SYSTEMIC TREATMENT STRATEGIES IN SEVERE SARCOIDOSIS

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SYSTEMIC TREATMENT STRATEGIES IN SEVERE SARCOIDOSIS

Systemische behandelstrategieën voor ernstige sarcoidose
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op vrijdag 20 februari 2015 des middags te 12.45 uur

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CONTENTS

CHAPTER 1

GENERAL INTRODUCTION AND OUTLINE OF THE THESIS

DEFINITION AND AETIOLOGY

Sarcoidosis is a granulomatous disease with a wide variety of clinical features, which has been recognised over 120 years ago, but still remains a puzzling disease with many grey areas [1]. Although the considerable amount of research performed over the past century has improved the understanding of the pathogenesis of sarcoidosis, it has not yet unveiled the true cause of the disease [2]. Studies support the hypothesis that sarcoidosis might be the result of an exaggerated granulomatous reaction after exposure to unidentified antigens in individuals who are genetically susceptible [1]. The clinical heterogeneity of sarcoidosis suggests that multiple microbial, organic or inorganic antigens can trigger the inflammatory process [3,4]. Several types of immune cells such as Th1-lymphocytes, monocytes and macrophages are thought to play a role in sarcoidosis [5], and more recently an important role for Th17-lymphocytes and B-cells in the pathogenesis of sarcoidosis has been found [6,7]. The increased incidence amongst the other halfs of identical twins with sarcoidosis together with familial clustering has revealed a genetic component for susceptibility to sarcoidosis [8-10]. The descriptive definition of the disease, as reported by the American Thoracic Society/ European respiratory society/ World association for Sarcoidosis and Other Granulomatous Disorders Statement on Sarcoidosis states: “Sarcoidosis is a multisystem disorder of unknown cause. It commonly affects young and middle-aged adults and frequently presents with bilateral hilar lymphadenopathy, pulmonary infiltration, and ocular and skin lesions. The liver, spleen, lymph nodes, salivary glands, heart, nervous system, muscles, bones, and other organs may also be involved. The diagnosis is established when clinicoradiological findings are supported by histological evidence of noncaseating epithelioid cell granulomas. Granulomas of known causes and local sarcoid reactions must be excluded. Frequently observed immunological features are depression of cutaneous delayed-type hypersensitivity and a heightened Thl- immune response at sites of disease. Circulating immune complexes, along with signs of B cell hyperactivity, may also be found. The course and prognosis may correlate with the mode of the onset and the extent of the disease. An acute onset with erythema nodosum or asymptomatic bilateral hilar lymphadenopathy usually heralds a self-limiting course, whereas an insidious onset, especially with multiple extrapulmonary lesions, may be followed by relentless, progressive fibrosis of the lungs and other organs.” [11] This definition allows us to diagnose sarcoidosis, but does not differentiate between the various clinical evolutions and corresponding treatment regimes.

EPIDEMIOLOGY

Sarcoidosis is a global disease, affecting patients of all ages, race groups and both genders. Incidence rates vary globally with the highest rates reported in Scandinavian and African-American individuals and low rates in Japan [12-14]. In African-Americans in the United States the incidence rate was approximately 3.8 fold higher than in Caucasians (35 per 100 000 and 11 per 100 000 per year, respectively) [15]. Besides different incidence rates, also phenotypical differences between ethnic groups stand out. Löfgrens syndrome, the acute form of sarcoidosis, is more common in Scandinavian countries and rare in patients of African or Asian origin. In contrast, cardiac

sarcoidosis is most prevalent in Japan and is associated with increased mortality [16,17]. Although precise numbers are not available, the prevalence of sarcoidosis in the Netherlands is estimated at 30-40 per 100 000 [18] with incidence rates of 10-20 per 100 000 per year. Sarcoidosis is classified as an orphan disease affecting less than one in 2000 inhabitants in the Netherlands [19].

