of clinical signs during the use of anti-TNF-α therapy. Although this is a retrospective analysis in a selected patient population, these results seem to confirm our hypothesis that ^{18}F-FDG uptake in sarcoidosis represents active disease. At the same time, this study might suggest the use of ^{18}F-FDG PET as a management tool during therapy although additional research is necessary to validate this hypothesis.

In one patient, a pulmonary improvement was exhibited though ^{18}F-FDG PET did not adequately reflect this effect (Figure 2). However, the initial effect of infliximab achieved in this patient was considered to be limited. Previously increased ACE, sIL-2R, serum aminotransferase and bilirubin remained unchanged and despite less fatigue there was no improvement of the other symptoms. The lack of clinical response was considered to justify a higher dosage of infliximab (7.5 mg/kg bodyweight per 4 weeks) which resulted in an improvement of all symptoms, a significant decrease or normalisation of ACE, sIL-2R, serum aminotransferase and bilirubin, and an increase in VC of 13% and DLCO of 6%. Although the initial minor pulmonary improvement was not properly reflected by ^{18}F-FDG PET, ^{18}F-FDG PET performed after the second series of infliximab, demonstrated an improvement at all sites with previously increased metabolic activity. Because of the abovementioned course of the disease, we believe that this imaging technique, even in this specific patient, adequately reflects the actual activity state.

Because the results of ^{18}F-FDG PET in these 12 patients correlate with the analysis of conventional activity markers in a considerate extent, the additional role of this imaging technique appears to be limited. However, the difficulty of sarcoidosis activity assessment is illustrated when the effect of infliximab is evaluated in the individual patient.
ACE showed an average decrease of 39%, although 6 patients (50%) already showed a normal ACE level prior to infliximab therapy. sIL-2R showed an average decrease of 47% and seemed somewhat more compatible with the patients sarcoidosis activity state. Overall, pulmonary function tests showed a significant improvement of 5.4% for VC and 3.3% for DLCO. However, in 6 patients the positive change in VC was accompanied by a negative change in DLCO or vice versa. In addition, this study illustrates the limitations of chest radiography as 6 patients showed an improvement of symptoms and pulmonary function tests without any change in chest radiography. Chest radiographic stages did not change in any of the 11 clinically responding patients.
Even though infliximab therapy resulted in an overall decrease in biological markers and improvement of symptoms and pulmonary function tests, the individual results can be misleading as illustrated in our patient population. This dictates cautiousness in interpreting the effect of infliximab based on conventional parameters because this might lead to the underestimation of a potentially useful treatment in a sometimes devitalizing disease.

Baughman et al. performed a randomized, double blind, placebo controlled study to evaluate infliximab therapy in sarcoidosis patients.[5] An increase in FVC of 2.5% was found as well as a decrease in reticulonodular opacities on chest radiography. Subgroup analysis showed a larger improvement in patients with longer disease duration, lower FVC or more symptoms. In addition, infliximab was more effective in patients receiving immunosuppressive drugs, higher doses of corticosteroids or in patients with multiple extra pulmonary lesions.
Differences in response to infliximab were also observed in patients with ankylosing spondylitis. In this patient population, three types of responders were observed during treatment: early clinical remission, persistent low disease activity and limited improvements.[4]
In order to evaluate the effect of infliximab in sarcoidosis patients and perhaps customise treatment, a tool to monitor the effect of this expensive therapy might

be desirable. In non-Hodgkin's lymphoma patients, ^{18}F-FDG PET has already proven to be a guide in therapy management by evaluating the chemotherapy effect after 1 or 2 cycles.[18, 19] The metabolic response in non-Hodgkin's lymphoma patients is correlated with treatment failure and disease free survival.[19] To assess the potential guiding role of ^{18}F-FDG PET during infliximab treatment in sarcoidosis, a prospective and blinded study with a larger patient population is needed.

In conclusion, changes in ^{18}F-FDG PET correlate with signs of clinical improvement in sarcoidosis patients during infliximab treatment to a great extent. There is even a significant correlation between the decrease in SUV_{max} of the lung parenchyma and improvement of VC. The current study seems to confirm that ^{18}F-FDG uptake in sarcoidosis represents active disease and might warrant additional research to evaluate the potential role of ^{18}F-FDG PET in sarcoidosis management.

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Chapter 7

^{18}F-FDG PET based classification of sarcoidosis

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Submitted

Abstract

Introduction

In sarcoidosis, various phenotypic presentations are known, all correlating with a certain clinical outcome and thus patient management. Adequate establishment of the phenotypes is therefore important. ^{18}F-FDG PET has proven to be reliable in depicting active sarcoidosis. This whole body imaging technique may therefore contribute to the development of a classification system, representing the sarcoidosis phenotype. In this study, we introduce an ^{18}F-FDG PET based classification system for sarcoidosis.

Methods

In 78 sarcoidosis patients, ^{18}F-FDG PET was retrospectively categorized by three reviewers, based on four patterns. Type I was defined as 'metabolic activity in mediastinal and hilar lymph nodes' (thoracic lymph node type), type II as 'metabolic activity in the lung parenchyma with or without mediastinal and hilar lymph nodes' (lung parenchymal type), type III as 'metabolic activity in lymph nodes throughout the body' (lymphogenic type) and type IV as 'metabolic activity in organs other than the lungs' (organ type). Inter observer agreement was determined by Cohen's kappa and subsequently, consensus was reached.

Results

Based on consensus, sarcoidosis was limited to thoracic lymph nodes in 27 patients (35%). The lung parenchymal type was found in 34 patients (44%). Only four patients (5%) demonstrated a lymphogenic type while 13 patients (17%) had organ involvement. The inter observer agreement was good (kappa 0.69-0.71).

Conclusion

Classification of sarcoidosis based on four major ^{18}F-FDG PET patterns demonstrates a high reproducibility and may therefore be of scientific and clinical significance, warranting future research.

Introduction

Sarcoidosis is a multisystem granulomatous disease of unknown cause. It commonly affects young and middle-aged adults and frequently presents with bilateral hilar and mediastinal lymphadenopathy.[1, 2] Pulmonary infiltration, skin and ocular lesions as well as involvement of the heart, liver, spleen, bones, muscles and nervous system may be found. The diagnosis of sarcoidosis is based on the presence of noncaseating granulomas with exclusion of other granulomatous disorders and associated clinical and radiological findings.[1] Not only the granulomas are non-specific for sarcoidosis, also the clinical and radiological findings are part of a broad differential diagnosis.[3] Assessment of disease severity is as important as the description of the extent of sarcoidosis. For example, a small area of inflammation in the myocardium may have devastating consequences, while more extensive inflammation in the skin is usually not fatal.[4]

Although the causes of sarcoidosis remain unknown, the recognition of race as an important risk factor, occasional familial clustering and association of the major histocompatibility complex with specific forms of disease presentation clearly points out towards genetic predisposition.[4-6] Investigators have relied mainly on candidate gene association studies to search for susceptibility genes.[1] The ongoing refinement of genetic marker maps, genotyping technology, and statistical analyses makes genomic exploration for sarcoidosis genes appealing.[7]

Accurate assessment of organs involved in sarcoidosis activity could potentially be valuable in gene studies since gene linkage with specific sarcoidosis phenotypes may result in stronger associations than with the sarcoidosis population as a whole. Recently, a strong association between Löfgren's syndrome and variants of genes on chromosome 3 in the vicinity of CCR2 have been described.[8, 9] It would be very interesting to define more sarcoidosis phenotypes and associate these phenotypes with genetic profiles.[9] Defining sarcoidosis phenotypes implies proper techniques to locate all organ manifestations. Traditional sarcoidosis classification systems rely on the chest radiograph Scadding stage. Like recently developed scoring systems with high-resolution computed tomography (HRCT) these techniques focus only on pulmonary disease.[4, 9] Clinical comprehensive scoring systems are mainly focusing on disease severity and less on disease extension.[4, 10, 11]

Recently, several studies and many case reports have been published about the

use of Fluor-18 deoxyglucose (^{18}F-FDG) PET in the assessment of disease activity of sarcoidosis. In these articles, an excellent sensitivity was found which varied between 97% and 100%.[12-17] Unlike other imaging modalities, ^{18}F-FDG PET clearly demonstrated many sites with extra pulmonary sarcoidosis activity.

