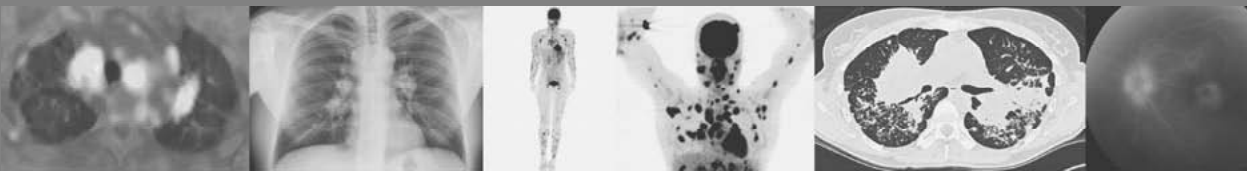
Chapter 8

Summary, general discussion and directions for future research



Summary

The clinical course of sarcoidosis is extremely variable. Although the assessment of inflammatory activity is helpful to monitor the course of the disease and guide therapeutic strategies, this assessment remains a challenge for clinicians managing sarcoidosis patients with unexplained persistent disabling symptoms. Each of the currently available markers has its shortcomings regarding sensitivity, specificity, costs and risks or possible side-effects for the patient. Another complicating factor is that specific organ involvement may be beyond the scope of the diagnostic tools used. It is therefore difficult in clinical practice to decide which investigations are required, if the aim is to spare patients invasive testing and limit costs.

The studies presented in this thesis evaluated the relationship between the currently available markers of inflammatory activity, and the relationship between these markers and parameters of disease severity in sarcoidosis. The studies included patients with sarcoidosis who had had unexplained persistent disease-related disabling symptoms for at least one year and were referred to a tertiary care center in the Netherlands. Persistent disabling symptoms were defined as the presence of more than one symptom that had substantial influence on quality of life, and that could not be explained from the results of routine investigations, including the absence of lung functional or chest radiographic deterioration. These symptoms included fatigue (Fatigue Assessment Scale (FAS) ≥22)[1], symptoms compatible with small fiber neuropathy (SFN; SFN Screenings List (SFNSL) score ≥11)[2], arthralgia and/or muscle pain, dyspnea (MRC dyspnea scale ≥3), exercise intolerance or coughing. In addition, we assessed the response of inflammatory signs and clinical characteristics to treatment with adalimumab in sarcoidosis patients with refractory chronic uveitis. The findings are summarized and discussed.

Chapter 1, the general introduction, presents a summary of the clinical features and pathogenesis of sarcoidosis, as well as a brief review of the available markers of inflammatory activity.

Chapter 2 provides an overview of the knowledge about and limitations of the use of metaiodobenzylguanidine (MIBG) scintigraphy in cardiac and pulmonary diseases. ^{123}I-radiolabeled MIBG reflects adrenergic neuron integrity. The uptake of MIBG is diminished by injury to adrenergic neurons, for instance induced by inflammation, and increased sympathetic activity is associated with a more rapid rate of MIBG loss. Hence, radiolabeled MIBG is able to reflect neuronal dysfunction in different organs. MIBG neuronal images of the lungs and heart can show heterogeneous distribution patterns involving either diminished or increased MIBG uptake and/or wash-out, reflecting changes in endothelial integrity, neuronal innervations, and clearance of norepinephrine. Interest is growing in the role of neurotransmitter involvement and the relation between endothelial cell integrity and vascularization, and it is of the utmost importance to understand the effect on the pathophysiology of diseases. Currently, however, there is no added clinical value to be gained by routine use of

MIBG scanning of the lungs and heart. This is partly due to the fact that the exact mechanism of MIBG uptake and wash-out under normal physiological circumstances, and the factors which influence them, have not been fully clarified. Nevertheless, the technique has tremendous potential, as it can unravel previously unknown relationships between innervation, vascularization, and endothelial integrity. Other diagnostic tools like magnetic resonance imaging (MRI) and computed tomography (CT) do not have this ability, so the future looks bright for these new neuronal imaging techniques.

Chapter 3 reports on the assessment of the presence of inflammatory activity using ^{18}F-FDG PET in 89 sarcoidosis patients with unexplained persistent disabling symptoms, and evaluates the association between PET findings and serological inflammatory markers. Positive PET findings were classified as thoracic and/or extrathoracic. The serological markers of inflammatory activity we considered were angiotensin-converting enzyme (ACE), soluble interleukin-2 receptor (sIL-2R), and neopterin. The majority of sarcoidosis patients with persistent disabling symptoms, even those with radiographic stage IV, had positive PET findings. Remarkably, 80% of the patients had extrathoracic lesions. In 20% of the patients, PET was positive without signs of serological inflammatory activity. PET appeared to offer added value in assessing inflammatory activity in patients with persistent symptoms in the absence of signs of serological inflammatory activity, as well as in detecting extrathoracic lesions.

Chapter 4 describes the prevalence and distribution pattern of bone and bone marrow involvement as detected by ^{18}F-FDG PET/CT in 94 sarcoidosis patients with positive PET findings. Their PET/CT scans were screened to localize any bone/bone marrow involvement. Additionally, low-dose CT scans were screened for other causes of increased bone uptake. Relevant clinical data were gathered retrospectively.

More than one-third of PET/CT-positive sarcoidosis patients had osseous abnormalities on PET/CT. The majority of these lesions (94%) could not be detected on low-dose CT. No single preferred location was found. These preliminary results stress the value of PET/CT imaging in the assessment of bone/bone marrow involvement in sarcoidosis patients. Its clinical relevance needs to be explored in future studies.