CLINICAL FEATURES AND DIAGNOSTIC TOOLS

The clinical characteristics of sarcoidosis patients vary strongly. Certain clinical and radiological features are characteristic of sarcoidosis, but none are specific for the diagnosis [2]. Although a schematic reproduction of the diagnostic process of sarcoidosis is helpful (Figure 1), in reality diagnosing sarcoidosis is often not easy. The diagnosis is usually made when the statistical likelihood of alternative diagnoses becomes too small to justify further investigation. Therefore sarcoidosis is a diagnosis per exclusion, and confidence in the diagnosis can be strengthened with time [2]. The three major criteria for diagnosis are: 1) compatible clinical and radiological presentation; 2) confirmation of non-caseating granulomas in biopsy or typical cell profile in the bronchoalveolar lavage fluid; and 3) exclusion of an alternative diagnosis associated with granuloma formation [11].

Most common symptoms are fatigue, fever, dry cough, dyspnoea, chest pain, malaise and weight loss [16]. Pulmonary involvement or enlargement of hilar lymphnodes is seen in approximately 90% of patients and can be asymptomatic. Because virtually all organs can be involved in this disease; extrapulmonary localisations may vary from skin manifestations, to cardiac sarcoidosis, neurosarcoidosis, uveitis and hypercalcaemia [20,21]. The acute form of sarcoidosis, Löfgren's syndrome presents with erythema nodosum, bilateral hilar lymphadenopathy and arthritis. Another typical presentation of the disease is Heertfort's syndrome, which is characterised by uveitis, parotid gland swelling, fever and in some cases facial nerve palsy. In these two scenarios diagnosis relies on the specific presentation of the disease and biopsy is not needed, while other scenarios require a systematic work-up to fulfil the major criteria for diagnosis as mentioned above (Figure 1) [1].