Our goal was to develop a sarcoidosis classification system based on ^{18}F-FDG PET that would represent the different phenotypic presentations of the disease. If the proposed system is reproducible, ^{18}F-FDG PET patterns could be associated with disease outcome and genetic marker maps in future studies.

Materials and Methods

Patients

In this retrospective analysis, 78 consecutive patients with histologically proven sarcoidosis were evaluated. Patients with normal ^{18}F-FDG PET scans were not included. All patients were seen at the department of pulmonology of the St. Antonius Hospital between January 2004 and August 2007. The diagnosis of sarcoidosis was based on clinical findings, supported by histological evidence and after the exclusion of other known causes of granulomatosis in accordance with the consensus statement on sarcoidosis of the American Thoracic Society (ATS) / European Respiratory Society (ERS) / World Association of Sarcoidosis and other Granulomatous Disorders (WASOG).[1] Patients underwent ^{18}F-FDG PET as part of their routine analysis. Chest radiographic stages according to Scadding were evaluated.[18]

^{18}F-FDG PET

Patients fasted for at least six hours and before the intravenous injection of ^{18}F-FDG, 5 milligram of diazepam was administered to reduce muscle activity and accumulation of ^{18}F-FDG in brown fat. In order to reduce radiation exposure and accelerate ^{18}F-FDG excretion by the kidneys, 20 milligrams of furosemide was injected intravenously. Subsequently, 295-400 MBq ^{18}F-FDG (Covidien, Petten, the Netherlands) was administered intravenously. 295 MBq ^{18}F-FDG was given to patients with a body weight less than 80 kilograms. When the body weight exceeded 80 kilograms a calculated dose was used (body weight /10 * 37 MBq) with a maximum of 400 MBq ^{18}F-FDG. PET was performed using the Philips Allegro PET system with external Cesium-137 source for transmission scanning (Philips Medical Systems, Eindhoven, the Netherlands). Sixty minutes after administration of ^{18}F-FDG, transmission scan was started. Transmission time was 23 seconds per bed position. Emission scan was performed from the subinguinal region to the head with an acquisition time of three minutes per bed position. Reconstruction was performed in accordance with the 3D-RAMLA protocol applying 4 iterations with a 144 x 144 matrix.[19]

^{18}F-FDG PET classification

Based on ^{18}F-FDG PET, patients were categorized by three readers. In Figure 1 to 4, the suggested categories are presented. Type I is defined as 'metabolic activity exclusively in mediastinal and hilar lymph nodes' (thoracic lymph node type) and type II as 'metabolic activity in the lung parenchyma with or without (extra) thoracic lymph nodes' (lung parenchymal type). In the latter, other organs are not affected. Type III is defined as 'metabolic activity in lymph nodes throughout the body' (lymphogenic type) and type IV as 'activity in organs other than the lungs, *i.e.* spleen, bone marrow, muscles'(organ type). In type IV, thoracic disease might be present.

Two experienced nuclear medicine physicians interpreted ^{18}F-FDG PET, followed by a pulmonologist, specialized in interstitial lung diseases. All physicians reviewed the anonymous ^{18}F-FDG PET images independently. In a subsequent meeting, consensus was reached in patients in which disagreement was present.

Statistical analysis

The statistical evaluation was performed using SPSS 16 (SPSS Inc, Chicago, IL, USA). Cohen's kappa was used to determine the inter observer agreement in categorizing ^{18}F-FDG PET. The strength of agreement, expressed as kappa value, can be subdivided into 5 categories: < 0.20 (poor), 0.21-40 (fair), 0.41-0.60 (moderate), 0.61-0.80 (good) and 0.81-1.0 (very good).[20]

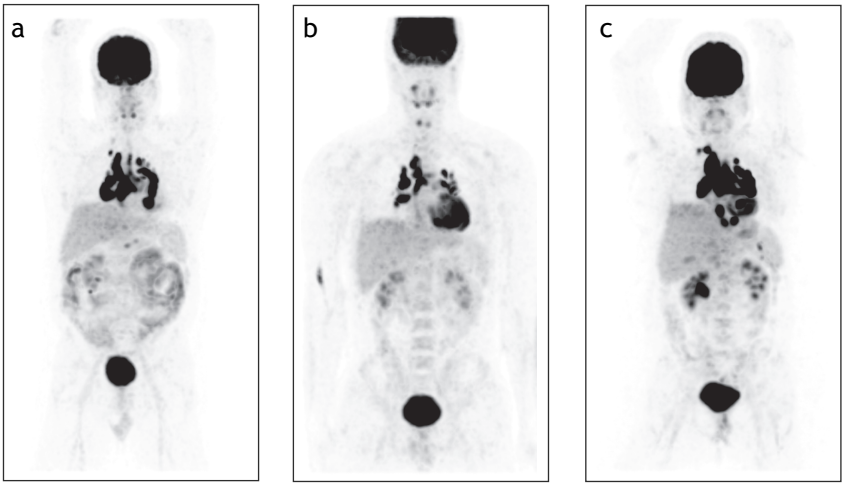

Figure 1 ^{18}F-FDG PET type I is defined as metabolic activity in mediastinal and hilar lymph nodes without extra pulmonary disease.

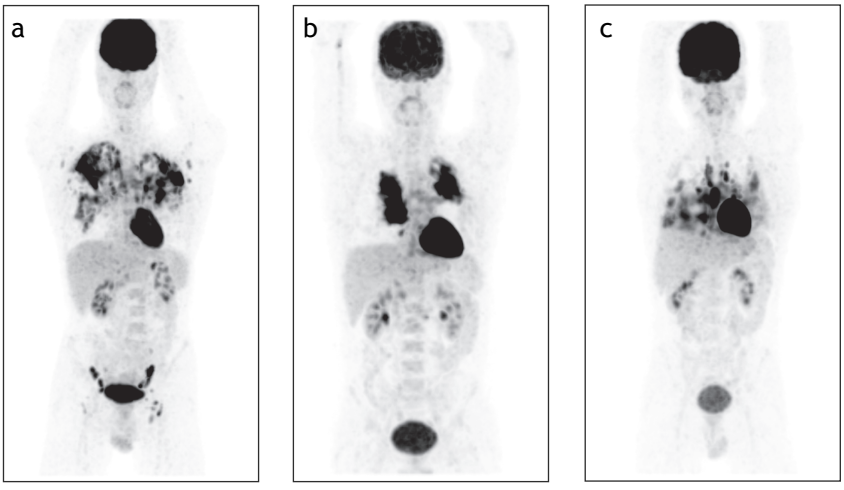

Figure 2 ^{18}F-FDG PET type II is defined as metabolic activity in the lung parenchyma with or without mediastinal, hilar and extra thoracic lymph nodes. Organs other than the lung are not affected.