Chapter 5 evaluates the association between severity of pulmonary involvement and ^{18}F-FDG PET activity in persistently symptomatic sarcoidosis patients. Over a 5-year period, relevant clinical data, including laboratory- and lung function test results, were gathered from the medical records of 95 sarcoidosis patients who underwent both PET and high-resolution CT (HRCT) because of unexplained persistent disabling symptoms. The HRCT scans were classified using a semi-quantitative HRCT scoring system. PET findings were classified as positive or negative. The patients with positive pulmonary PET findings (n=56) demonstrated significantly higher total HRCT scores, but lower values of the diffusion capacity for carbon monoxide (DLCO) and forced vital capacity (FVC) compared to the patients with negative pulmonary PET findings (n=39).

The severity of the pulmonary involvement in sarcoidosis as assessed by HRCT features and lung function parameters was associated with PET activity. The majority of patients with radiological fibrotic changes on HRCT (85%) demonstrated pulmonary inflammatory activity. Moreover, a surprisingly high number of these patients also demonstrated extrathoracic PET-positive lesions (82%) and serological signs of inflammatory activity (73%).
The question can be raised which patients might benefit from having a PET scan for the assessment of inflammatory activity. Until now, there have been no guidelines for selecting those patients with unexplained persistent disabling symptoms for whom PET might offer added value in the assessment of inflammatory activity.
Chapter 6 makes an attempt to develop a prediction rule that can be used to identify symptomatic sarcoidosis patients for whom there is a high probability that ^{18}F-FDG PET will show the presence of inflammatory activity. A clinical prediction rule, based on sIL-2R levels and HRCT scoring results, was derived and internally validated, and appeared to be useful to identify sarcoidosis patients with a high probability of inflammatory activity. Hence, using this rule may be helpful to identify sarcoidosis patient in whom a PET might be of additional value to assess inflammatory activity. These results may affect patient care by providing supportive evidence for more effective use of PET scan in the assessment of inflammatory activity in sarcoidosis.
Chapter 7 presents the results of a prospective case series that included 26 sarcoidosis patients with refractory chronic uveitis. The included patients were systematically followed for 12 months after the initiation of adalimumab 40 mg sc once a week. The study evaluated the effect of adalimumab on intraocular inflammatory signs and other relevant clinical manifestations (lung function, serological inflammatory parameters, and fatigue) of sarcoidosis. Adalimumab appeared successful in the treatment of sarcoidosis patients with refractory chronic non-infectious uveitis, showing improvement in intraocular inflammatory signs as well as in the other relevant clinical indicators of disease activity that were evaluated. Future randomized studies are needed to determine the optimal dosage, dose interval and duration of therapy in refractory multisystemic sarcoidosis.

In conclusion, this thesis describes the spectrum of techniques to assess inflammatory activity in persistent symptomatic sarcoidosis. It presents an overview of the knowledge about and limitations of the use of MIBG scintigraphy in cardiac and pulmonary diseases, and discusses the relationship between PET findings and serological inflammatory markers, lung function test results and radiologic parameters. PET appeared to offer added value in assessing inflammatory activity in patients with persistent symptoms in the absence of serological inflammatory activity signs. The serological inflammatory parameters that were considered were ACE, neopterin, and sIL-2R. Combining several serological inflammatory markers increased the sensitivity to inflammatory activity as detected by PET. Severity of pulmonary involvement as assessed by HRCT features and lung function parameters was

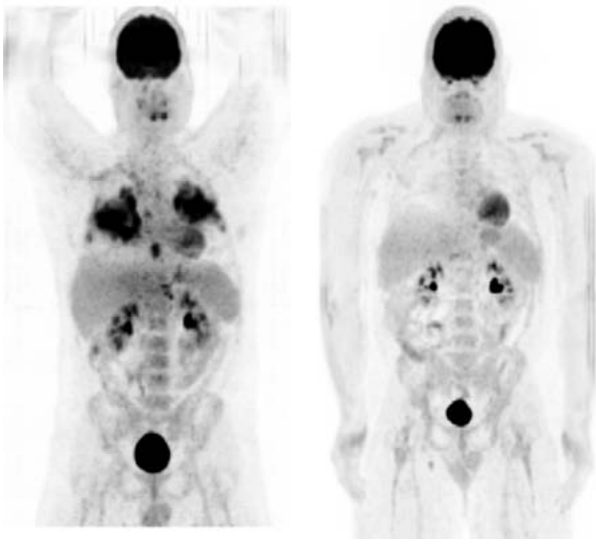
associated with PET activity. Based on these findings, a clinical prediction rule that can be used to identify symptomatic sarcoidosis patients for whom there is a high probability that PET will show the presence of inflammatory activity was derived and internally validated, and proved to be able to distinguish between patients with PET-positive and PET-negative findings. The value of PET/CT imaging in the assessment of bone/bone marrow involvement in sarcoidosis patients was demonstrated by the presence of osseous abnormalities on PET/CT in more than one-third of the PET-positive patients, which was much higher than expected according to most previously published studies. Finally, adalimumab appeared successful in the treatment of sarcoidosis patients with refractory chronic non-infectious uveitis, showing improvement in intraocular inflammatory signs as well as in the other relevant clinical indicators of disease activity that were evaluated.

General discussion

A problem frequently encountered in the clinical setting is illustrated by the following case of a 40-year-old female patient with sarcoidosis. She had been treated with prednisone for two years, mainly because of restrictive lung function loss with a moderate decrease in DLCO. After 6 months, methotrexate was added, due to the lack of amelioration. Despite this therapy, she was still suffering from disabling symptoms, including fatigue, limited exercise capacity, dyspnea at exercise and coughing. No changes were found on the chest X-ray (CXR) (persistently bilateral consolidations) and the pulmonary function impairment remained unchanged. All serological inflammatory markers (ACE, sIL-2R, and neopterin) were negative. The question was raised whether the disease had become quiescent under methotrexate therapy, and the persistent symptoms were a result of previously caused damage, or whether ongoing inflammatory activity was the cause of the persistent symptoms. Several studies have shown that therapy with tumor necrosis factor (TNF)-α inhibitors decreases inflammatory activity, and that this correlates with clinical signs of improvement.[3,4] However, judging whether this patient might benefit from TNF-α inhibitor treatment was difficult, since in this case the clinician could not rely on the above-mentioned traditional tools. The question is therefore what additional diagnostic tools would be helpful in assessing the presence of inflammatory activity in sarcoidosis?

