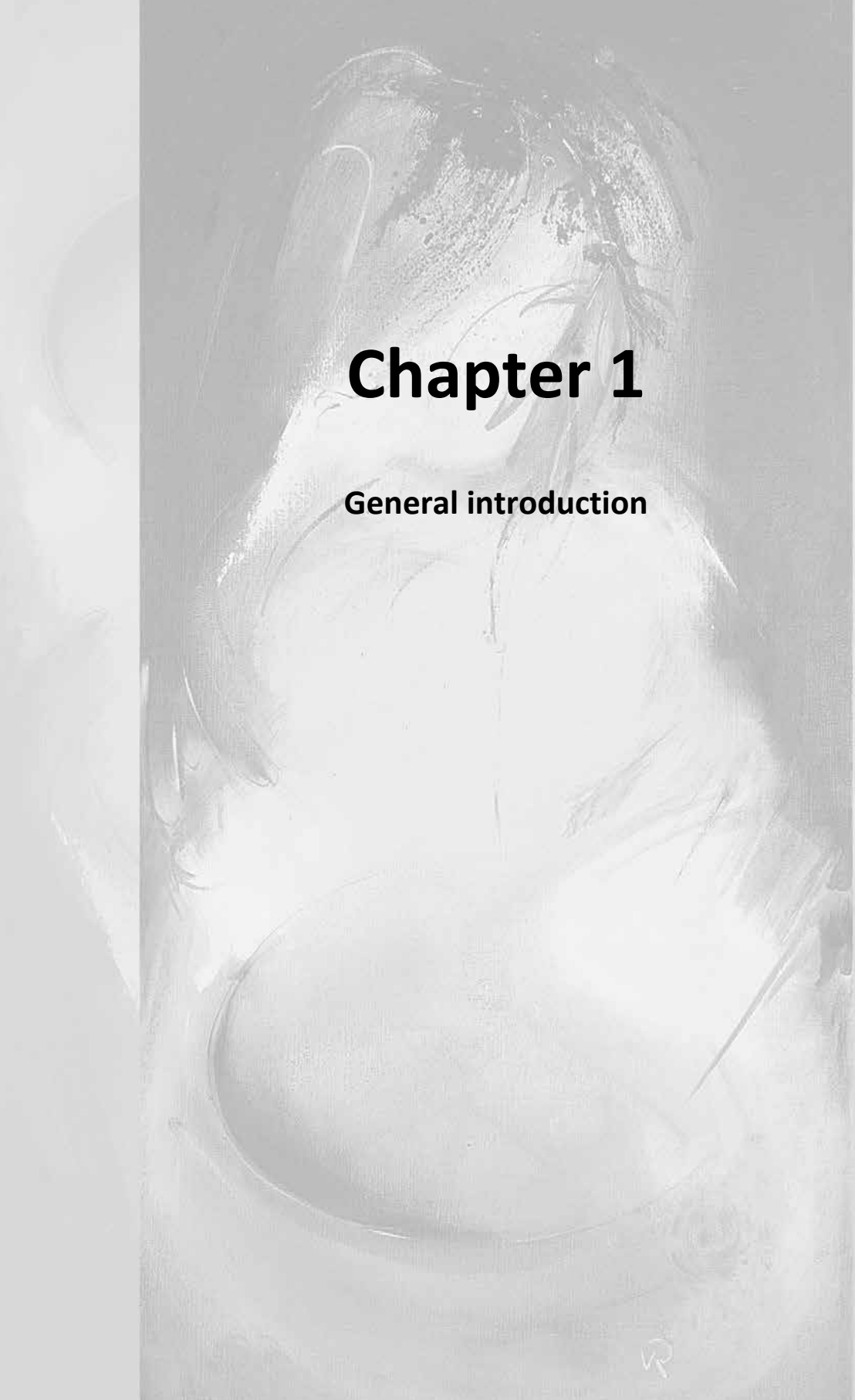

Chapter 1

General introduction

Sarcoidosis

Sarcoidosis is a multiorgan disease of unknown cause, characterized by inflammatory activity with formation of noncaseating granulomas in various organ systems.[1] Sarcoidosis has been known for more than 100 years; it was first described in 1877 by the English physician Hutchinson, and several years later by two dermatologists, Besnier and Boeck.[1] It primarily affects the lungs and the lymphatic system, but virtually any organ system can be involved, such as the liver, skin, eyes, heart, and nervous system.[1]

The disease occurs throughout the world, affecting both genders, and all ages and races.[1] However, epidemiologic studies show that it most commonly affects individuals in the third to fourth decade of their lives.[2] Furthermore, the highest prevalence rates have been found in Scandinavian European countries and African-Americans in the United States had about a 3.8-fold higher age-adjusted annual incidence rate (35.5 cases per 100 000) compared with Caucasians (10.9 per 100 000).[1,3,4] The prevalence in the Netherlands is thought to be about 30–40 per 100 000.[5]

Pathogenesis

Although there has been tremendous progress in understanding the pathogenesis, the cause of sarcoidosis remains unclear.[6] Sarcoidosis is probably the end result of a process consisting of an immune response to a variety of environmental triggers in a genetically susceptible individual, in which also oxidative stress appears to play an important role.[2,6-8]

Immunopathogenesis

The development and accumulation of granulomas constitute the fundamental abnormality in sarcoidosis.[8] Granulomas are compact collections, characterized by a core of monocyte-derived epithelioid histiocytes and multinucleated giant cells encircled by CD4+ T lymphocytes (Figure 1.1).[6] In more mature granulomas, fibroblasts and collagen encase the ball-like cluster of cells, sometimes with sclerosis and altered organ architecture and function.[8] The presence of granulomas is not specific for sarcoidosis, but can also occur in other lung diseases such as hypersensitivity pneumonitis, drug reactions, and tuberculosis, or other diseases such as common variable immunodeficiency, and Crohn's disease.[1,9]