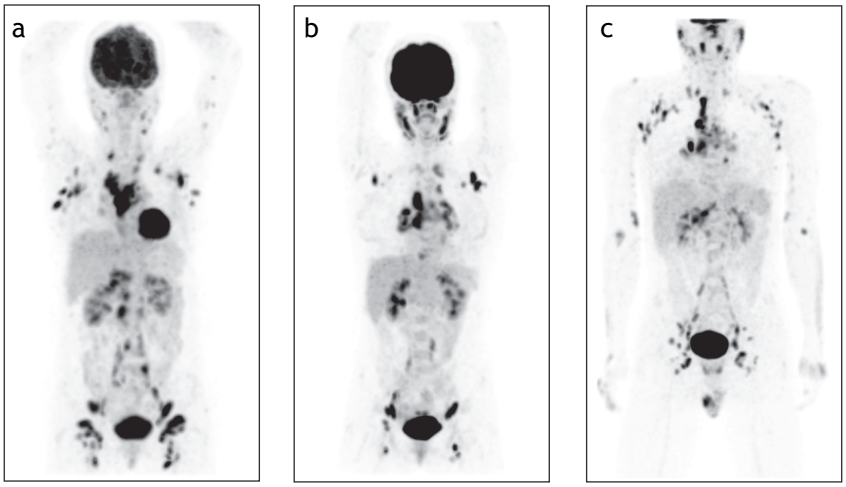

Figure 3 ^{18}F-FDG PET type III is defined as metabolic activity in mediastinal, hilar and extra thoracic lymph nodes. The lung parenchyma and other organs are not involved.

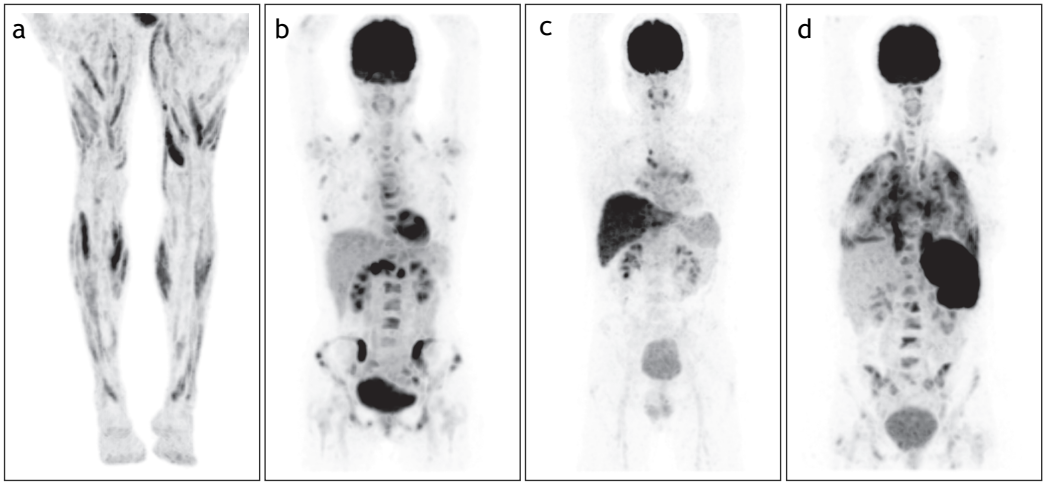

Figure 4 ^{18}F-FDG PET type IV is defined as metabolic activity in organs other than the lung although the lung might be involved. Sarcoidosis activity is present in muscles (a), bone marrow (b), liver (c) and spleen (d). In the latter, the lung parenchyma, lymph nodes in mediastinum and hila and bone marrow are affected as well.

Results

Patients

^{18}F-FDG PET of 78 sarcoidosis patients were retrospectively analyzed. Patients' characteristics are summarized in Table 1. In all patients, sarcoidosis was histologically confirmed. The time interval between histological confirmation of sarcoidosis and ^{18}F-FDG PET was 21.6 months (range: 2 days - 13.2 years). In 59 patients, sarcoidosis was diagnosed within 2 years of ^{18}F-FDG PET. In 19 patients, the time interval between histology and ^{18}F-FDG PET exceeded two years. None of these patients were diagnosed with Löfgren's syndrome.

Table 1 Patient characteristics

	n = 78
Age (years)	49 (range 26-79)
Sex	
Female	31
Male	47
Disease duration	
< 2 years	59
> 2 years	19
Chest radiographic stage	
0	6
I	20
II	34
III	12
IV	6

According to the chest radiographic stages, there were 6 patients with stage 0 disease. In 20 patients, sarcoidosis was limited to hilar lymph nodes (radiographic stage I) and 46 patients did have parenchymal abnormalities (radiographic stage II/III). Six patients had signs of fibrosis (radiographic stage IV).

^{18}F-FDG PET classification

In Table 2, the ^{18}F-FDG PET classification results of the 3 observers are shown as well as the consensus. In 27 patients (35%), the 'thoracic lymph node type' was found. Lung parenchymal disease was present in 34 (44%). Out of these patients, 18 (23%) demonstrated extra thoracic lymph nodes. In only 4 patients (5%), the 'diffuse lymph node' type was present, while the 'organ type' was found in 13 patients (17%). Thus, extra thoracic disease in organs and lymph nodes was present in 35 patients (45%).

Table 2 ^{18}F-FDG PET based classification of sarcoidosis, *n* = 78

		Observer 1	Observer 2	Observer 3	Consensus
Type I	Thoracic lymph nodes	30 (39%)	34 (44%)	31 (40%)	27 (35%)
Type II	Lung parenchyma	28 (36%)	25 (32%)	35 (45%)	34 (44%)
Type III	Diffuse lymph nodes	5 (6%)	11 (14%)	4 (5%)	4 (5%)
Type IV	Organs	15 (19%)	8 (10%)	8 (10%)	13 (17%)

Inter observer agreement

Cohen's kappa between the nuclear medicine physicians was 0.70. Cohen's kappa between the pulmonologist and the two nuclear medicine physicians was 0.69 and 0.71, respectively. In 22 patients (28%), the reviewers disagreed.

Table 3 Chest radiographic stages per ^{18}F-FDG PET type

		n	Stage 0	Stage I	Stage II	Stage III	Stage IV
Type I	Thoracic lymph nodes	27	3 (11%)	16 (59%)	6 (22%)	2 (7%)	-
Type II	Lung parenchyma	34	1 (3%)	1 (3%)	19 (60%)	8 (24%)	5 (15%)
Type III	Diffuse lymph nodes	4	1 (25%)	1 (25%)	2 (50%)	-	-
Type IV	Organs	13	1 (8%)	2 (15%)	7 (54%)	2 (15%)	1 (8%)

Stage 0 = no abnormalities, stage I = bihilar lymphadenopathy, stage II = bihilar lymphadenopathy and parenchymal infiltrates, stage III = parenchymal infiltrates, stage IV = signs of fibrosis

Discussion

Sarcoidosis is known for its variety in phenotypic presentation. Each phenotype correlates with a certain clinical outcome and consequently with patient management. It is therefore of importance that different phenotypes are adequately determined.
Traditionally, the first choice in diagnostic imaging is conventional chest radiography and HRCT. In contrast with these techniques, ^{18}F-FDG PET reveals the overall involvement of the human body and secondly, it demonstrates sarcoidosis activity, *i.e.* reversible disease.[12, 14, 21] In the present study, ^{18}F-FDG PET patterns in sarcoidosis patients were categorized based on the presence and extent of organ involvement. The involvement of thoracic lymph nodes and the lung parenchyma were taken into account as well as the presence of extra thoracic disease.