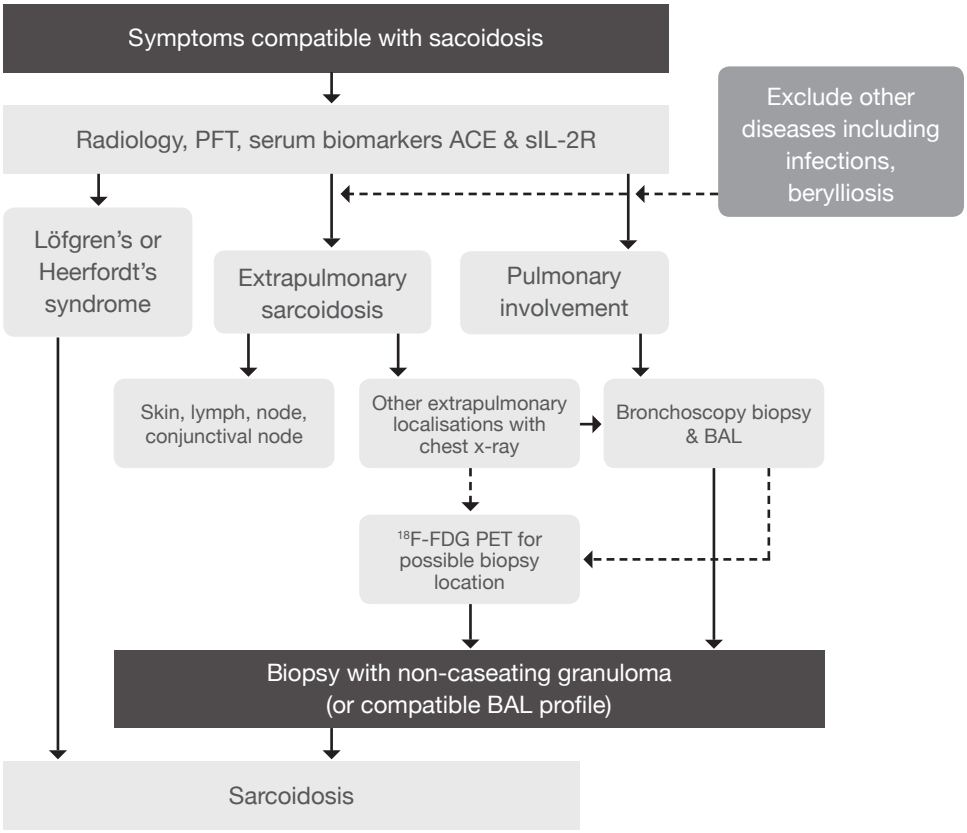


Figure 1 Diagnostic pathway of sarcoidosis
Solid line indicates usual practice. Dotted line indicates alternative practice.
PFT= Pulmonary function tests, ACE= Angiotensin Converting Enzyme, sIL-2R= soluble Interleukin-2 receptor, BAL= bronchoalveolar lavage, ^{18}F-FDG PET = 18F-fluorodeoxyglucose by positron emission tomography

HISTOLOGY

The diagnosis of sarcoidosis can be made in case of a suitable clinico-radiological presentation with presence of non-necrotising granulomas at biopsy and after exclusion of other causes of non-necrotising granulomas such as beryllium disease, Crohn's disease but also infections [11]. The granulomas found in sarcoidosis are compact collections of cells with a centre of monocyte-derived epithelioid histiocytes and multinucleated giant cells surrounded by CD4+ T lymfocytes (Figure 2A-C).

In case of need for biopsy, the best biopsy site depends on accessibility, safety, and potential yield of the procedure (Figure 1). Most easily accessible sites such as skin lesions (other than erythema nodosum) or superficial lymph nodes should be considered before other sites such as endobronchial tissue or mediastinal lymph nodes [1].

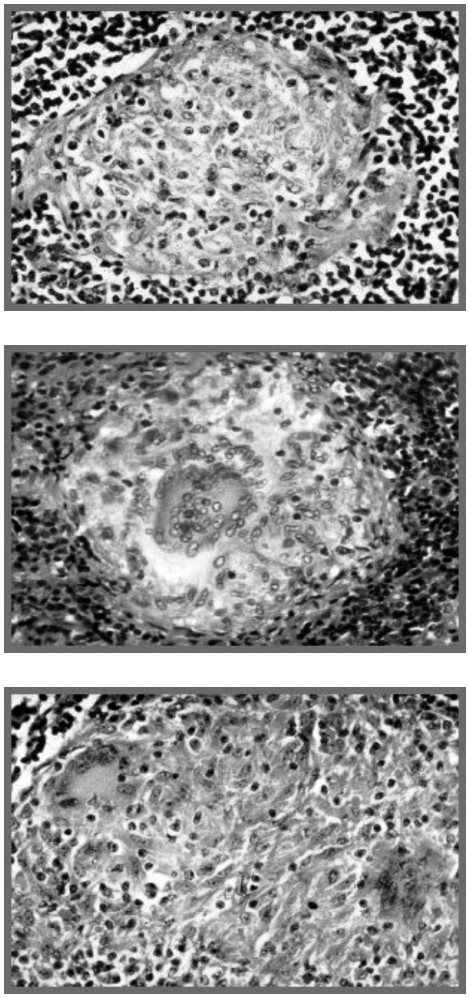


Figure 2a-c Histopathology of sarcoidosis
A Non-necrotising granuloma in sarcoidosis
B Non-necrotising granuloma with giant cell in a sarcoidosis patient
C Non-necrotising granulomas can also occur in infectious diseases such as tuberculosis
(http://granuloma.homestead.com/morphology.html)

BRONCHOALVEOLAR LAVAGE

To support the diagnosis of sarcoidosis, especially in patients without confirmation from biopsy, bronchoalveolar lavage (BAL) can be performed [11]. In 80% of sarcoidosis patients, BAL shows a moderate (20–50%) lymphocytosis. Furthermore, a T lymphocyte CD4/CD8 ratio higher than 3.5 is found in 50% of cases [22]. The combined use of CD4/CD8 ratio and number of CD103 positive CD4 cells within the CD4 cell population provides more power to discriminate between sarcoidosis and other interstitial lung diseases than CD4/CD8 ratio alone [23]. Although BAL has given valuable insights into the pathogenesis of sarcoidosis with recognition of characteristic T cell profile and proinflammatory mediators, the value of BAL for determination of prognosis appears low, as results of studies have so far been conflicting [24,25].