Assessing inflammatory activity is hampered by the lack of a gold standard. In recent years, PET has been shown to be a very sensitive technique to assess inflammatory activity in sarcoidosis by detecting and quantifying the level of inflammatory and granulomatous reactions that occur in the lungs and elsewhere in the body.[5-8] This means that a technique has become available that is able to evaluate the presence of inflammatory activity in each organ system. In view of the radiation dose and costs involved in PET, however, it is essential to define appropriate indications for PET.

Comparative evaluation of the currently used markers for inflammatory activity and the results obtained with PET could be helpful to clarify the value of the latter for the assessment of inflammatory activity and to define a phenotype of patients for whom PET would offer added value. The presence of inflammatory activity can be regarded as highly likely in patients with newly diagnosed acute, symptomatic sarcoidosis.[5,9] In contrast, it is often rather difficult to answer the question whether inflammatory activity is still present in sarcoidosis patients without deteriorating lung function or radiological deterioration, but with unexplained persistent disabling symptoms. Hence, our aim was to evaluate the value of the various available tools for the assessment of inflammatory activity in a population of sarcoidosis patients with persistent disabling symptoms without a clear clinical explanation.



A B

Figure 8.1 PET findings of the sarcoidosis patient who is discussed in the text (p126).

a. Coronal PET image showing pathologically increased FDG uptake in the pulmonary parenchyma and para-esophageal lymph nodes.

b. Coronal PET image after therapy with infliximab showing resolution of the pathologically increased FDG uptake in the pulmonary parenchyma and para-esophageal lymph nodes.

Assessment of inflammatory activity

Inflammatory activity as assessed by positive PET results was present in the majority (75%) of the sarcoidosis patients with unexplained persistent disabling symptoms in our sample, even those with radiographic stage IV (Chapter 3). As described in the same chapter, five patients with an obstructive sleep apnea syndrome (OSAS) who

were adequately treated with continuous positive airway pressure (CPAP) still suffered from disabling symptoms, including fatigue. Hypermetabolism detected with PET appeared to be present in these five patients, supporting the assumption that inflammatory activity was still present and probably explained the persistent fatigue. An example of a sarcoidosis patient with pronounced fatigue and multiple PET-positive lesions in the muscles of the lower extremities is shown in Chapter 1, Figure 1.6.

Serological inflammatory markers for the assessment of inflammatory activity

Previous studies have investigated the value of individual serological inflammatory markers for assessing disease activity.[9-14] Several studies found that neopterin and sIL-2R levels were significantly elevated in progressive, and thus active, disease.[9,12] Keijsers et al.[5] showed that elevated ACE and sIL-2R levels correlated with positive PET findings, though the reported sensitivity of these markers was low. Neopterin levels were not evaluated in their study. Among the serological inflammatory parameters evaluated in the study described in Chapter 3, positive sIL-2R showed the strongest association with PET positivity. Nevertheless, the highest sensitivity as regards the detection of inflammatory activity demonstrated by PET was achieved by combining the three serological inflammatory markers (ACE, sIL-2R, and neopterin). This was a novel finding, as the value of combining serological inflammatory markers had not been studied before. The sensitivity of the combined serological inflammatory markers (80%) as reported in Chapter 3 is probably an underestimation of the real sensitivity, since neopterin values were only available for 70% of the patients, and the sensitivity for the subgroup of patients for whom the values of all three serological inflammatory markers were available (n=62) was calculated to be 86%. The positive predictive value of the combined serological inflammatory markers appeared to be excellent, though the negative predictive value was moderate. We concluded that PET appears to offer added value in assessing inflammatory activity in patients with persistent symptoms in the absence of signs of serological inflammatory activity.

Inflammatory activity in sarcoidosis originates from several different mechanisms, viz. ongoing T-cell and macrophage activity and granuloma formation.[10,11,14-16] In accordance with this, we found that all of the serological inflammatory markers we used offered added value in detecting inflammatory activity. In the subgroup of patients for whom values of all three serological inflammatory markers were available, increased levels of only one serological inflammatory marker were found in 12 of the 36 patients with positive combined serological inflammatory markers. ACE, sIL-2R, and neopterin were the single increased marker in 4 of these patients each. ACE is mainly produced by activated granuloma cells (epitheloid cells),[17] while sIL-2R is a marker of T-cell activity[10,14,18] and neopterin represents activation of the monocyte/macrophage system.[12] Other relevant inflammatory pathways, not

detected by the serological inflammatory markers we used, probably account for the fact that some of the PET-positive patients had negative serological inflammatory markers. As explained above, missing neopterin values presumably also reduced the sensitivity of the combined serological inflammatory markers in detecting inflammatory activity in our population. This sensitivity will most likely increase if complete results for all serological inflammatory markers are available in future prospective trials.