In the current study, 4 recognizable and straightforward ^{18}F-FDG PET patterns were used, called the 'thoracic lymph node type' (I), 'lung parenchymal type' (II), 'diffuse lymph node type' (III) and 'organ type' (IV). In a substantial part of the studied population (45%), extra thoracic disease was present in lymph nodes or organs, demonstrating the value of ^{18}F-FDG PET compared to conventional radiographic imaging. Independent evaluation of the ^{18}F-FDG PET images by three reviewers demonstrated a good inter observer agreement (Cohen's kappa 0.70), implying that the introduced classification system is reproducible.

Sarcoidosis is known for its high spontaneous remission rate.[22] However, parenchymal disease, splenomegaly and involvement of more than three organ systems is associated with a poor prognosis.[23, 24] These 3 parameters could easily be assessed by ^{18}F-FDG PET and are, in part, represented by the currently used classification. Type IV of our ^{18}F-FDG PET classification system was defined as 'organ involvement', but a precise identification of the affected organ was not provided. Therefore, a classification incorporating the involved organ is desired in future studies. Such studies are required to assess whether the introduced ^{18}F-FDG PET types indeed correlate with clinical outcome and predict disease remission.
Certain HLA genes are associated with the prognosis of sarcoidosis. For example, HLA-DQB1*0201 correlates with a good prognosis while HLA-DQB1*1501 and HLA-

DQB1*0602 have been associated with chronic and severe disease.[25, 26] In line with the previously suggested research, a point of interest would be whether ^{18}F-FDG PET patterns have a genetic base and demonstrate HLA associations. When the suggested correlations can be found, ^{18}F-FDG PET might contribute to the understanding of the pathogenesis of sarcoidosis.
In the currently used system the sum of metabolic activity per organ could not be incorporated. The fact is, that quantitative analysis programs assessing the amount of ^{18}F-FDG in a specific organ are not available to date. Such analysis might be relevant, particularly in patients with lung parenchymal disease. Clinical outcome may differ in patients with subtle metabolic activity in the lung parenchyma compared to those with high activity involving all lobes. It would therefore be interesting to compare the amount and extent of lung parenchymal activity with sarcoidosis severity and lung functional outcome.

In conclusion, the ^{18}F-FDG PET based classification of sarcoidosis into four major types, demonstrated a good inter observer agreement. Future studies correlating these types with disease outcome and the need for therapy will be performed to assess the clinical usefulness of this promising technique.

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Chapter 8

Summary, discussion and future perspectives

Summary

^{18}F-FDG PET is an in-vivo, non invasive imaging technique. It has obtained a crucial role in oncology and its application is still evolving. In this thesis, the utility of ^{18}F-FDG PET in the assessment of sarcoidosis activity was evaluated. The search for an instrument to improve the accurate assessment of active disease, was initiated by the lack of a gold standard for sarcoidosis activity. Currently, a combination of clinical signs and symptoms, biomarkers, chest radiography, bronchoalveolar lavage and pulmonary function tests are used to assess the presence of active disease. ^{18}F-FDG PET was therefore compared with the aforementioned activity parameters and its use in evaluating treatment efficacy was determined.

In **Chapter 1**, the immunopathogenesis and genetics of sarcoidosis are described, as well as the consensus statement on sarcoidosis activity. Radiological imaging of sarcoidosis is described, as well as the three possible nuclear imaging techniques in sarcoidosis, *i.e.* ^{67}Ga scintigraphy, ^{18}F-FDG PET and somatostatin receptor scintigraphy.

For many years, ^{67}Ga scintigraphy was used in the assessment of sarcoidosis activity. However, the resolution of ^{67}Ga scintigraphy is low, its radiation dose is high and image acquisition is only possible 48 hours after injection of the radiopharmaceutical. At the end of the 20th century, ^{18}F-FDG PET demonstrated a high sensitivity for active sarcoidosis. Compared to ^{67}Ga scintigraphy, the radiation dose is reduced with 60% and its resolution is higher. Moreover, ^{18}F-FDG PET is completed within 2 hours. **Chapter 2** describes the prospective analysis of ^{67}Ga imaging and ^{18}F-FDG PET with regard to the sensitivity of sarcoidosis activity and the inter observer agreement of both techniques. Disease activity in the lung parenchyma was equally visualised, while ^{18}F-FDG PET revealed more lesions in the mediastinum, hila, lymph nodes and extra pulmonary regions in general. Furthermore, the inter observer agreement was higher for ^{18}F-FDG PET. In ^{67}Ga scintigraphy, SPECT appeared to have an unacceptable inter observer agreement, particularly for the lung parenchyma. From our results, we can conclude that ^{18}F-FDG PET is the nuclear imaging technique of choice in the assessment of sarcoidosis activity.

Angiotensin-converting enzyme (ACE) is the most widely used serological marker for sarcoidosis activity. It is produced by epitheloid cells and macrophages and is thought to represent the total granuloma load. Furthermore, it is recommended for therapy monitoring. Until recently, sensitivity of ACE was only moderate but improved significantly after the discovery of the insertion/deletion polymorphisms. Soluble interleukin-2 receptor (sIL-2R) derives from activated lymphocytes and is used to estimate the total inflammatory activity of sarcoidosis. In **Chapter 3**, the sensitivity of genotype corrected ACE and sIL-2R is compared with ^{18}F-FDG PET in newly diagnosed sarcoidosis patients. ACE and sIL-2R had a sensitivity of only 36% and 47%, respectively, while this was 94% for ^{18}F-FDG PET. As representatives of the total amount of active disease, ACE and sIL-2R were compared with the extent of metabolic activity, expressed as standardized uptake value (SUV). The maximum and average SUV did however, not correlate with the two biomarkers. Our data suggest that ^{18}F-FDG PET may be considered in patients with normal serum markers, particularly in those patients who are still suspected of having active disease.

Bronchoalveolar lavage (BAL) is recommended during the diagnostic process of sarcoidosis. Specific BAL features may indicate the diagnosis of sarcoidosis and exclude other interstitial lung diseases. BAL lymphocytes exceeding 15%, represent a lymphocytic alveolitis and is very sensitive. $CD4^+$ T lymphocytes are the dominating cell type, represented by an increased $CD4^+/CD8^+$ ratio. Lymphocytes and the $CD4^+/CD8^+$ ratio do not have a predictive value, but neutrophils correlate with the occurrence of fibrosis. Potentially more discriminative are the $CD103^+$ T cells, a subset of $CD4^+$ T cells. The $CD103^+CD4^+/CD4^+$ ratio is usually low in sarcoidosis, but appears somewhat higher in patients with parenchymal disease. In **Chapter 4**, BAL cell profiles and ^{18}F-FDG PET are compared in newly diagnosed, pulmonary sarcoidosis patients. ^{18}F-FDG PET was positive in 97% of the patients, with increased thoracic activity in 95%. In patients with exclusive mediastinal/hilar activity, BAL lymphocytes were increased in 91%, $CD4^+/CD8^+$ ratio in 65% and neutrophils in 13% while $CD103^+CD4^+/CD4^+$ ratio was decreased in 74%. In patients with metabolic activity in the lung parenchyma, BAL lymphocytes were increased in 72%, $CD4^+/CD8^+$ ratio in 60% and neutrophils in 34% with a decreased $CD103^+CD4^+/CD4^+$ ratio in 70%. Overall, a moderate, though significant correlation was found between SUV_{max} in the mediastinum/hila and the $CD4^+/CD8^+$ ratio. Additionally, SUV_{max} in the lung

parenchyma correlated with the percentage of neutrophils. Like lymphocytes and the $CD4^+/CD8^+$ ratio, SUV_{max} in the mediastinum/hila was inversely related with the chest radiographic stage. SUV_{max} in the lung parenchyma showed a positive correlation with the chest radiographic stage, like the $CD103^+CD4^+/CD4^+$ ratio and neutrophils. These data imply that ^{18}F-FDG PET represents the $CD4^+/CD8^+$ ratio in BAL as well as the number of neutrophils.