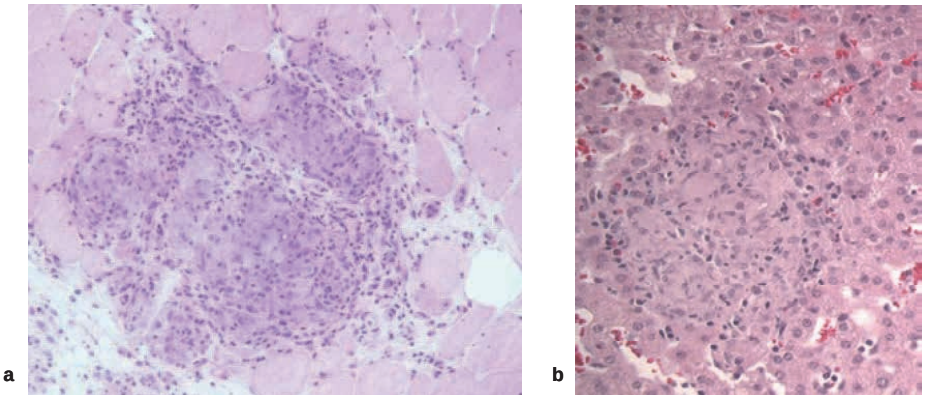

Figure 1.1 **a.** Noncaseating granuloma present in a biopsy obtained from the left quadriceps muscle of a sarcoidosis patient (H/E, magnification 10x). **b.** Noncaseating granuloma present in a liver biopsy section of a sarcoidosis patient (H/E, magnification 20x). Both granulomas consist of epithelioid histiocytes and giant cells surrounded by a rim of lymphocytes. H/E, haematoxylin and eosin.

Granuloma formation and maintenance is initiated by the recruitment of macrophages and monocytes, which internalize antigens and become antigen presenting cells.[2] CD4+ T cells interact with the major histocompatibility complex class II molecules on these antigen presenting cells.[2,8] The activated CD4+ T cells differentiate into T helper 1 (Th1) like cells and secrete predominantly interleukin (IL)-2 and interferon-gamma (IFN-γ), augment macrophage tumor necrosis factor-alpha (TNF-α) production, and amplify the local cellular immune response.[8] IL-2 acts as a local growth factor for T lymphocytes, whereas IFN-γ enhances the accessory and cytotoxic functions of T cells and regulates the secretion of other lymphokines.[2] TNF-α, IL-12, and IL-18 play critical roles in supporting the Th1 cell response by inducing IFN-γ production and enhancing T cell cytotoxicity.[2,10] This facilitates the formation of granulomas. The formed granuloma can resolve spontaneously, but may persist and can lead to a chronic inflammatory status.[6] In case of ongoing antigen presentation, a shift from cytokines produced by Th1 cells to cytokines produced by Th2 cells (e.g. transforming growth factor-β), appears to stimulate fibroblast proliferation and collagen production, leading to fibrosis.[8] Persistent granulomatous inflammation may in part be due to failure of immune regulatory mechanisms to resolve the inflammatory process.[6] Figure 1.2 shows a schematic presentation of granuloma formation in sarcoidosis.

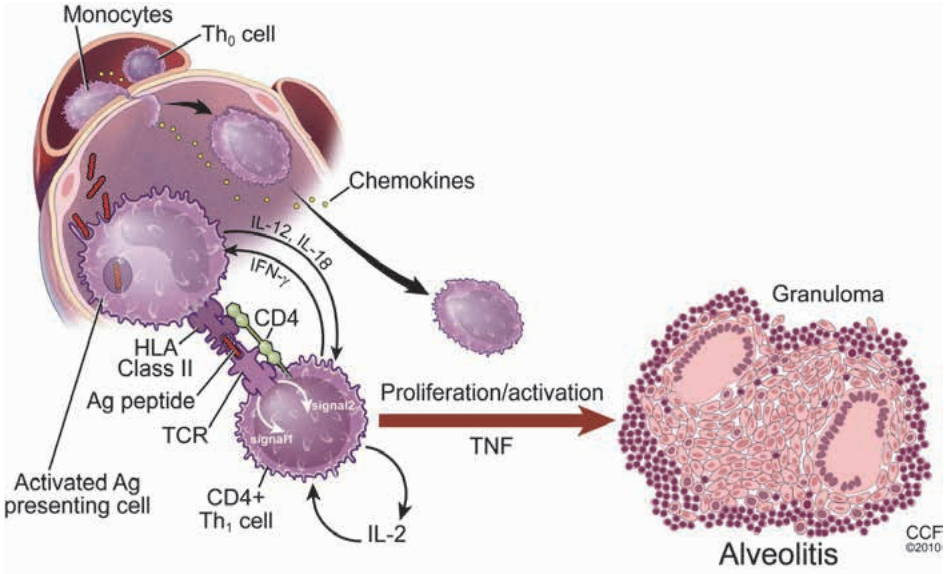

Figure 1.2 A schematic presentation of the inflammatory response with granuloma formation in sarcoidosis. An antigen induces antigen-specific, Th1 mediated granulomatous inflammation with production of Th1 cytokines (IFN-γ, IL-2). Granuloma formation is set in motion by activated macrophages and T cells along with other effector cells (e.g. fibroblasts) under the regulatory influence of local cytokine production. Removal of the antigen allows down regulation of the immune response. Alveolar macrophages activated in the context of a predominant Th2 response appear to stimulate fibroblast proliferation and collagen production, leading to progressive fibrosis.[8] Ag, antigen; HLA, human leukocyte antigen; IFN-γ, interferon-gamma; IL, interleukin; TCR, T cell receptor; Th, T helper; TNF, tumor necrosis factor. Adapted from Baughman et al.[6]