RADIOLOGY

Since the landmark report of Scadding in 1961 [26], chest radiographic staging has continued to provide useful prognostic information (Table 1). Between 85 and 95% of sarcoidosis patients show abnormalities on chest radiography. The most common diagnostic sign on chest radiographic examination are bilateral intra-thoracic hilar lymphadenopathy, which occurs in up to 80% of newly diagnosed patients, and diffuse micro-nodular parenchymal infiltration [27]. In general, patients with lower radiographic stages (stage I or II) are more likely to have resolution of symptoms and chest radiography abnormalities. However, many patients do not show a stepwise progression from stage I to stage IV as their pulmonary disease develops [27]. And importantly, a disadvantage of the Scadding staging system is poor inter-observer agreement [28]. Furthermore, although correlations exist between Scadding stage and spirometry data in larger groups of patients, the Scadding system has limited applicability in individual patient evaluation due to large individual variation [29]. Moreover, the staging system was shown to only weakly correlate with the level of dyspnoea [30]. Moreover, Scadding stages do not correspond with the type and extend of extra-pulmonary organ involvement.

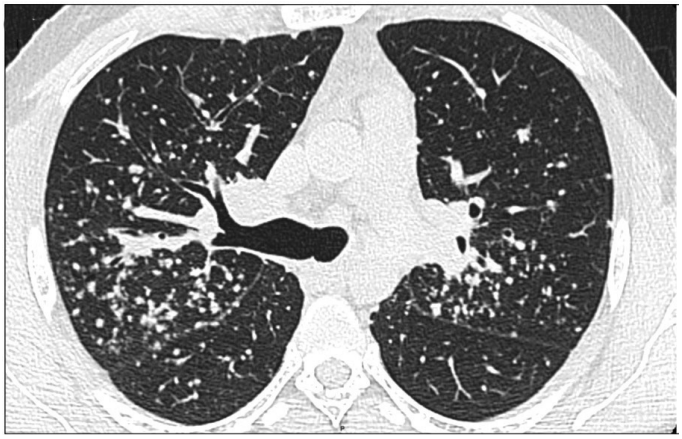
Table 1 Scadding Staging system on chest radiography [26]

Chest Radiograph Stage	Appearance	% at Diagnosis
Stage 0	Normal Chest radiograph	5-15
Stage I	Nodal enlargement	45-65
Stage II	Nodal enlargement with parenchymal infiltrates	30-40
Stage III	Parenchymal infiltrates (without nodal enlargement)	10-15
Stage IV	Parenchymal fibrosis	5

High Resolution Computed Tomography (HRCT) was proven superior to con¬ventional CT or chest X-ray for assessing subtle parenchymal abnormalities in sarcoidosis [27]. Common features that can be assessed into more detail on HRCT than normal X-ray are peribronchovascular thickening, diffuse micronodular pulmonary infiltration which is associated with a typical lymphatic distribution and sometimes the galaxy sign (Figure 3) [31]. Because HRCT can show lung abnormalities at earlier stages than normal chest X-ray, the prognostic value of the Scadding system for chest X-rays can not be translated to HRCT directly. For example patients with minor parenchymal abnormalities detected on HRCT, which would not be seen on chest X-ray, still have a good prognosis [31].