Ongoing inflammatory activity in the lung parenchyma can be reflected by a decline in pulmonary function tests (PFT). However, before a decrease in PFT becomes evident, sarcoidosis needs to progress first, potentially accompanied by a clinical deterioration.

Chapter 5 describes the significance of metabolic activity in the lung parenchyma imaged by ^{18}F-FDG PET. In newly diagnosed sarcoidosis patients, baseline ^{18}F-FDG PET was correlated with the change in PFT during one year follow-up. Untreated patients with diffuse metabolic activity in the lung parenchyma showed a significant decrease in diffusion capacity of lung for carbon monoxide (DLCO). A change in vital capacity (VC) or forced expiratory volume in one second (FEV_1) could not be found. Immunosuppressive therapy was required in 36% of the patients before follow-up was completed.

Patients with diffuse lung parenchymal activity, receiving immunosuppressive therapy after ^{18}F-FDG PET, demonstrated a significant improvement in DLCO, VC and FEV_1. In these patients, ^{18}F-FDG PET demonstrates that metabolic activity represents active, reversible disease and that improvement can be achieved.

In untreated patients with metabolic activity in the mediastinum and hila but without activity in the lung parenchyma, no change in DLCO, VC or FEV_1 could be found. The results of this study suggest that diffuse parenchymal disease imaged by ^{18}F-FDG PET, predicts a future deterioration of DLCO when untreated, while absent metabolic activity in the lung parenchyma justifies a wait-and-see policy.

Tumor necrosis factor-α (TNF-α) is one of the cytokines involved in the formation of granulomas in sarcoidosis. Anti-TNF-α therapy might be effective in patients with severe pulmonary and extra pulmonary disease. Several parameters are currently used for therapy decisions and to evaluate treatment efficacy, such as a change in symptoms, ACE, sIL-2R, chest radiography and pulmonary function tests. However, none of these parameters can be used as a gold standard. In **Chapter 6**, changes in ^{18}F-FDG PET were correlated with standard sarcoidosis activity parameters during

infliximab treatment. Twelve patients with refractory sarcoidosis received 6 cycles of infliximab. Prior to therapy, ACE was increased in 6 patients (50%), while sIL-2R was increased in 11 patients (92%).
Clinical improvement as judged by conventional parameters was seen in all patients, though with a minor response in one. Symptoms improved in 11 out of 12 patients but the chest radiographic stages did not change. A significant decrease in ACE and sIL-2R was found with an improvement of VC and DLCO. ^{18}F-FDG PET revealed either improvement or normalization in 11 out of 12 clinically responding patients. The overall decrease in SUV_{max} was 55%, but an increase of 34% was seen in the patient with a limited response. Furthermore, the decrease in SUV_{max} of the lung parenchyma correlated with an improvement of VC.
From these data, we conclude that changes imaged by ^{18}F-FDG PET during infliximab treatment in sarcoidosis patients correlate with signs of clinical improvement.

In sarcoidosis, various phenotypic presentations are known, all correlating with a certain clinical outcome and thus patient management. Adequate establishment of the phenotypes is therefore important. In **Chapter 7**, an ^{18}F-FDG PET based classification system for sarcoidosis is introduced. ^{18}F-FDG PET was categorized by three reviewers, based on four patterns. Type I was defined as 'metabolic activity in mediastinal and hilar lymph nodes' (thoracic lymph node type), type II as 'metabolic activity in the lung parenchyma with or without mediastinal and hilar lymph nodes' (lung parenchymal type), type III as 'metabolic activity in lymph nodes throughout the body' (lymphogenic type) and type IV as 'metabolic activity in organs other than the lungs' (organ type).
Based on consensus of the reviewers, sarcoidosis was limited to thoracic lymph nodes in 35% of the patients. The lung parenchymal type was found in 44%. Only 5% of the patients demonstrated a lymphogenic type while 17% had organ involvement. The additionally evaluated inter observer agreement showed a kappa of 0.70, demonstrating that classification of sarcoidosis based on four major ^{18}F-FDG PET patterns is reproducible.

Conclusions

The following conclusions may be drawn from the studies described in this thesis:

- Compared to ^{67}Ga scintigraphy, ^{18}F-FDG PET is more sensitive in assessing sarcoidosis activity and demonstrates a higher inter observer agreement
- ^{18}F-FDG PET appears more sensitive than genotype corrected ACE and sIL-2R in determining the presence of active disease
- The degree of metabolic activity in the pulmonary tract imaged by ^{18}F-FDG PET and expressed as SUV_{max}, correlates with the $CD4^+/CD8^+$ ratio and neutrophils in BAL
- In untreated patients, the absence of parenchymal activity imaged by ^{18}F-FDG PET correlates with stable pulmonary function tests after one year, while diffuse parenchymal activity predicts a decrease in DLCO
- In patients with diffuse parenchymal activity imaged by ^{18}F-FDG PET, pulmonary function tests improve by using immunosuppressive therapy, suggesting that ^{18}F-FDG PET presents the possible improvement that can be achieved
- In chronic sarcoidosis patients treated with infliximab, changes in ^{18}F-FDG PET correlate with the clinically observed response
- In chronic sarcoidosis patients, the change in SUV_{max} of the lung parenchyma correlates with the change in VC attained by infliximab therapy
- An ^{18}F-FDG PET based classification system of sarcoidosis incorporating four major phenotypic presentations is reproducible

Discussion

In this thesis, the value of ^{18}F-FDG PET is demonstrated in newly diagnosed sarcoidosis patients. One might discuss the necessity of ^{18}F-FDG PET in the overall sarcoidosis population and how ^{18}F-FDG PET should be implemented in clinical practice.

The presence of active disease is most obvious in patients with the initial diagnosis of sarcoidosis. However, assessment of activity remains laborious in those with previously diagnosed disease and persistent symptoms.

In general, the tests performed at the onset of the disease will be repeated when sarcoidosis recurs or becomes progressive, with the exception of bronchoalveolar lavage and histology. Biomarkers might be normal due to the use of immunosuppressive therapy and symptoms remain present in a substantial part of the patients, even when the disease has become inactive. Chest radiography may not change or show signs of fibrosis. In such patients, the clinician is hampered by the absence of an accurate test to assess sarcoidosis activity. As described in this thesis, similar patients with chronic sarcoidosis were evaluated by ^{18}F-FDG PET. Not only the presence of ongoing inflammatory activity was demonstrated, but ^{18}F-FDG PET also correlated with the improvements induced by infliximab therapy. The change in SUV_{max} of the lung parenchyma was related to the change in VC, demonstrating that ^{18}F-FDG PET represents the potential improvement that can be achieved by initiating therapy.