Evaluating the extent of disease by assessing extrathoracic inflammatory activity

Remarkably, extrathoracic PET-positive lesions were found in 80% of the PET-positive patients, which stresses the value of whole-body evaluation. Grutters et al.[10] found an association between sIL-2R levels and extrathoracic involvement, though this was not confirmed in our population. A possible explanation for this discrepancy might be the higher prevalence of extrathoracic involvement in our study, as discussed in Chapter 3. In turn, this might be the result of our use of PET for the assessment of extrathoracic involvement, Grutters et al.[10] used clinical, functional, and radiologic data. The prevalence of extrathoracic involvement in the PET-positive patients in our study was comparable to that found in studies using PET in active sarcoidosis.[5-7,19] The extrathoracic organs we found to be involved included peripheral lymph nodes, bone, spleen, muscle, liver, parotid glands, skin, and the central nervous system. The extrathoracic organ involvement was higher than we had suspected before performing PET. In almost two-thirds of the patients with extrathoracic PET-positive lesions, these lesions had not been suspected beforehand. The high frequency of extrathoracic lesions we established shows that this is another merit of using PET (to assess the extent of the disease). This was underlined by the presence of osseous abnormalities on PET/CT in more than one-third of the PET-positive patients, as described in Chapter 4. In a large cohort study of 137 sarcoidosis patients, Teirstein et al.[6] found that PET established the presence of increased extracardiac FDG uptake which had not been identified by physical examination, chest radiography or CT scans. Our results were in accordance with these findings. Hence, PET may uncover occult sarcoidosis localizations or multiple organ involvement. Moreover, it can establish the most appropriate location for a biopsy to obtain histological evidence for the diagnosis. And detecting extrathoracic lesions may also provide an explanation for (mainly extrathoracic) symptoms. In addition, the assessment of a PET-positive lesion can be useful in monitoring the treatment effect.

Pulmonary involvement

The CXR is the most commonly used radiologic imaging technique to evaluate patients with pulmonary sarcoidosis. The Scadding radiographic staging system provides general information regarding the prognosis of the pulmonary disease over time. In

general, patients with lower CXR stages have a higher chance of resolution of their radiographic findings.[20,21] However, the CXR stage has been shown to correlate only weakly with the level of dyspnea; no significant correlation was demonstrated between CXR findings and either lung function test results or 6-minute walk distance.[15,20-23] Moreover, one major issue concerning the radiographic staging system has been interobserver variability, as a result of which it has limited applicability in individual patient assessments, including treatment decisions.[21] A prospective study of 36 patients compared clinical status and spirometry during flares of pulmonary sarcoidosis with CXR findings as described by the CXR International Labour Organization profusion score.[24] It found there was too much variation for a cut-off to be identified that would reliably diagnose exacerbations. Approximately one-half of the radiographic readings showed an improvement or no change during a significant exacerbation. In line with this, we found no clear relation between CXR stages and PET findings.

The presence and extent of parenchymal abnormalities on HRCT has been found to correlate with functional impairment in sarcoidosis.[25-29] Previous follow-up CT studies in patients with pulmonary sarcoidosis have shown that nodular opacities represent potentially reversible findings.[30,31] As regards parenchymal consolidations, however, it is not possible to differentiate between fibrotic or granulomatous components in the consolidations on the basis of HRCT findings. Due to pre-existing major abnormalities, radiological features are frequently of limited value for the assessment of inflammatory activity in sarcoidosis patients with fibrotic disease. HRCT is a morphological imaging technique that provides only indirect information on the underlying metabolic changes. To the best of our knowledge, no studies on the radiological substrate of parenchymal FDG uptake, as detected by PET, had been performed previously in a population of sarcoidosis patients. Consequently, no comparison between PET findings and HRCT was available. Achieving a reliable comparison of results requires a standardized scoring system of abnormal findings on HRCT and PET. Previous studies found that the intra- and inter-reader reliability of a semiquantitative HRCT scoring system described by Oberstein et al.[32] was good, and the HRCT features of this scoring system were associated with respiratory functional impairment in sarcoidosis.[25] The inter-observer agreement of the PET scoring system we used proved to be very good, as reported in Chapter 3. As described in Chapter 5, the severity of pulmonary involvement as assessed by HRCT features and lung function parameters correlated with PET activity in sarcoidosis. The semiquantitative HRCT scoring system we used appeared to offer a better ability to identify patients with positive pulmonary PET findings than the traditionally used radiographic staging system introduced by Scadding. It is also important to emphasize that a proportion of the patients with normal CXR findings (stage 0) as well as the majority of patients with signs of fibrosis on CXR (stage IV) or HRCT had positive pulmonary PET findings. An example of positive pulmonary PET findings in a sarcoidosis patient with fibrotic changes on HRCT is shown in Figure 8.2.

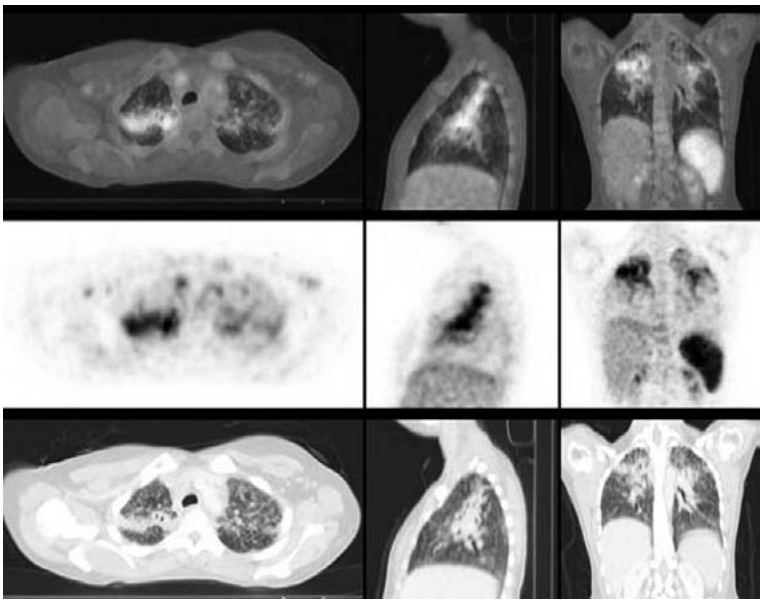


Figure 8.2 Sarcoidosis patient with signs of pulmonary fibrosis and ^{18}F-FDG PET activity.
Top: transversal (left), sagittal (middle) and coronal (right) positron emission tomography/computed tomography (PET/CT) fusion images showing that the increased ^{18}F-fluorodeoxyglucose (FDG) uptake in the pulmonary parenchyma corresponds with the parenchymal consolidations. The abdominal increased FDG uptake corresponds with the spleen.
Middle: transversal (left), sagittal (middle) and coronal (right) PET images showing increased FDG uptake in the pulmonary parenchyma and in the spleen (coronal image).
Bottom: transversal (left), sagittal (middle) and coronal (right) CT images showing parenchymal consolidations with hilar retraction and architectural distorsion of the pulmonary parenchyma.