Environmental causes

Sarcoidosis probably requires exposure to one or more exogenous antigens.[6] It is quite possible that the triggering antigen varies depending on ethnicity, geographic location, and individual genetic background.[6] Mycobacterial, fungal, and other microbial antigens, are suspected as possible causative agents.[6,8,11] Furthermore, environmental and occupational exposures are associated with the development of sarcoidosis.[11] A Case Control Etiologic Study of Sarcoidosis (ACCESS) study identified several exposures associated with sarcoidosis risk, including insecticides and working in musty environments with bioaerosol exposure.[12] Also exposure to inorganic particulate matter can promote an inflammatory response leading to granuloma formation.[11] Occupational studies have shown positive associations with service in the United States Navy, metalworking, firefighting, and the handling of building supplies.[8]

Genetic features

Familial clustering of sarcoidosis has been demonstrated.[13] In the ACCESS study, sarcoidosis patients were almost five times more likely than controls to report a sibling or a parent with a history of sarcoidosis.[14] There is a higher familial relative risk in whites compared with African-Americans.[14] A range of different genes and polymorphisms have been investigated and found to have an influence on the disease. Genetics studies have focused on human leukocyte antigen (HLA) genes.[1] HLA are cell surface proteins that are essential for immune recognition and function. For example, carriage of HLA-DRB1*1101 and HLA-DPB1*0101 alleles is found to be a risk factor for sarcoidosis,[15] whereas HLA-DQB1*0201 and HLA-DRB1*03 are strongly associated with acute disease and a good prognosis.[16-18] Of the *TNF* genes, the *TNF-α* polymorphisms have been extensively studied.[18] The presence of a *TNF-α* -308A variant allele is associated with a favorable prognosis.[18] Furthermore, the presence of a single nucleotide polymorphism in the butyrophilin-like 2 (*BTNL2*) gene, which normally reduces proliferation and cytokine production from activated T cells, increases the risk of developing sarcoidosis and doubles the risk of progressing to persistent pulmonary sarcoidosis.[19-21] These findings illustrate that genetic predisposition appears not to be dependent upon a single gene and/or polymorphism.

Oxidative stress

An important role for oxidative stress in the etiology of sarcoidosis has also been proposed, as the consequence of an imbalance between the presence of and the protection against reactive oxygen species (ROS).[7,22-25] ROS are capable of reducing endogenous defence levels and enhancing inflammation.[7] It has been shown that oxidized proteins are elevated in the bronchoalveolar lavage (BAL) fluid of sarcoidosis patients.[26,27] Another consequence of the occurrence of oxidative stress is a reduced redox state, as is reported in the erythrocytes of female sarcoidosis patients.[24] Furthermore, the transcription factor nuclear factor-kappa beta (NF-κB), known to be activated by radical damage, is increased in alveolar macrophages and mononuclear blood cells of active sarcoidosis patients compared to those of healthy controls.[25] And finally, the total antioxidant capacity in sarcoidosis patients is approximately 75% of that of matched controls.[7]

Clinical presentation

The presentation and the course of sarcoidosis are highly variable, depending on the ethnicity, duration of the illness, the specific site and extent of organ involvement, and the fluctuating activity of the granulomatous process.[1,2] Sarcoidosis activity can lead to a wide range of disease severity, varying from minimal involvement to derangement of organ physiology with functional impairment.[1,2,6,28]

Patients can present with various clinical signs and symptoms depending on the organs involved.[1,2] Furthermore, nonspecific constitutional symptoms, such as fever, malaise, and weight loss, occur in about one-third of patients.[1] In addition, patients can also suffer from generic disease symptoms, such as fatigue, exercise intolerance and muscle weakness, with substantial impact on the quality of life of patients and their families.[1,29-32]

The acute form of sarcoidosis, i.e. Löfgren's syndrome, is characterized by fever, polyarthritis, erythema nodosum, and bilateral hilar lymphadenopathy.[1] In general, the prognosis of an acute onset of disease is good with usually spontaneous resolution within 2 years.[1,33,34] However, in 10–30% of the cases sarcoidosis has an insidious onset, and in these patients the disease can become chronic with an often relapsing course in which spontaneous remission is less likely.[2,34] A worse prognosis is associated with certain patient and disease characteristics, like black race, extrapulmonary (especially neurological, cardiac and osseous) involvement, and advanced pulmonary disease (Table 1.1).[1,33-38] The mortality rate of sarcoidosis is approximately 5%, usually the result of cardiac or neurological involvement, or respiratory failure due to pulmonary fibrosis.[2]

Table 1.1 Adverse prognostic factors in sarcoidosis.[1,33-38]

Age ≥40 years at onset
African-American
Requirement for glucocorticosteroids, especially within the first 6 months of diagnosis
Extrapulmonary involvement
Cardiac involvement
Chronic uveitis
Hepatomegaly
Hypercalcaemia
Lupus pernio
Nasal mucosal involvement
Neurological involvement (except isolated cranial nerve palsy)
Osseous involvement
Splenomegaly
Pulmonary involvement
Bronchoalveolar lavage neutrophilia at presentation
Moderate to severe dyspnea on presentation
Pulmonary hypertension
Significant lung function impairment
Stage III–IV chest radiograph (absence of lymphadenopathy)[a]

[a] According to the Scadding radiographic staging system: stage 0, normal chest radiography; stage I, bilateral hilar lymphadenopathy (BHL); stage II, BHL and parenchymal abnormalities; stage III, parenchymal abnormalities without BHL; and stage IV, advanced lung fibrosis.[1] Adapted from Lazar and Culver.[35]

Diagnostic procedures

The diagnosis of sarcoidosis is supported by a compatible clinical and radiographic presentation together with histological evidence of noncaseating granulomas on biopsy.[1,2] Important procedures available for the diagnostic work-up include assessment of laboratory parameters, pulmonary function and exercise capacity tests, radiographs and high-resolution computed tomography (HRCT) of the chest, fluorine18-fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG PET/CT or PET) and magnetic resonance (MR) imaging to complement more routinely used imaging techniques, and biopsy.[1,6] Both pulmonary and extra-pulmonary localizations should be assessed.[1]

Laboratory parameters

Inflammatory sarcoidosis activity is reflected by an increase in serological markers of inflammatory activity, i.e. angiotensin-converting enzyme (ACE), soluble-interleukin2-receptor (sIL2R), C-reactive protein (CRP), and neopterin.[6,39-42] These serological markers are not specific for sarcoidosis and are not used for establishing the diagnosis, but for the assessment of inflammatory activity.[6]