Accordingly, patients suspected of having sarcoidosis recurrence or progression may benefit from the introduction of ^{18}F-FDG PET as it can aid in the accurate assessment of disease activity and thus in optimizing patient management.

The prognosis of sarcoidosis is associated with several clinical variables. Splenomegaly, the involvement of more than 3 organ systems, stage III pulmonary disease, black race, disease onset after the age of 40, symptoms lasting for more than 6 months and the absence of erythema nodosum correlate with a poorer prognosis. ^{18}F-FDG PET is able to assess the extent of the disease, *i.e.* the number of affected organ systems, as well as parenchymal involvement. Unlike conventional chest radiography and HRCT of the chest, functional imaging by ^{18}F-FDG PET reveals the overall involvement of the human body and might therefore be of prognostic significance.

In this thesis, an ^{18}F-FDG PET based classification system of sarcoidosis was introduced.
Refining this ^{18}F-FDG PET system would incorporate the ability to 1) discriminate between lung parenchymal disease activity and/or mediastinal and hilar lymph nodes, 2) give a quantitative calculation of the amount of disease activity in the lung parenchyma and 3) subdivide extra pulmonary activity into lymph nodes and in organs with or without direct therapeutic consequences. Adequate staging of the extent and severity of sarcoidosis could be used in future studies assessing ^{18}F-FDG PET with regard to clinical outcome. In addition, such a classification system facilitates the association between ^{18}F-FDG PET phenotypes and (non) HLA genes.

The above requires prospective cohort studies, assessing the significance of pulmonary and extra pulmonary ^{18}F-FDG PET findings with regard to clinical outcome. As long as these studies haven't been performed, ^{18}F-FDG PET could be advised in patients suspected of having active disease with normal conventional activity markers.

Future perspectives

Elaborating upon the currently available ^{18}F-FDG PET studies in sarcoidosis, the following research could be performed:

- Prospective cohort studies assessing the value of ^{18}F-FDG PET with regard to clinical outcome
- Quantifying the amount of ^{18}F-FDG in the lung parenchyma to correlate with pulmonary function tests during follow-up
- Evaluating ^{18}F-FDG PET in patients with longstanding sarcoidosis
- Autoradiographic studies of granulomas in sarcoidosis to understand the uptake mechanism of ^{18}F-FDG and gain insights into the evolving granuloma
- Incorporating the extent and severity of sarcoidosis activity in an ^{18}F-FDG PET based classification system of sarcoidosis
- Correlate the ^{18}F-FDG PET based classification system of sarcoidosis with (non) HLA genes
- The assessment of active cardiac and neurosarcoidosis by (^{18}F-FDG) PET
- ^{18}F-FDG PET in evaluating treatment efficacy and tailored therapy

Samenvatting in het Nederlands

Sarcoïdose is een ziekte die gekenmerkt wordt door de aanwezigheid van granulomateuze ontstekingen. Deze ontstekingen kunnen ontstaan in alle organen, maar de longen, huid en ogen zijn het meest frequent aangedaan. Het is niet bekend hoe de ziekte ontstaat, maar men vermoed dat blootstelling aan een onbekende organische of anorganische stof dan wel een infectieus agens leidt tot een afweer reactie van het lichaam. Deze afweer reactie leidt tot granuloomvorming.
Sarcoïdose ontstaat meestal vóór het 40e levensjaar en komt iets vaker voor bij vrouwen. De incidentie van sarcoïdose varieert van 10 tot 40 per 100.000 personen en komt met name voor in de Verenigde Staten, Japan en de noordelijke Europese regio's. De mortaliteit is 1% tot 5%, merendeels veroorzaakt door destructie van de longen.
Sarcoïdose kent een hoge kans op spontane remissie, maar toch treedt bij een derde van de patiënten enige vorm van orgaan beschadiging op. Sommige klinische bevindingen correleren met een slechtere prognose van de ziekte zoals een vergrote milt, betrokkenheid van meer dan 3 orgaan systemen, afwijkingen in het long parenchym, de afwezigheid van erythema nodosum, afro-amerikaanse komaf, aanvang van de ziekte na het 40e levensjaar en symptomen die langer duren dan 6 maanden.
Behandeling met corticosteroïden is geïndiceerd wanneer er sprake is van ernstige aantasting van longen, hart, ogen en hersenen alsook bij de aanwezigheid van een verhoogd calcium. De keuze voor andere medicatie hangt af van de ernst van de ziekte alsook de effectiviteit en bijwerkingen van corticosteroïden.

^{18}F-FDG PET is een in vivo, niet invasieve beeldvormende techniek. ^{18}F-FDG PET heeft een belangrijke rol in de oncologie verkregen en de toepassing van deze techniek breidt zich steeds verder uit. In dit proefschrift wordt het gebruik van ^{18}F-FDG PET bij het vaststellen van sarcoïdose activiteit geëvalueerd. De zoektocht naar een techniek die actieve sarcoïdose adequaat kan vaststellen werd geïnitieerd door het ontbreken van een goud standaard voor het vaststellen van ziekte activiteit. Momenteel wordt een combinatie van klinische bevindingen, symptomen, serum markers, X-thorax, bronchoalveolaire lavage en long functie testen gebruikt om de aanwezigheid van actieve ziekte vast te stellen. Om te kunnen bepalen of ^{18}-FDG PET ook in staat is om actieve sarcoïdose weer te geven werd deze techniek

vergeleken met de eerdergenoemde activiteitsparameters en tevens werd het effect van therapie geëvalueerd middels deze techniek.

In **Hoofdstuk 1** wordt de ontstaanswijze van sarcoïdose besproken, de genetische aspecten die hierbij van invloed kunnen zijn en de consensus voor het vaststellen van ziekte activiteit. Radiologische technieken om sarcoïdose weer te geven worden uiteengezet alsook de drie mogelijke nucleair geneeskundige technieken, te weten ^{67}Ga scintigrafie, ^{18}F-FDG PET en somatostatine receptor scintigrafie.

Gedurende vele jaren is ^{67}Ga scintigrafie gebruikt ter bepaling van de sarcoïdose activiteit. Echter, de resolutie van ^{67}Ga scintigrafie is matig en de stralingsdosis is hoog. Daarnaast is het vervaardigen van de beelden pas mogelijk 24 uur na toediening van de radioactiviteit wat betekent dat de patiënt twee keer naar het ziekenhuis moet komen.
Aan het einde van de 20^{e} eeuw werd bekend dat ^{18}F-FDG PET goed in staat is om sarcoïdose activiteit vast te stellen. In vergelijking met ^{67}Ga scintigrafie, is de stralenbelasting 60% lager, de resolutie hoger en het onderzoek is afgerond binnen 2 uur.
In **Hoofdstuk 2** wordt een prospectieve analyse beschreven van ^{67}Ga scintigrafie en ^{18}F-FDG PET in nieuw gediagnosticeerde sarcoïdose patiënten met betrekking tot het vaststellen van actieve ziekte. Tevens wordt de mate van overeenstemming tussen twee beoordelaars besproken. ^{67}Ga scintigrafie en ^{18}F-FDG PET bleken gelijkwaardig in het vaststellen van ziekte activiteit in de longen, maar ^{18}F-FDG PET liet meer laesies zien in het mediastinum, hili, extra thoracale lymfklieren en in organen anders dan de longen. De overeenstemming tussen de beoordelaars was hoger voor ^{18}F-FDG PET dan voor ^{67}Ga scintigrafie. Omdat ^{18}F-FDG PET drie dimensionaal wordt weergegeven, werden er ook bij de ^{67}Ga scintigrafie 3-D opnames gemaakt van de thorax, zogenaamde SPECT beelden. Ondanks dat SPECT de interpretatie van beelden moet vereenvoudigen, bleek de overeenstemming van de beoordelaars voor deze SPECT beelden zeer laag. Uit deze studie kunnen we concluderen dat ^{18}F-FDG PET als nucleaire techniek de voorkeur heeft bij het bepalen van sarcoïdose activiteit.