Unfortunately, HRCT is not a good indicator of disease activity. In a large study of 95 patients, HRCT findings did not reliably distinguish between active disease (i.e., high serum ACE levels or a BAL lymphocytosis) and inactive disease. Furthermore, correlation between individual HRCT patterns and lung function indices were rather poor [32] . A scoring system by Drent and colleagues did

have good inter-observer agreement and correlated with lung function impairment in sarcoidosis [33]. Still, this scoring system did not find its way into practice on a wide scale.



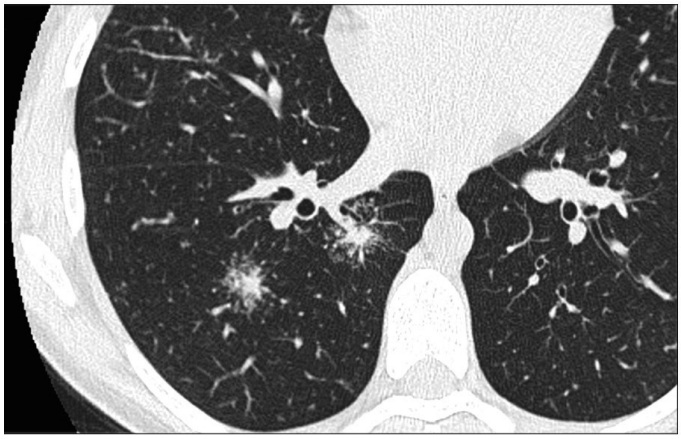


Figure 3a and b: HRCT of typical features of pulmonary sarcoidosis (Valeyre et al [1])
A Shows typical perilymphatic distribution of micronodules with fissural spreading
B Shows typical nodules with irregular margins and satellite micronodules known as the galaxy sign.
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FUNCTION

In interstitial lung diseases, it has been long recognised that pulmonary function tests (PFTs) reflect underlying disease severity more accurately than chest X-rays or symptoms [34]. Because granulomas can be located in the alveolar walls, small lymphatics, the larger airways and respiratory muscles, lung function abnormalities may arise in any compartment, resulting in a wide spectrum of functional impairment patterns. Although restrictive physiology with impaired forced vital capacity (FVC) is most often observed [2,16,35], airflow obstruction is found in a significant percentage of patients [36,37]. An obstructive pattern may be the result of endobronchial disease or stenosis, airway hyperreactivity or parenchymal airway distortion [38,39]. A reduced diffusion capacity may be related to restrictive disease, but a disproportionate reduction in gas transfer is suggestive of pulmonary hypertension [40-42]. Due to the high inter-patient variability, it is advised to perform the full range of routine testing PFTs, including spirometric volumes, plethysmographic volumes, and measures of gas transfer at initial evaluation [27].

In contrast to findings in Idiopathic Pulmonary fibrosis, FVC at presentation does not correlate directly with mortality [27]. Furthermore, only a modest correlation between FVC and the level of patient reported dyspnoea exists [30].

Functional testing is challenging in extrapulmonary sarcoidosis. In cardiac sarcoidosis, left ventricular ejection fraction can be guiding to estimate treatment effect [43]. For many other forms of extrapulmonary sarcoidosis e.g. neurosarcoidosis or small fibre neuropathy, no specific functional tests exist. The difficult task of monitoring treatment effect or disease progression should in these cases be evaluated by the treating physician based on imaging, inflammatory activity, symptoms and physical examination.

MONITORING DISEASE ACTIVITY

Because sarcoidosis is such a heterogeneous disease with a wide spectrum of clinical evolutions, clinicians are in constant quest to find which marker best reflects disease activity. Whereas repeated functional tests such as spirometry can provide insight into pulmonary disease progression or remission, they are mostly measures of disease severity instead of reflecting the inflammatory status of the disease. End stage fibrotic pulmonary disease will have severely deflected spirometry loops, but does not necessarily represent an active inflammatory state. Furthermore, the absence of a proper functional test for many manifestations of extrapulmonary sarcoidosis such as neurosarcoidosis or cutaneous sarcoidosis underlines the need for finding usable markers or composite scores for disease activity.