Elevated calcium levels can be found in both serum and independently in the urine of sarcoidosis patients, resulting from overproduction of 1,25-dihydroxyvitamin D3 by activated macrophages in granulomas.[8,43] Furthermore, laboratory abnormalities as a consequence of specific organ involvement can be found, such as liver test abnormalities, anaemia, leucocytopenia, and thrombocytopenia.[8,44,45]

Pulmonary function and exercise capacity tests

Depending on the severity of the sarcoidosis process in the lung, deterioration of pulmonary function with a wide spectrum of lung function abnormalities can occur.[46,47] Abnormal pulmonary function tests, including the diffusing capacity for carbon monoxide (DLCO) and forced vital capacity (FVC), are used as indicators for treatment.[46]

A substantial number of sarcoidosis patients are hindered by reduced exercise capacity, as established using the 6-minute walk distance (6MWD).[30,48] Exercise intolerance is shown to correlate among others with reduced peripheral muscle strength.[30,49] Information about body composition profiles in sarcoidosis is lacking. However, a trend towards lower levels of fat-free mass (FFM) in sarcoidosis patients with reduced leg muscle strength was found.[30] Furthermore, cardiopulmonary exercise testing, including maximal oxygen uptake (VO_2max), offers added value in monitoring impaired gas exchange during exercise in sarcoidosis patients with disabling symptoms, even in those with normal DLCO at rest.[50]

Imaging techniques

The majority of sarcoidosis patients (between 85 and 95%) have chest radiograph abnormalities.[47] According to the Scadding radiographic staging system, five stages of radiographic abnormality can be recognized: stage 0, normal chest radiography; stage I, bilateral hilar lymphadenopathy (BHL); stage II, BHL and parenchymal abnormalities; stage III, parenchymal abnormalities without BHL; and stage IV, advanced lung fibrosis.[1,51]

HRCT makes it possible to view the lung structure in detail and detect abnormal changes of the lung parenchyma at earlier stages than with chest radiographs.[46,47,52-54] Furthermore, HRCT is more valuable in making a specific diagnosis compared with chest radiography.[55] A HRCT scoring system, adapted from one previously described by Oberstein et al.[56], demonstrated to have a good agreement in intra- and inter-reader reproducibility and moreover, appeared to predict the presence of respiratory functional impairment in sarcoidosis.[55]

In sarcoidosis, PET can be of added value in patients with unexplained persistent disabling symptoms, especially in those without serological signs of inflammatory activity (Figure 1.3), in patients with radiological signs of fibrosis, and in the detection of active cardiac sarcoidosis.[41,57-62] Furthermore, PET is useful in the assessment of the extent of disease with specific organ involvement to uncover a suitable location for biopsy (for diagnosis) or to explain persistent symptoms.[41,57-62]

MR imaging can be useful for assessment of extrapulmonary sarcoidosis.[62] Its value in cardiac sarcoidosis and neurosarcoidosis has been demonstrated.[62-66] In cardiac sarcoidosis, the combined use of PET and cardiac MR imaging may provide optimal detection of the disease by differentiating between patients with active granulomatous inflammation and those with fibrous lesions.[62] Furthermore, MR imaging can also be helpful when assessing bone marrow and muscular sarcoidosis involvement.[62]

Biopsy

In the presence of a compatible clinicoradiographic picture, the establishment of granulomas by biopsy can ascertain the diagnosis sarcoidosis.[1] A biopsy is not required in all cases.[6] A presumptive diagnosis without tissue biopsy can also be made in some special situations, which include the presence of bilateral hilar adenopathy on the chest radiograph of an asymptomatic patient, the presentation with a classic Löfgren's syndrome, and Heerfordt syndrome (uveitis, parotiditis, and fever).[1,6]

A typical clinicoradiographic picture can be combined with the results of BAL fluid analysis to establish the diagnosis of sarcoidosis, without the need for histological confirmation by biopsy.[1] Furthermore, BAL is also useful in the differential diagnosis of interstitial lung diseases.[67] In sarcoidosis, the majority of patients have an increased number of lymphocytes and a normal amount of eosinophils and neutrophils in BAL fluid.[67,68] Furthermore, in most patients an increased CD4/CD8 ratio can be found, which has a high specificity (92–94%), but low sensitivity (55–59%).[1] Since the CD4/CD8

ratio in BAL can also be normal or decreased, it is of limited diagnostic value in the individual patient.[67,68]

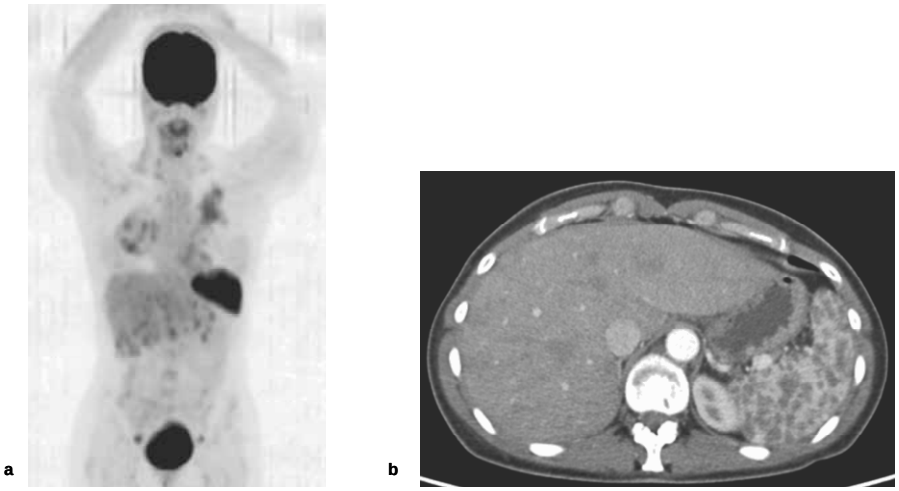

Figure 1.3 A 42-year-old woman with a 2-year history of biopsy-proven sarcoidosis had been suffering from disabling symptoms, such as fatigue, exercise intolerance and muscle weakness for more than a year, without abnormal inflammatory serum parameters. A ^{18}F-FDG PET scan was performed. **a.** The whole-body PET scan showed very high metabolic activity in the spleen and less intense activity in the liver (black). Abnormalities were also seen in the lungs as well as in the para-aortic, para-iliacal and inguinal lymph nodes. Normal physiological glucose uptake was seen in the brain and bladder. **b.** CT scan showed multiple hypodense lesions in the liver and especially in the spleen.