Assessment of disease activity through lung function tests requires evidence of progression between two measurements, and so does not reflect the current status. Moreover, lung function testing cannot distinguish between reversible granulomatous lesions and irreversible fibrotic changes, and correlates only modestly with the level of dyspnea reported by patients.[15,21] The assessment of the global lung burden as traditionally performed by lung function testing in combination with CXR abnormalities therefore seems insufficient for sarcoidosis patients with unexplained persistent disabling symptoms. A more accurate and global measure of both pulmonary and extrapulmonary disease may be achieved by the use of combined PET/CT in these patients.

Increased FDG uptake has also been observed in patients with idiopathic pulmonary fibrosis (IPF).[33-36] All of the models proposed for the pathogenesis of pulmonary fibrosis involve a central role for fibroblasts, which are known to express glucose transporter-1.[33,37] It could be speculated that the elevated FDG uptake in patients with fibrotic changes, including honeycombing, is a reflection of increased fibroblast

metabolism and not due to inflammatory activity sensu strictu.[33] However, the majority of the sarcoidosis patients with fibrotic changes in our population showed extrathoracic PET-positive findings (80%) and increased serological inflammatory markers (78%). Furthermore, the mean maximum standardized uptake values (SUVmax; 7.2±4.1) in these patients were higher than the values reported by two studies with IPF patients[33,36] (0.99±0.29 and 2.9±1.1, respectively). These findings strongly suggest that PET-positive findings in sarcoidosis patients with CXR stage IV are related to inflammatory activity. The extrathoracic findings can be helpful to differentiate between sarcoidosis and other interstitial lung diseases like IPF in patients presenting with pulmonary fibrosis.

Predicting the presence of inflammatory activity

Based on the results reported above, a clinical prediction rule, based on sIL-2R levels and HRCT scoring results, was derived and internally validated, as reported in Chapter 6. This clinical prediction rule was useful to identify patients for whom there is a high probability that PET will show the presence of inflammatory activity. If only patients with scores below a chosen cut-off value of the prediction rule score are referred to PET for further evaluation, the use of this prediction rule can substantially reduce the number of referrals to PET scanning. As discussed in Chapter 6, Table 6.3, for example, PET would be indicated for less than a third of the patients in our study if a positive predictive value for the presence of inflammatory activity (PET positivity) of ≥90% is considered acceptable for clinical decision making without referral to PET. This predictive value was reached with a positive sIL-2R result alone or with a total HRCT score of ≥6 points. The clinical prediction rule we derived is a first attempt to identify patients with a high probability that PET will show the presence of inflammatory activity. It shows that such easily applicable predictive models can be developed for clinical use. This has the potential to increase the cost-effectiveness of diagnostic investigation, as performing PET for the assessment of inflammatory activity is unnecessary in patients with a high predicted probability. An additional advantage is that the assessment of inflammatory activity could become more standardized. Future prospective studies are warranted to develop more sophisticated models, and the search for new inflammatory parameters should continue as well.

Further improvement might be achieved by genotyping the sarcoidosis patients, since different genotypes might account for the variable results of the tools currently available for the assessment of inflammatory activity. The use of genotype-corrected ACE levels, human leukocyte antigens (HLA) genotypes, TNF polymorphisms and butyrophilin-like 2 (BTNL2) variant alleles is of potential value to improve the prediction of the probability of the presence of inflammatory activity and/or to assess the prognosis more accurately.[17,38-45] This requires further clinical phenotype-genotype studies that could lead to a better classification of sarcoidosis patients.

Prognostic value of inflammatory activity

The ability to identify patients at increased risk of progressive disease or complications is crucial, since they require a different management and monitoring approach. Rothkrans et al.[14] found that sIL-2R was useful for monitoring respiratory disease severity in sarcoidosis. Other studies showed that sIL-2R and neopterin levels were elevated in sarcoidosis patients with progressive disease, requiring steroid treatment.[9,12] These levels were particularly elevated in patients with acute disease, although patients with persistent disease also showed a moderate but significant increase.[12] This indicates the potential value of sIL-2R and neopterin as suitable parameters for predicting disease progression and severity in sarcoidosis patients who have no indication for therapy at the time of diagnosis.[9,12,14]