SERUM MARKERS

Possible options to measure disease activity are serum biomarkers such as angiotensin-converting enzyme (ACE) and soluble IL-2 receptor (sIL-2R).

ACE has been proposed as a marker of disease activity in sarcoidosis over 30 years ago [44,45], but the value of this marker is still under debate [27]. ACE is produced by epitheliod cells in

granulomas and serum ACE level is thought to reflect total granuloma burden [44]. At time of diagnosis, ACE is elevated in 30-80%. Although it does not correlate with chest radiographic stage [46], one report did find a correlation between ACE and abnormalities on HRCT [47]. Furthermore, high serum levels of ACE at time of diagnosis could predict disease progression after two years [48]. A weakness of ACE in sarcoidosis is the low sensitivity of the test due to large biological inter-individual variation in levels even in the healthy population. Approximately 50% of the variation is due to a genomic insertion/deletion (I/D) polymorphism in the ACE gene [49]. Genotyping for the I/D polymorphism can increase the value of serum ACE significantly [50], although most studies investigating ACE genotyping have been performed in healthy controls instead of sarcoidosis patients. Furthermore, serum ACE levels are not reliable in patients using ACE-inhibitors. To date, the value of ACE as a follow up marker to predict response to therapy has not been determined.

Another serum marker that was found to be associated with sarcoidosis inflammatory activity and used in many sarcoidosis clinics is sIL-2R. IL-2 promotes T-cell proliferation through binding at its receptor IL-2R. SIL-2R is released mainly by activated T-lymfocytes and found to be increased in serum and BAL fluid of patients with sarcoidosis [51-53]. SIL-2R was proposed as a disease activity marker as it was found to correlate with the CD4+T lymphocyte subset in BAL (R=0.53) [54]. Furthermore, sIL-2R serum level was found to correlate with parenchymal infiltration in pulmonary sarcoidosis at disease presentation and might therefore function as prognostic marker at diagnosis [55,56]. Longitudinal data of sIL-2R levels in sarcoidosis patients are scarce and to date it is unknown whether sIL-2R levels correlate with response to treatment.

^{18}-FDG PET-SCAN

In addition to serum markers, nuclear imaging has found a role in activity monitoring of sarcoidosis. ^{18}F-fluorodeoxyglucose by positron emission tomography (^{18}F-FDG PET) uses a synthesized glucose analogue and is well known for its application in a wide variety of clinical conditions such as cancer. Another application of ^{18}F-FDG PET is visualising metabolic activity of inflammation as macrophages and T-lymphocytes have high numbers of glucose-receptors [57]. ^{18}F-FDG PET has previously demonstrated to be a predictor for decline in lung function in untreated patients. Furthermore, ^{18}F-FDG PET is more sensitive than ^{67}Galium imaging in the assessment of sarcoidosis activity in mediastinal and pulmonary areas [58,59]

Another asset of ^{18}F-FDG PET is its capacity to reveal specific organ involvement, which can also direct towards a possible biopsy location (Figure 1). The combined use of ^{18}F-FDG PET with CT (^{18}F-FDG PET/CT) directly shows which areas of the lung or lymph nodes are involved and can be repeated after treatment (Figure 4) [57].

A way to quantify ^{18}F-FDG PET results and to objectively measure changes between scans is the Maximum Standardised Uptake Value (SUVmax). Change of SUVmax during infliximab therapy correlated with lung function improvement afterwards [60].

It must be acknowledged that ^{18}F-FDG PET should not be performed repetitively, because of radiation exposure and associated health care costs. Furthermore, not all treatment centres may

have access to an ^{18}F-FDG PET and qualified nuclear physicians with specific knowledge in the field of sarcoidosis.

The value of ^{18}F-FDG PET in clinical treatment decision making has not yet been studied.