Therapeutic options

Most sarcoidosis patients show spontaneous resolution of the disease and do not require systemic therapy.[34,51] However, for patients with a severe disease course and poor prognosis a timely implementation of a potent individual treatment regimen is important to avoid or slow down the development of complications and to alleviate the disease burden.[51] Individual series of cases of sarcoidosis have found that the need for systemic therapy ranges from 20 to 70% of patients.[34,69-71] Of those patients who have commenced therapy, at least half appear to require systemic treatment for more than 2 years.[33,34,70]

Factors which are associated with a good prognosis without the need for systemic therapy are the presence of hilar adenopathy alone, the presence of Löfgren's syndrome and the absence of needing systemic therapy within the first 6 months of

diagnosis.[34] While these patients in general do well, approximately 10% will need long-term systemic therapy.[33,34,70] Patients with adverse prognostic factors (Table 1.1) are more likely to become chronic, requiring long-term treatment.[1,33-38] However, the majority of these patients can be maintained on a relatively low-dose immunosuppression regimen.[34,72] A subset of patients require more aggressive treatment.[34] In a survey of sarcoidosis clinics across the world, this represented approximately 10% of all patients who were still being seen 5 years after their original diagnosis.[34,73]

The published data on the different treatment options in sarcoidosis are limited and the treatment remains therefore mostly empiric.[34,74] In particular, there have been only a few high quality placebo controlled, double blind studies of the classical drugs used to treat sarcoidosis.[74] One of the difficulties has been the lack of standardized validated follow-up measures to assess response to therapy.[74] However, recommendations for clinical evaluation of the outcome measurements have recently been established.[74,75]

Several pharmacological options exist for patients who require therapy. However, none of these drugs are curative. In cutaneous sarcoidosis, topical treatment can be sufficient to control the disease.[6] Nonsteroidal anti-inflammatory drugs (NSAIDs) can be effective for symptom relief in patients with arthralgia/arthritis.[76,77] Furthermore, the antioxidant quercetin is shown to have beneficial effects in sarcoidosis.[22] This flavonoid offers protection against ROS-induced oxidative damage and has anti-inflammatory capacities by a reducing effect on among others TNF-α.[22]

The decision on whether to start systemic immunosuppressive treatment or not should be based on the patient's symptomatology, including those that affect quality of life, as well as the extent of compromised organ function.[6,34,74,78] Although several immunosuppressive drugs are available, there is a lack of standardized management strategies for sarcoidosis.[78]

First-line therapy: glucocorticosteroids

In general, glucocorticosteroids are considered the first-line therapy for acute and chronic pulmonary and extrapulmonary sarcoidosis.[74,78,79] Glucocorticosteroid therapy has been the standard therapy for over 50 years.[74] Prolonged glucocorticosteroid use is associated with significant side-effects, such as weight gain/obesity, diabetes mellitus or osteoporosis, making glucocorticosteroids undesirable for chronic disease management.[78,80] Tapering to the lowest effective dose is an ultimate treatment goal and alternative glucocorticosteroid-sparing treatment agents are needed.[6,34,74,78] Furthermore, in steroid-refractory cases, second-line nonsteroidal drugs, which are referred to as disease-modifying antisarcoid drugs (DMASDs), and third-line therapies, offer alternative strategies.[6,34,74,78]

Second-line therapy: disease-modifying antisarcoid drugs

Several agents have been used as DMASDs in both pulmonary and extrapulmonary sarcoidosis. Table 1.2 summarizes the most important second- and third-line therapeutic agents. Knowledge of second-line agents in sarcoidosis is based on only a few placebo controlled, double blind, randomized trials, but mainly is derived from retrospective clinical trials and case reports.[74,78]

Table 1.2 Overview of second-line and third-line agents for the treatment of sarcoidosis.[81-112]

Second-line therapeutic agents	Third-line therapeutic agents
Methotrexate (MTX)	TNF-α inhibitors
Azathioprine (AZA)	Infliximab
Leflunomide (LEF)	Adalimumab
Mycophenolate mofetil (MMF)	New experimental treatment modalities
Pentoxifylline (POF)	Rituximab (RTX)
Thalidomide	Vasoactive intestinal peptide (VIP)
Cyclosporine A	Apremilast
Cyclophosphamide	Armodafinil (in fatigue)
(Hydroxy)chloroquine ((H)CQ)	Methylphenidate (in fatigue)
	Intravenous immunoglobulins (in SFN)
	ARA 290 (in SFN)

SFN, small fiber neuropathy; TNF-α, tumor necrosis factor-alpha.