There are several arguments for the prognostic value of PET-positive findings. Keijsers et al.[46] found that increased FDG uptake in the pulmonary parenchyma correlated with the number of neutrophils and the $CD103^{+}CD4^{+}/CD4^{+}$ ratio in bronchoalveolar lavage (BAL) fluid of sarcoidosis patients. In agreement with this, we also found signs of inflammation in the BAL fluid (increased numbers of lymphocytes as well as polymorphonuclear neutrophils, indicating disease severity) of patients with PET-positive findings (Chapter 3). In earlier studies, an increase in the number of neutrophils in BAL fluid was found to be associated with an unfavourable outcome.[9,47] Another study showed that diffuse parenchymal activity in sarcoidosis patients, as imaged by ^{18}F-FDG PET, predicts a future deterioration of DLCO when medical treatment is withheld.[48] The same study also found that treatment with corticosteroids or immunosuppressive drugs improved lung function significantly.[48] PET also enables a more accurate clinical assessment of prognostic factors by demonstrating and monitoring organ involvement, like bone involvement, which is associated with a chronic course of the disease.[49,50] We showed in Chapter 4 that PET/CT is an excellent modality to detect bone/bone marrow involvement compared to more conventional modalities, suggesting that physiological changes precede morphological changes, which is a concept known from PET/CT in oncology.[51] Nevertheless, no clear data exist on the prognostic significance of PET/CT-positive osseous findings in sarcoidosis patients or on whether there is an increased risk of fracture at these sites.[49] However, since follow-up PET/CT after therapy in our study showed improvement of the osseous involvement, PET/CT may play a role in monitoring osseous involvement in sarcoidosis. Based on the above findings, it is tempting to speculate that increased FDG uptake not only demonstrates inflammatory activity, but also has prognostic value.

The prognostic value of intraocular inflammatory signs is well-established, as ocular manifestations of sarcoidosis have significant impact on visual prognosis.[52,53] The presence of posterior or panuveitis in particular is a risk factor indicating a poor prognosis.[54,55]

Detection of cardiac sarcoidosis is of the utmost importance with respect to the prognosis, since this is a major cause of serious morbidity and mortality in

sarcoidosis.[17,56,57] Fasting cardiac PET seems to be the most sensitive test for detecting active sarcoid lesions and their response to treatment, but there is no thoroughly evaluated diagnostic algorithm to screen for cardiac sarcoidosis.[57-59] As described in Chapter 2, changes in MIBG uptake due to cardiac autonomic dysfunction can appear in sarcoidosis.[60-63] Myocardial inflammatory activity may play a role, and autonomic dysregulation might contribute to fatal arrhythmias and unexplained sudden death.[64] Small fiber neuropathy occurs frequently in sarcoidosis, and it is known from patients with neuropathy that the involvement of small autonomic nerve fibers is a predictor of cardiovascular mortality.[65-67] Active granulomatous infiltration and the resulting myocardial fibrosis are considered to be the substrate. Cardiac sympathetic dysfunction, assessed by means of myocardial ^{123}I-MIBG scanning, appears to be heterogeneous in sarcoidosis patients and depends on the presence or absence of small fiber neuropathy (SFN).[68] Cardiac sympathetic nervous system abnormalities detected by ^{123}I-MIBG have predictive value for ventricular tachyarrhythmia, and can be used to assess the prognosis of patients with chronic heart failure, as well as the risk of sudden death, as shown in recent studies.[69-71] This indicates the potential of ^{123}I-MIBG for the assessment of cardiac involvement and its prognostic value in sarcoidosis. Further studies in this field are warranted.

Implications of inflammatory activity for therapy

The indications for treatment of individual patients depend on many factors, not just whether the patient is symptomatic but also whether there is evidence of asymptomatic significant disease, especially in vital organs. The majority of patients with signs of fibrosis on CXR (stage IV) or HRCT in our population had positive pulmonary PET findings. The presence of potential partial reversibility in patients with radiological signs of fibrosis may have therapeutic consequences.[6] Inflammatory activity can indicate persistent evolution of the disease and may therefore be a target for therapy. Deciding which sarcoidosis patients with fibrotic disease may benefit from pharmacological treatment remains a challenge to clinicians, as it is not always clear whether respiratory symptoms in these patients are a result of organ damage or due to ongoing inflammation or both. Careful consideration also needs to be given to the likely benefits of any therapy, set against the risk of adverse events, since adding the burden of medication like corticosteroids to disabled sarcoidosis patients with pulmonary fibrosis might harm them even further. To date, there is no medication with the proven capability of reversing fibrosis, but there is hope that treatment can arrest fibrosis of reversible granulomas that persist among the fibrotic elements.[72] This is in line with the results of the post-hoc analysis in the Sarcoidosis Investigators study,[73] which suggested a greater benefit of infliximab therapy in patients with more severe disease, including CXR stage IV. Techniques that are purported to differentiate between fibrotic tissue and granulomatous tissue with inflammatory activity are therefore of importance. There is little evidence to support corticosteroid or

immunosuppressive treatment of fibrotic lung disease unless the presence of inflammatory activity can be demonstrated.[72]

Several reports have demonstrated a significant reduction of FDG uptake after the initiation or modification of treatment in sarcoidosis patients.[3,4,6,7] Keijsers et al.[3] demonstrated that changes in PET imaging in a small cohort of sarcoidosis patients treated with infliximab considerably correlated with clinical signs of improvement, including reduced fatigue. Teirstein et al.[6] described that the improvement of symptoms, conventional imaging findings, and physiological data paralleled the therapy-related decrease in SUVmax as seen on the PET scans in most patients, including three patients with CXR stage IV. A decrease in FDG uptake after therapy was also found in patients with refractory sarcoidosis treated with adalimumab.[4] The same study found that the patients' quality of life improved, which is in agreement with a previous study, which found a positive effect of treatment with TNF-α inhibitors on cognition and fatigue.[74] The adalimumab study found no effect on pulmonary function tests and ACE levels in patients with refractory sarcoidosis.[4] Possible explanations for this are that the number of included patients was small (n=10), some patients had nearly normal lung function which could thus not be expected to improve, and further deterioration in lung function may have been prevented by the adalimumab therapy. Furthermore, it was unclear how many patients had increased ACE levels at baseline. We studied a larger population (n=26; Chapter 7) and found that treatment with adalimumab decreased intraocular inflammatory signs, laboratory inflammatory parameters (CRP and sIL-2R), and fatigue. In the same study, DLCO improved after therapy with adalimumab in those cases with an impaired DLCO at baseline. PET/CT was repeated after a change in therapy in three of the patients with bone involvement discussed in Chapter 4, and showed a decrease in the number of bone lesions and the SUVmax, respectively.