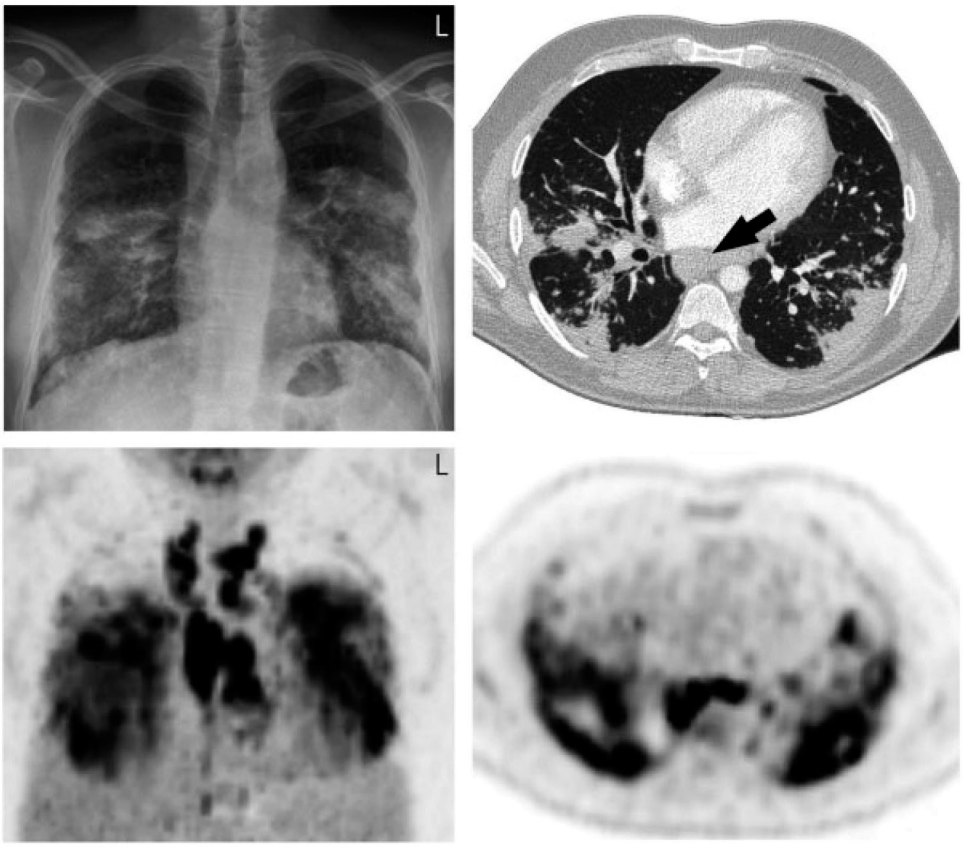

Figure 4: Comparison of Chest X-ray, HRCT and ^{18}F-FDG PET findings in pulmonary sarcoidosis (Adams et al [57])

Chest radiograph on the top left shows typical pulmonary infiltrates without pronounced bihilar lymphadenopathy in a patient with severe sarcoidosis (Scadding stage III). High-resolution CT (top right) in the same patient shows confluent nodular densities with peribronchovascular, perifissural, and subpleural distribution. CT also demonstrates subcarinal (arrow) and paratracheal enlarged lymph nodes. ^{18}F-FDG PET on the bottom left and right demonstrates a highly active lung parenchyma and also active lymph nodes in the mediastinum. *(Reprint with permission of publisher Thieme.)*

SEVERITY AND PROGNOSIS

Sarcoidosis can be a self-limiting disease and spontaneous remission occurs within 2-3 years in the majority of patients but it can also have a chronic character [61]. These different outcomes have led to the classification of sarcoidosis into acute (≤2 years) and chronic (≥3–5 years) phenotypes [11]. Refractory sarcoidosis refers to patients progressing despite treatment [62].
Löfgren's syndrome, in general has a good prognosis. Moreover, an inverse correlation between chest radiographic stage at diagnosis and the probability of spontaneous recovery exists (Table 3) [63].