Methotrexate

Methotrexate (MTX) has been the most widely studied second-line therapeutic agent for sarcoidosis.[74] The efficacy of MTX has been demonstrated in pulmonary sarcoidosis, but also in cutaneous, ocular, musculoskeletal, liver, and neurological sarcoidosis.[82-84,113-118] MTX is often considered the first-choice DMASD used for patients with sarcoidosis, with 80% of physicians reporting MTX as their preferred second-line option (Figure 1.4).[34,119]

Initially, the anti-inflammatory effects of MTX were ascribed to its inhibition of cellular proliferation through the inhibition of the enzyme folate reductase, the cytostatic mechanism of action in the treatment of cancer.[120,121] However, current evidence has shown that its anti-inflammatory action differs from its cytostatic activity.[120] A proposed mechanism of action of MTX is the reduction of intracellular glutathione levels by an oxidant-associated mechanism, which leads to inhibition of macrophage recruitment and function.[121] A third mechanism is the inhibition of the synthesis of the potentially toxic compounds spermine and spermidine (transmethylation products).[121] However, the most likely explanation of the anti-inflammatory actions of MTX is the stimulation of adenosine release.[120,121] Adenosine suppresses the inflammatory functions of neutrophils, macrophage/monocytes, dendritic cells and lymphocytes.[120,121]

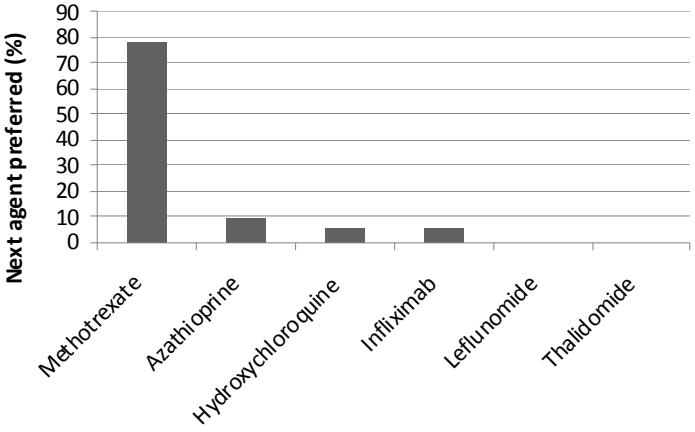

Figure 1.4 Results of a Delphi analysis, showing which agent is preferred as the next agent chosen to add to or to replace glucocorticosteroids for sarcoidosis patients experiencing an inadequate response to glucocorticosteroid use. Data taken from Schutt et al.[34,119]

Many of the typical side-effects of MTX are due to its antifolate properties, including leucocytopenia, anaemia, stomatitis, and alopecia.[120] Administration of folic acid reduces gastrointestinal and hepatic toxicity without affecting efficacy.[122,123] Adenosine release may also help explain the MTX induced hepatic toxicity, since adenosine, acting at A_1 and A_{2B} receptors, stimulates hepatic steatosis and adenosine, acting at A_{2A} receptors, plays an important role in the development of hepatic fibrosis.[120] MTX can also lead to pulmonary toxicity; unexplained cough should lead to evaluation for MTX toxicity (Figure 1.5).[74] However, the frequency of life threatening interstitial lung disease from MTX is only 1%.[74,124]

Azathioprine

Azathioprine (AZA) has been reported as effective in treating pulmonary and extrapulmonary sarcoidosis.[74] The reports usually have been case series. Compared to MTX, AZA demonstrates a similar efficacy in pulmonary and extrapulmonary sarcoidosis.[78,82] Both drugs have been shown to be effective steroid-sparing agents in pulmonary sarcoidosis, but more infections were reported with AZA.[82]

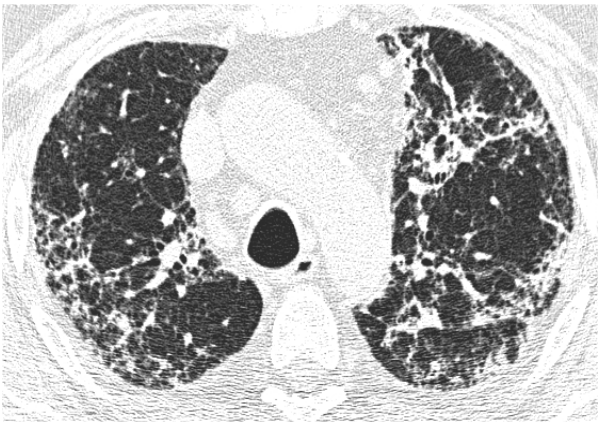

Figure 1.5 High-resolution computed tomography study showing the consequences of pulmonary toxicity caused by methotrexate. There is an irregular reticular pattern with areas of confluent opacities, small cystic changes and a few traction bronchiectasis. These abnormalities are predominantly located in the dorsal and basal part of both lungs. Although on this slice there is some extension of disease in the anterior part of the left lung.

AZA inhibits nucleotide synthesis by feedback inhibition in the early stages of purine metabolism and it prevents T cell and B cell proliferation.[125] AZA is metabolized by the enzyme thiopurine S-methyltransferase (TPMT) to 6-mercaptopurine.[74] AZA is associated with nausea and leucocytopenia.[74] Furthermore, it can lead to severe hepatotoxicity.[74,126] Patients with low or deficient TPMT levels are at higher risk of developing drug-related toxicity.[127] Heterozygous individuals with intermediate enzymatic activity comprise 5–15% of patients, while approximately 0.3% are homozygous, with very low or absent enzymatic activity.[128] Therefore, measuring TPMT before initiating AZA therapy is recommended.[127]

Leflunomide

Leflunomide (LEF) is used as an alternative or in addition to MTX.[74,78] However, there are no studies directly comparing the effectiveness and side-effects of LEF and MTX. LEF has been reported as effective in two observational case series.[88,89] A complete or partial response for cutaneous, ocular, and sinonasal involvement was seen, but LEF was less effective for neurological and musculoskeletal manifestations.[78]

LEF inhibits the enzyme dihydroorotase in the pyrimidine synthesis.[78] Major toxicities are similar to MTX.[74,78] There is no difference in hepatic or haematologic changes during long-term monitoring in patients treated with both LEF and MTX compared with patients who were treated with MTX alone.[74] Worrisome side-effects reported for LEF include severe weight loss and peripheral neuropathy.[78,88,89] Pulmonary toxicity has been reported with LEF, but at a lower rate than that reported

with MTX.[78,124] A recently reported safety issue with LEF is silent fibrosis.[78] Patients with rheumatoid arthritis who received concomitant LEF and MTX for more than 6 months had an increased risk of silent liver fibrosis.[78,129]

Mycophenolate mofetil

Mycophenolate mofetil (MMF) is an inosine monophosphate dehydrogenase inhibitor that has an antiproliferative effect on lymphocytes and profoundly attenuates the production of autoantibodies by B cells.[78] A recent study, which evaluated MMF as a steroid-sparing agent in 10 patients with chronic pulmonary sarcoidosis, found significantly reduced daily glucocorticosteroid doses.[78,90] Furthermore, in four patients a reduction of pulmonary symptoms and radiological signs and improvements in pulmonary function were seen; whereas, the other six patients' diseases remained stable.[90] MMF might also have a role in neurosarcoidosis affecting the central nervous system.[130] Combining MMF with systemic glucocorticosteroids did not cause any severe side-effects.[90,130] Adding MMF to glucocorticosteroids may be a viable and safe treatment option in chronic pulmonary and central nervous system sarcoidosis, but additional studies are necessary.