Several studies have suggested that infliximab might be more beneficial to patients with multi-organ extrapulmonary involvement,[73,75] underlining the importance of assessing the extent of disease and the possible role of PET for this purpose. Research into various inflammatory diseases, including pulmonary sarcoidosis, has found that CRP levels can predict the response to anti-TNF-α therapy.[76-80] In line with this, we reported in Chapter 7 that CRP levels had decreased after six months as well as after one year of treatment with adalimumab. The CRP levels appeared to be higher in the PET-positive patients compared with the PET-negative patients in the studies discussed in this thesis. The exact importance of the presence of inflammatory activity for treatment decisions obviously needs to be established in future studies, although the above-mentioned limited data support the prognostic value of PET-positivity and the implications for therapy.

The value of PET in the follow-up of therapy is another topic of interest. It is still not clear how long therapy, including anti-TNF-α agents, should be continued. Further studies are required to investigate if PET could be helpful in guiding the duration of treatment. If this is the case, PET could also reduce the costs by avoiding unnecessarily

prolonged treatment with this expensive medication, and avoid long-term side-effects. Based on the above-mentioned results of studies with follow-up PET during treatment,[3,4,6] a reduction in inflammatory activity can be presumed to have occurred in patients with clinical signs of improvement during treatment. Follow-up PET scans therefore seem unnecessary in these patients.

Improvement of cardiac sympathetic function, assessed with ^{123}I-MIBG single photon emission computed tomography (SPECT) after treatment with carvedilol has been reported in sarcoidosis (Figure 8.3).[60]
This is in accordance with previous findings in patients with idiopathic dilated cardiomyopathy.[81] Carvedilol has not only adrenergic receptor blocking effects but also antioxidant activity,[82] and future studies are required to explore the clinical relevance of the relations between oxidative stress, antioxidant therapy and cardiac dysfunction in sarcoidosis. Cardiovascular autonomic function in sarcoidosis has also been reported to improve after therapy with infliximab, but no trial has been conducted until now.[83]

Concluding remarks on a strategy for the assessment of inflammatory activity: which tests for which patients?

Minimally invasive testing is required to shorten radiation exposure and reduce costs, and to keep the inconvenience for patients to a minimum. As explained above, the presence of inflammatory activity in patients with newly diagnosed acute, symptomatic sarcoidosis is virtually universally accepted.[5,9] During the follow-up of sarcoidosis patients, the presence of inflammatory activity is indicated by deterioration in terms of lung-function or radiological findings, hypercalcemia, hypercalciuria, progressive cutaneous involvement or uveitis.[15,17,84,85] The absence of these features allows a wait-and-see policy in asymptomatic patients. In sarcoidosis patients with unexplained persistent disabling symptoms, however, the absence of these features will not be reassuring, and both patients and clinicians will not feel satisfied without further exploration of possible treatable conditions. Initially, depending on the presenting symptoms, comorbidity like obstructive sleep apnea syndrome, depression or hypothyroidism should be excluded.[86,87,88] If these do not provide an explanation for the symptoms, further assessment of inflammatory activity is warranted, since this seems to have prognostic value and might have implications for therapy, as discussed above. PET is a sensitive method to assess inflammatory activity and the extent of disease in sarcoidosis.[5-8] PET is not indicated in the standard work-up, but can be of great value to complement more routinely used techniques.

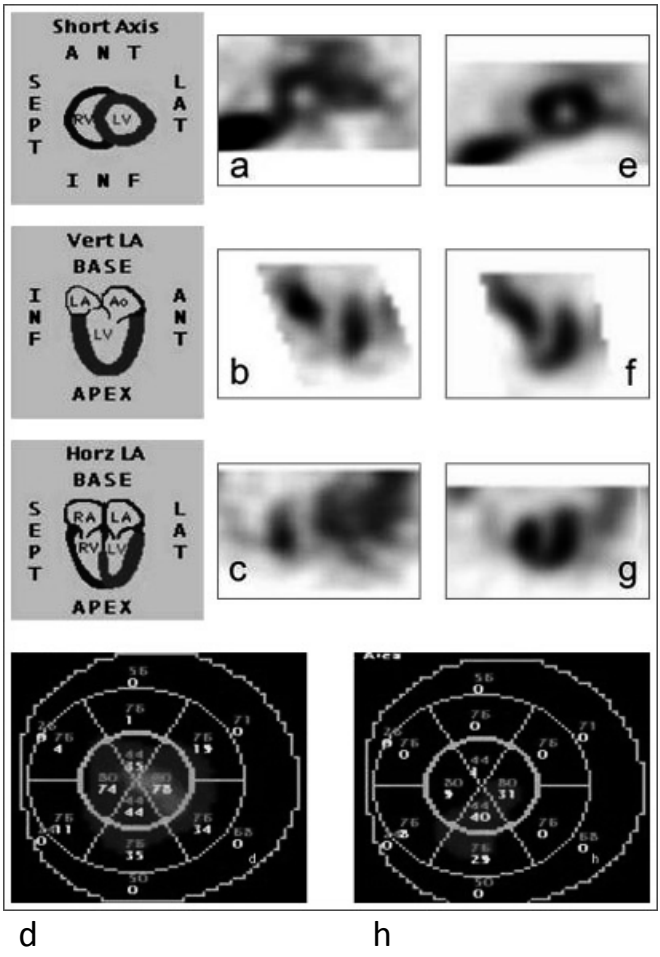


Figure 8.3 ^{123}I-metaiodobenzylguanidine single photon emission computed tomography (^{123}I-MIBG SPECT) study of a 48 year-old man known with pulmonary sarcoidosis (disease duration 5 years) and small fiber neuropathy, who presented with cardiac arrhythmia and dizziness that had lasted two weeks. The images demonstrate no uptake of ^{123}I-MIBG in the apical inferior wall of the myocardium (**a, b, c**). Bulls' eye quantification showed a defect size of 16.3% (**d**). A myocardial perfusion SPECT scintigraphy with ^{201}Thallium showed normal perfusion in the apical inferior wall. Echocardiography and magnetic resonance imaging (MRI) of the heart were also judged normal. He was treated with carvedilol for five months and his clinical condition stabilized and no activity of sarcoidosis could be found. He did not recall having had any further heart rhythm disturbances. The ^{123}I-MIBG SPECT study was repeated and showed improvement of the uptake in the apical inferior wall (**e, f, g**), with a defect size of only 3.9% (**h**).[60] This case illustrates that ^{123}I-MIBG is useful to monitor treatment effects.