Angiotensine converting enzym is wereldwijd de meest gebruikte serologische marker voor het bepalen van sarcoïdose activiteit. Het wordt geproduceerd door

epitheloid cellen en macrofagen, twee celtypen aanwezig in het granuloom. De hoogte van het ACE zou correleren met het aantal granulomen in het lichaam en ACE wordt aanbevolen om het effect van medicatie te monitoren. De sensitiviteit van ACE om sarcoïdose activiteit te meten was tot recent matig, maar steeg door de ontdekking van verschillende ACE genotypen. Soluble interleukine-2 receptor (sIL-2R) is afkomstig van geactiveerde lymfocyten en wordt gebruikt om de totale ontstekingsactiviteit van sarcoïdose te bepalen. In **Hoofdstuk 3** wordt de sensitiviteit van genotype gecorrigeerd ACE en sIL-2R vergeleken met ^{18}F-FDG PET in nieuw gediagnosticeerde sarcoïdose patiënten. ACE en sIL-2R hadden een sensitiviteit van respectievelijk 36% en 47%, terwijl ^{18}F-FDG PET een sensitiviteit van 94% liet zien. Omdat ACE en sIL-2R representatief zouden zijn voor de uitgebreidheid van actieve ziekte, werden deze parameters vergeleken met de mate van metabole activiteit zichtbaar middels ^{18}F-FDG PET, uitgedrukt als Standardized Uptake Value (SUV). Echter, zowel de maximale als gemiddelde SUV correleerden niet met de hoogte van deze twee markers. Onze data suggereren dat ^{18}F-FDG PET kan worden overwogen in patiënten met een normaal ACE en sIL-2R en waarbij klinisch de verdenking op actieve sarcoïdose bestaat.

Broncho-alveolaire lavage (BAL) wordt aanbevolen in het diagnostische proces van sarcoïdose. Specifieke BAL parameters kunnen wijzen op sarcoïdose, maar middels BAL kunnen ook andere longziekten worden uitgesloten. Een lymfocyten percentage boven de 15% indiceert een lymfocytaire alveolitis en is zeer sensitief. In sarcoïdose zijn de $CD4^+$ T lymfocyten het overheersende celtype in de BAL, wat wordt weergegeven in een verhoogde $CD4^+/CD8^+$ ratio. Hoewel het aantal lymfocyten en de $CD4^+/CD8^+$ ratio geen voorspellende waarde hebben, correleert het aantal neutrofielen met het optreden van long fibrose. $CD103^+$ T cellen zijn een type $CD4^+$ T cel. Deze $CD103^+$ T cellen zouden wellicht beter in staat zijn om sarcoïdose te diagnosticeren. De $CD103^+CD4^+/CD4^+$ ratio is meestal laag in sarcoïdose, maar is iets hoger in patiënten waarbij het long parenchym is aangedaan. In **Hoofdstuk 4** werden de verschillende BAL cel typen vergeleken met ^{18}F-FDG PET in nieuw gediagnosticeerde, pulmonale sarcoïdose patiënten. ^{18}F-FDG PET was positief in 97% van de patiënten en toonde in 95% van de patiënten tekenen van actieve pulmonale ziekte. In patiënten met actieve lymfeklieren in hili en mediastinum zonder parenchym afwijkingen, was het aantal BAL lymfocyten verhoogd in 91% van de patiënten, de $CD4^+/CD8^+$ ratio in 65% en het aantal neutrofielen in 13%

terwijl de $CD103^+CD4^+/CD4^+$ ratio verlaagd was in 74%. In patiënten met metabole activiteit in het long parenchym, toonde BAL in 72% van de patiënten een verhoogd aantal lymfocyten, in 60% een verhoogde $CD4^+/CD8^+$ ratio en in 34% een verhoogd aantal neutrofielen. Een verlaagde $CD103^+CD4^+/CD4^+$ ratio werd gezien in 70%. In de gehele populatie werd een matige, maar significante correlatie gezien tussen SUV_{max} in het mediastinum en hili en de $CD4^+/CD8^+$ ratio. De SUV_{max} in het long parenchym correleerde met het percentage neutrofielen.
Tevens werden de verschillende BAL cel typen en de SUV_{max} waarden gecorreleerd met het radiologische stadium van sarcoïdose. Het percentage lymfocyten, de $CD4^+/CD8^+$ ratio en de SUV_{max} in het mediastinum en hili bleken af te nemen naarmate het radiologische stadium toenam, terwijl de SUV_{max} in het long parenchym en het percentage neutrofielen hoger bleek bij een hoger radiologisch stadium. Deze data suggereren dat ^{18}F-FDG PET de $CD4^+/CD8^+$ ratio in de BAL weergeeft alsook het percentage neutrofielen.

Voortdurende ontstekingsactiviteit in het long parenchym kan worden teruggezien in een achteruitgang van de longfunctie. Echter, voordat een achteruitgang van de longfunctie zichtbaar wordt moet de ziekte blijven voortschrijden wat gepaard kan gaan met een klinische achteruitgang van de patiënt. **Hoofdstuk 5** beschrijft de betekenis van metabole activiteit in het long parenchym weergegeven middels ^{18}F-FDG PET. In nieuw gediagnosticeerde sarcoïdose patiënten werden de bevindingen van ^{18}F-FDG PET, vervaardigd ten tijde van de diagnose, gecorreleerd met de veranderingen in de longfunctie gedurende een follow-up van één jaar. Onbehandelde patiënten met diffuse metabole activiteit in het long parenchym toonden een significante daling van de diffusie capaciteit van de long voor koolmonoxide (DLCO). Een verandering in vitale capaciteit (VC) of geforceerd expiratoir volume in één seconde (FEV_1) werd niet gezien. Immunosuppressieve medicatie moest worden gestart in 4 van de 11 patiënten (36%) voordat de follow-up was beëindigd.
Patiënten met een diffuse metabole activiteit in het long parenchym, die werden behandeld na het vervaardigen van de ^{18}F-FDG PET lieten een significante verbetering zien van de DLCO, VC en FEV_1. In deze patiënten populatie correleert de aanwezigheid van diffuse metabole activiteit in het long parenchym met actieve en reversibele sarcoïdose en toont ^{18}F-FDG PET derhalve de potentiële, longfunctionele verbetering.

In onbehandelde patiënten met metabole activiteit in mediastinum en hili, maar niet in het long parenchym werd na één jaar geen verschil in de longfunctie gemeten. De resultaten van deze studie suggereren dat diffuse parenchym activiteit zichtbaar middels ^{18}F-FDG PET een verslechtering van de DLCO voorspelt wanneer dit onbehandeld blijft. In patiënten zonder afwijkingen in het long parenchym lijkt een afwachtende houding gerechtvaardigd.