Pentoxifylline

Pentoxifylline (POF), a methylxanthine derivative, inhibits phosphodiesterase resulting in reduction of inflammation by inhibition of a number of pro-inflammatory cytokines, including TNF-α.[74] Some studies reported POF to be an effective therapeutic option in acute pulmonary sarcoidosis, with improvement of DLCO and a trend for the drug to be steroid-sparing.[91,92] The drug is associated with significant gastrointestinal toxicity, which limits its use in treatment of chronic sarcoidosis.[35,74]

Thalidomide

Thalidomide is a synthetic derivate of glutamic acid and has several molecular targets. The most important function in sarcoidosis is the inhibition of TNF-α production.[125] It was first reported as an effective treatment in chronic cutaneous sarcoidosis.[131] In pulmonary sarcoidosis, thalidomide has been proven effective in improving pulmonary function, whereas in some cases the drug was steroid-sparing.[93,94] Recent in vitro research showed inhibitory effects of thalidomide on lung fibroblasts, which needs further investigation in vivo.[132] Thalidomide has severe teratogenic effects. Side-effects include peripheral neuropathy, hypersomnulance, and an increased risk for deep venous thrombosis and pulmonary embolism.[94]

Cyclosporine A

Cyclosporine is a fungal peptide that suppresses T cell activation.[125] In some case reports it was thought to be effective for refractory disease.[74,133] However, a double blind, randomized trial of chronic pulmonary sarcoidosis found no significant benefit from adding cyclosporine A to glucocorticosteroids therapy in terms of pulmonary function or steroid-sparing potency.[95] The drug is associated with major side-effects, including hypertension, renal dysfunction, increased risk for opportunistic infections, and malignancy.[95] Therefore, cyclosporine A is rarely used to treat sarcoidosis.[74]

Cyclophosphamide

Cyclophosphamide is metabolized in the liver into active alkylating metabolites, which inhibit lymphocyte number and function with suppression of both cellular and humoral immunity.[78,125] In sarcoidois, cyclophosphamide is typically used for cardiac sarcoidosis or neurosarcoidosis.[78,134-136] Adverse events are generally mild, mainly consisting of gastrointestinal symptoms.[78] However, major side-effects, like hemorrhagic cystitis, recurrent pneumonia, and liver test abnormalities, require cyclophosphamide discontinuation.[137] It also possesses the risk of ovarian failure in women of reproductive age.[78]

Chloroquine and hydroxychloroquine

The antimalarial agents, chloroquine (CQ) and hydroxychloroquine (HCQ), have demonstrated efficacy in sarcoidosis, most likely as a result of their immunomodulatory properties.[74,78] CQ has been used for both cutaneous and pulmonary sarcoidosis, whereas HCQ is mostly effective for cutaneous sarcoidosis.[74,78,96,138,139] CQ is associated with significant gastrointestinal and ocular toxicity, which is dose dependent.[74,140] HCQ has been preferred, but monitoring for ocular toxicity is still necessary.[74,141]

Third-line therapy: TNF-α inhibitors and new experimental treatment modalities

For some patients with sarcoidosis, glucocorticosteroids and DMASDs may not control disease.[74] In certain patients, excessive production of TNF-α by alveolar macrophages retrieved by BAL has been reported.[142] Several biological agents which specifically inhibit TNF-α have become available and have demonstrated efficacy in sarcoidosis, especially in patients refractory to other treatments.[74,78] Furthermore, some new experimental treatment modalities can form alternative third-line therapeutic options (Table 1.2).[74,78]

TNF-α inhibitors

TNF-α inhibitors might be a valuable treatment option for patients with pulmonary and/or extrapulmonary sarcoidosis refractory to conventional therapy, on the condition that they are used with caution.[78] Infliximab is a biological chimeric monoclonal antibody that binds to free TNF-α, blocking its interaction with the TNF-receptor, and sometimes to cell surface TNF-α.[125] Two double blind, placebo controlled trials of infliximab in patients with chronic pulmonary sarcoidosis showed a significant improvement of FVC[98,99] and in one study improvement in chest radiographs.[98] Judson et al.[143] used an extrapulmonary physician organ severity tool for evaluation of specific organ involvement in sarcoidosis. Although the sample size was low, their results suggest that infliximab may be beneficial in extrapulmonary sarcoidosis. A recent retrospective study investigated the sustainability of infliximab response in pulmonary and extrapulmonary sarcoidosis for up to 85 months and concluded that infliximab has the capacity to maintain improvements in sarcoidosis over time, based on the finding that 58,5% of the organs evaluated achieved improvement.[97]

Adalimumab is a recombinant fully human monoclonal antibody against TNF-α.[74] Prospective observational studies have shown effectiveness of adalimumab in treating refractory posterior uveitis[100], in decreasing total sarcoidosis disease activity as measured by PET[101], and in improving radiological abnormalities.[102] Adalimumab also has a positive effect on cognition and fatigue in sarcoidosis.[144] Furthermore, a recent study has shown adalimumab to be a possible effective and relatively safe treatment in cutaneous sarcoidosis.[145]