Based on the current findings, PET seems to offer added value in sarcoidosis patients with unexplained persistent disabling symptoms. PET appears especially helpful in those persistently symptomatic patients without serological signs of inflammatory activity and in patients with radiologic signs of fibrosis. The use of PET to assess the extent of disease can uncover a suitable location for biopsy to obtain histological evidence for the diagnosis or to explain (mainly extrathoracic) symptoms. Furthermore, the detection of extrathoracic involvement can offer prognostic value.

Impact for individual patients

The detection of inflammatory activity in sarcoidosis patients with unexplained persistent disabling symptoms might justify a trial with additional pharmacological treatment options. Conversely, the absence of inflammatory activity allows a conservative approach in terms of pharmacological treatment. This could avoid side-effects as well as redundant use of expensive medication, and would support the choice of other treatment options like rehabilitation. However, prospective evaluation of this approach is required. Apart from the value of PET imaging in treatment decisions or follow-up, simply establishing that there is an organic cause for the symptoms can be extremely reassuring to many patients. A standardized approach for the assessment of inflammatory activity is required, and developing a clinical prediction rule for this purpose appeared to be feasible. Such standardization is needed for clinical purposes but also to keep the costs manageable.

Limitations of the studies

The studies presented in this thesis were performed in a referral centre for sarcoidosis, so the refractory character of the disease may have been more severe than in a general sarcoidosis population. PET was not performed in every referred patient, which may have caused a selection bias in some of the studies discussed in this thesis. The questions asked by the referring physicians and the reasons for referring the patients to our interstitial lung disease service were very diverse, ranging from requests for therapeutic advice about features in refractory sarcoidosis, like hypercalcemia, uveitis or severe respiratory impairment not responding to corticosteroids, to questions about genetic issues or occupational exposures. In these patients, no PET was indicated to answer the questions appropriately. This does not mean the patients had less severe sarcoidosis, however. Though no other diseases that are able to cause PET-positive findings were diagnosed during the follow-up, other causes of PET-positive findings cannot be excluded completely. Since clinical data were gathered retrospectively in some of the studies, complete data were not available in some cases.

Directions for future research

Differences in terms of incidence and phenotype complicate comparisons of studies into the prognosis and effect of treatment in sarcoidosis, since patient populations are heterogeneous. Stratification of patients in clinical trials falls short of providing the ideal degree of individualization. The key to further progress in this field is to define disease types, predict progression and assess the need for therapy. Besides their clinical value, the results presented in this thesis could thus be helpful in defining these patient categories by providing a more standardized procedure for establishing the presence of inflammatory activity and organ involvement. Since a better definition of patient categories may now be possible, the need for multicenter studies is greater than ever, to ensure the inclusion of sufficiently large patient populations. Future studies aimed at improving patient management should attempt to standardize the work-up for sarcoidosis patients with persistent disabling symptoms.

Defining clinical phenotypes has become an important goal, as genetic studies have identified distinct genotypes associated with different clinical phenotypes.[38-41,44,45,89-91]

Several classification systems based on clinical outcome have been developed, but none of these have been prospectively validated.[12,91,92] Also, all of these classification system have disadvantages, like the need for a substantial follow-up period,[91] the assumption that the long-term outcome can always be predicted from clinical features at the time of presentation,[12] or interobserver variability.[92] There is a need to investigate whether such extended scoring systems can be improved by the inclusion of a flowchart (i.e. a clinical prediction rule) for the assessment of inflammatory activity.

So far, no instrument to establish the total burden of granulomatous inflammation is available. This could be achieved by quantification of the total FDG uptake in the body as a whole and for each individual organ system, as measured by the sum of the SUVs per region. This would allow a more precise comparison between different measurements and may enhance the accuracy of the definition of comparable patient populations. So far, the implementation is hampered by technical issues, e.g. the segmentation of the presence of FDG in the blood volume.

The use of several other nuclear techniques and tracers in sarcoidosis deserves further exploration. Prospective studies are needed to evaluate the prognostic value of cardiac sympathetic dysfunction in sarcoidosis, assessed with ^{123}I-MIBG scintigraphy, and its response to therapy with e.g. betablocking agents or TNF-α inhibitors. Scintigraphy using ^{99m}Technetium labeled anti-TNF-α (^{99m}Tc anti-TNF-α) monoclonal antibodies (infliximab) offers many research opportunities.[93,94]

^{99m}Tc-infliximab scintigraphy has been studied in patients with rheumatoid arthritis to detect levels of intra-articular TNF-α after local therapy with infliximab.[94] This technique might be able to visualize levels of TNF-α at specific localizations in sarcoidosis patients. Hypothetically, this could also offer therapeutic options by allowing targeted therapy.

Standardization of the assessment of inflammatory activity is warranted, as well as further evaluation of relevant prognostic features. It is my hope that the answers to the above-mentioned clinical questions may be helpful in understanding the impact of this often elusive disorder and hence improve the management of sarcoidosis patients.

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