Tumor necrosis factor-α (TNF-α) is één van de cytokines betrokken bij de ontwikkeling van het granuloom in sarcoïdose. Anti-TNF-α therapie kan effectief zijn in patiënten met ernstige pulmonale alsook extra pulmonale sarcoïdose. Het besluit om te starten met therapie wordt momenteel gebaseerd op verschillende parameters zoals een verandering van de klachten, ACE, sIL-2R, X-thorax en long functie testen. Deze parameters worden eveneens gebruikt om het effect van therapie te monitoren. Echter, geen van deze markers kan worden gebruikt als goud standaard om sarcoïdose activiteit te bepalen. In **Hoofdstuk 6** wordt de correlatie beschreven tussen veranderingen in ^{18}F-FDG PET en de standaard sarcoïdose activiteitsparameters gedurende de behandeling met infliximab. Twaalf patiënten met refractaire sarcoïdose werden 6 maal behandeld met infliximab. Voorafgaand aan de therapie was het ACE verhoogd in 6 patiënten (50%) en sIL-2R in 11 patiënten (92%). Klinische verbetering, gebaseerd op de conventionele activiteitsparameters, werd gezien in alle patiënten, echter met een beperkte respons in één patiënt. De klachten verbeterden in 11 van de 12 patiënten maar het radiologische stadium veranderde niet. Er werd een significante daling van het ACE en sIL-2R gezien alsook een verbetering van de VC en DLCO. ^{18}F-FDG PET toonde een verbetering of normalisering in 11 van de 12 klinisch responderende patiënten. De gehele populatie liet een gemiddelde daling in SUV_{max} zien van 55%, echter de patiënt met een beperkte klinische respons liet een stijging zien van 34%. Voorts bleek de afname van de SUV_{max} in het long parenchym te correleren met een verbetering van de VC.
Op basis van deze data kan worden geconcludeerd dat veranderingen weergegeven middels ^{18}F-FDG PET gedurende de behandeling met infliximab correleren met de klinische verbetering.

In sarcoïdose zijn verschillende fenotypische presentaties bekend, allen met een bepaalde klinische uitkomst en behandeling. Het adequaat vaststellen van het

fenotype is derhalve belangrijk. In **Hoofdstuk 7** werd een classificatie systeem voor sarcoïdose geïntroduceerd, gebaseerd op ^{18}F-FDG PET. ^{18}F-FDG PET werd gecategoriseerd door drie beoordelaars gebaseerd op vier patronen. Type I werd gedefinieerd als 'metabole activiteit in mediastinale en hilaire lymfeklieren' (thoracale lymfeklieren type), type II als 'metabole activiteit in het long parenchym al dan niet met mediastinale en hilaire lymfeklieren' (long parenchym type), type III als 'metabole activiteit in lymfeklieren verspreid door het lichaam' (lymfogene type) en type IV als 'metabole activiteit in organen anders dan de longen' (orgaan type). Gebaseerd op consensus van de beoordeelaars bleek sarcoïdose beperkt tot thoracale lymfeklieren in 35% van de patiënten. Het long parenchym type werd gezien in 44% van de patiënten. Slechts 5% van de patiënten toonde het lymfogene type terwijl 17% het orgaan type vertegenwoordigde. De tevens geëvalueerde overeenkomst tussen de beoordelaars toonde een kappa van 0.70 wat betekent dat het sarcoïdose classificatie systeem, gebaseerd op vier belangrijke ^{18}F-FDG PET patronen, reproduceerbaar is.

Conclusies

De volgende conclusies kunnen worden getrokken uit de studies gepubliceerd in dit proefschrift:

- In vergelijking met ^{67}Ga scintigrafie, is ^{18}F-FDG PET sensitiever in het vaststellen van sarcoïdose activiteit en toont ^{18}F-FDG PET een betere overeenkomst tussen de beoordelaars
- ^{18}F-FDG PET is sensitiever dan genotype gecorrigeerd ACE en sIL-2R in het vaststellen van sarcoïdose activiteit
- De mate van metabole activiteit in de long, weergegeven middels ^{18}F-FDG PET en uitgedrukt als SUV_{max}, correleert met de $CD4^+/CD8^+$ ratio en neutrofielen in BAL
- In onbehandelde patiënten correleert de afwezigheid van metabole activiteit in het long parenchym bij ^{18}F-FDG PET met stabiele long functie testen na één jaar, terwijl diffuse activiteit in het long parenchym correleert met een daling in de DLCO
- In patiënten met diffuse activiteit in het long parenchym bij ^{18}F-FDG PET leidt immunnosuppressieve therapie tot een verbetering van de long functie wat suggereert dat ^{18}F-FDG PET de potentiële longfunctionele verbetering weergeeft
- In patiënten met chronische sarcoïdose, behandeld met infliximab, correleren de veranderingen in ^{18}F-FDG PET met de klinisch geobserveerde respons
- In patiënten met chronische sarcoïdose, behandeld met infliximab, correleert een daling in SUV_{max} van het long parenchym met de stijging in VC
- Een op ^{18}F-FDG PET gebaseerd classificatie systeem voor sarcoïdose, onderverdeeld in vier belangrjike fenotypische presentaties, is reproduceerbaar

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Curriculum Vitae

Ruth Keijsers werd geboren op 16 november 1975 te Horst. Na het behalen van haar gymnasium diploma aan het Boschveld college te Venray, begon zij in 1994 met de studie Bedrijfskunde aan de Erasmus Universiteit Rotterdam. Eén jaar later startte zij in dezelfde stad met de studie Geneeskunde en behaalde zij het artsexamen in januari 2002. Aansluitend begon zij als AGNIO Interne Geneeskunde/Cardiologie in het Groene Hart Ziekenhuis te Gouda. In 2003 maakte zij de overstap naar de Cardiologie in het St. Antonius Ziekenhuis te Nieuwegein, alwaar de liefde voor de beeldvorming ontstond. Zo begon zij in datzelfde jaar met de opleiding Nucleaire Geneeskunde. Als onderdeel van de opleiding werkte zij onder meer op de afdeling Longziekten alwaar een begin werd gemaakt met het onderzoek leidend tot dit proefschrift. Sinds november 2007 werkt zij als nucleair geneeskundige in het St. Antonius Ziekenhuis te Nieuwegein.

Abbreviations

ACE	Angiotensin converting enzyme
APC	Antigen presenting cell
BAL	Bronchoalveolar lavage
CCL18	CC chemokine ligand 18
CD4	Cluster of differentiation 4: T helper cells
CD8	Cluster of differentiation 8: T cytotoxic cells
DLCO	Diffusion capacity of the lung for carbon monoxide
FEV_1	Forced expiratory volume in one second
GLUT	Glucose transporters
HLA	Human leukocyte antigen
HRCT	High resolution computed tomography
IFN-γ	Interferon gamma
IL	Interleukin
MCP-1	Monocyte chemotactic protein 1
MHC	Major histocompatibility complex
MIP-1	Macrophage inflammatory protein 1
PET	Positron emission tomography
PFT	Pulmonary function tests
RANTES	Regulated upon activation normal T cell expressed and secreted
sIL-2R	Soluble interleukin-2 receptor
SPECT	Single photon emission computed tomography
SRS	Somatostatin receptor scintigraphy
SUV_{max}	Maximum standardized uptake value
SUV_{mean}	Mean standardized uptake value
TGF-β	Transforming growth factor beta
Th0 cells	T helper cells type 0: naïve CD4+ T cells
Th1 cells	T helper cells type 1: activated CD4+ T cells
Th2 cells	T helper cells type 2: activated CD4+ T cells
TNF-α	Tumor necrosis factor alpha
VC	Vital capacity