Etanercept is a TNF-receptor antagonist.[74] It has been reported effective in some cases of refractory sarcoidosis, but in an open label trial of pulmonary sarcoidosis etanercept alone was associated with treatment failure in 12 of 17 cases.[103] Furthermore, the drug failed in a double blind, placebo controlled trial of patients with refractory sarcoidosis uveitis.[146] These results are in line with observations in Crohn's disease.[147]

For all TNF-α inhibitors similar toxicities have been reported.[148] These include allergic reaction to the agents, which is most likely to occur with infliximab since this agent is a chimeric antibody.[74] The concurrent use of MTX or other cytotoxic drugs has been advised to reduce the risk of antibody formation directed against infliximab.[149] There is a marked increased risk for reactivation of tuberculosis and increased severity of tuberculosis course associated with the use of TNF-α inhibitors.[78] Prior to starting anti-TNF-α therapy, screening for latent tuberculosis, using interferon-gamma release assays (IGRAs), is recommended.[74,78,150] Furthermore, there have been several reports of sarcoidosis like reactions during the use of TNF-α inhibitors in other diseases. Fortunately, the prognosis is good, with complete resolution of the event after discontinuation in most cases.[74,78,151,152]

New experimental treatment modalities

Rituximab (RTX), a chimeric monoclonal antibody that targets CD-20 cells and considerably reduces the number of mature B lymphocytes in the circulation.[74,78] It has been reported as effective for refractory sarcoidosis in case reports.[104-106,153,154] The drug has significant toxicity, including increased risk for viral infections.[74,106]

Inhaled vasoactive intestinal peptide (VIP) is reported to be associated with significant changes in the release of several cytokines by alveolar macrophages retrieved by BAL.[107] There was not a change in the FVC.[107] This study was small with a short follow-up time.[74] The drug was well tolerated, but due to a large number of treatments per day its application may be limited.[74]

A new phosphodiesterase inhibitor, apremilast, has been reported as effective for chronic cutaneous sarcoidosis.[74,78,108] It has not yet been reported in the treatment of pulmonary sarcoidosis.

Sarcoidosis can be accompanied by devastating fatigue and by serious symptoms caused by small fiber neuropathy (SFN). These features do not always respond to the treatment options discussed above. Reduction in sarcoidosis-associated fatigue was demonstrated in patients treated with the neurostimulant methylphenidate and the central nervous system stimulant armodafinil.[29,109,110] In SFN, standard treatment options, such as antidepressants, anticonvulsants, topical anesthetics, and opioids, are only effective for symptom relieve in 30–60% of patients.[78,111] Intravenous immunoglobulins seem to be promising, but require further study.[78,111] A novel therapy is ARA 290, a nonhaematopoietic erythropoietin analogue designed to activate the innate repair receptor, with potent anti-inflammatory and tissue protective properties.[111,112] Recently, the efficacy of ARA 290 was shown in a pilot study in patients with sarcoidosis suffering from SFN.[112]

Scope and aims of this thesis

In current clinical practice, there is a lack of standardized management strategies for sarcoidosis, despite the availability of several immunosuppressive therapeutic options. Evidence-based recommendations for the optimized use of the consecutive first-, second-, and third-line therapies are lacking. Sarcoidosis is a disease which can present to any one of a variety of organ specialists, which explains that therapeutic decisions are highly dependent on the individual specialist's expertise. Inexperience among physicians can lead to suboptimal application of pharmacotherapeutic possibilities.

Optimization of therapeutic options is important to avoid unnecessary exposure of patients to consecutive pharmacological agents. Furthermore, in view of the promising results of biological agents for refractory sarcoidosis patients, a trend seems to be emerging amongst the healthcare providers towards a low threshold for switching from second-line to third-line agents. However, the drawbacks of using biological agents on a

large scale are potential major side-effects and considerable costs. It is important to improve the use of less expensive, but effective DMASDs, in order to keep the healthcare system affordable and accessible to all. Biologicals should only be started for the correct indication and under experienced supervision. Since sarcoidosis is not one disease, but rather a mixed group of conditions, individualized therapy is inevitable guided by appropriate diagnostic procedures to assess the extent of the disease. Optimal use of pharmacotherapeutic options can serve to help pursue personalized medicine for the individual patient.

The aim of the studies presented in this thesis was to improve the pharmacotherapeutic management of sarcoidosis patients. Several aspects in terms of amelioration of treatment were investigated, with emphasis on the meaning of body composition profiling in sarcoidosis, the desirable handling of liver test abnormalities, the development of practical recommendations for the use of MTX and TNF-α inhibitors, and the exploration of possible factors identifying responders to TNF-α inhibitors.

Chapter 2 provides an overview of the extent, distribution and consistency of organ involvement detected by PET in 158 sarcoidosis patients with persistent disabling symptoms. **Chapter 3** reports on the prevalence of cachexia and muscle atrophy in 423 Dutch sarcoidosis patients, and the association of these body composition profiles with sarcoidosis disease activity and severity. **Chapter 4** describes the presence and severity of liver test abnormalities in 837 patients with confirmed sarcoidosis. Additionally, the association between severity of liver test abnormalities and histopathological abnormalities in hepatic sarcoidosis was evaluated. **Chapter 5** provides an overview of therapeutic options for sarcoidosis liver involvement, and combines these data into recommendations for the optimal therapeutic approach of hepatic sarcoidosis. **Chapter 6** presents the development of practical recommendations, on behalf of the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG), for the use of MTX in sarcoidosis by integrating the evidence obtained through a systematic literature review and the expert opinions of 113 sarcoidologists worldwide. **Chapter 7** describes the development of practical recommendations for the use of the TNF-α inhibitors infliximab and adalimumab in sarcoidosis by combining the evidence from both a systematic literature search and the opinions of the world's leading sarcoidosis experts. **Chapter 8** assesses the association between the presence of the *TNF-α* G-308A polymorphism and the response to TNF-α inhibitors amongst 111 patients with refractory sarcoidosis. **Chapter 9** summarizes the findings presented in this thesis and argues their implications. Finally, directions for future research are discussed.

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