Table 3 Outcome at 5 years based on initial chest radiographic staging (adapted from Scadding [26])

at Diagnosis	changes at 5 years (%)	(%)
Stage I	84	97
Stage II	58	63
Stage III	43	59
Stage IV	0	0

A subgroup of 10-30 % of sarcoidosis patients becomes chronic and can suffer from a wide variety of persisting symptoms e.g. dyspnoea, pain or fatigue, and may have endangered organ function. Loss of organ function is in some cases associated with ongoing or progressive parenchymal inflammation, but may also result from formation of fibrosis. In patients with chronic non self-limiting sarcoidosis, on-going granulomatous inflammation and/or fibrosis can be organ threatening and even fatal. These patients have an indication for systemic treatment with corticosteroids, and possibly second- or third-line therapeutics such as biologicals, to suppress the inflammation and stop further fibrosis formation. Clinical characteristics associated with worse prognosis are black race, extrapulmonary involvement and advanced pulmonary disease [63-65].
In contrast to patients with mild disease, patients with severe disease sometimes have to be hospitalised. In general, hospitalisations among sarcoidosis patients appear to have been increasing over the last decades, although the reason for hospitalisation is not always sarcoidosis itself [66]. Most common causes for hospitalisation of sarcoidosis patients are of cardiopulmonary nature such as respiratory failure and infections [67]. Sarcoidosis patients at risk for hospitalisation are more often of older age and black [68].

The mortality rates amongst sarcoidosis patients vary in literature due to the fact that some numbers are derived from cohort studies and some from data of unverified death certificates. Mortality rates are higher in referral centres (4.8%), in which patients have a less favourable prognosis than in population based studies (0.5%) [69]. An analysis of death certificates revealed that the underlying cause of death was the disease itself in 58.8% of patients with sarcoidosis and the risk of death

increased with age [70]. Age-adjusted mortality rates were consistently higher among blacks than whites [71]. The major causes of death in sarcoidosis include respiratory, cardiac, neurologic, and hepatic involvement [72]. Furthermore, sarcoidosis-associated pulmonary hypertension is strongly correlated with increased mortality [70,73]. Additionally, the presence of pulmonary fibrosis is an important risk factor of mortality in sarcoidosis [72,74].

Therapeutical management of sarcoidosis is very challenging due to the heterogeneity in disease manifestation, lack of randomised trial data, as well as the potential side-effects of treatment. In chapter 2 all currently available evidence on sarcoidosis treatment will be discussed into detail.

AIM

Although most sarcoidosis patients have limited disease, a group exists with severe and incapacitating disease requiring systemic therapeutic intervention. Due to various reasons, including low interest of pharmaceutical companies to invest in this orphan disease and the relatively low number of severe refractory patients, a large gap of knowledge remains in the field of sarcoidosis treatment.

The main goal of this thesis was to obtain an up-to-date picture of the efficacy of available second and third-line therapeutic options for sarcoidosis, and to gain new insight to predict which patients are most likely to respond to the various therapeutic interventions, including biological anti-TNFα agents that have recently appeared on the scene as rational therapy in severe and refractory cases.

OUTLINE OF THE THESIS

- **Chapter 2** gives an overview of knowledge on currently available therapy in sarcoidosis.
- Part I Second line therapy in sarcoidosis
  - In **chapter 3**, we further evaluated second-line therapy in the first study comparing the two most used drugs: methotrexate and azathioprine.
  - **Chapter 4** demonstrates the usefulness of ACE and sIL-2R as predictors of pulmonary function improvement during methotrexate therapy.
- Part II Third line therapy in sarcoidosis
  - **In chapter 5**, the off-label use of infliximab, a monoclonal antibody targeted against TNF was evaluated retrospectively as third-line therapeutic option for severe and refractory sarcoidosis.
  - **Chapter 6** describes our experience with formation of antinuclear antibodies during infliximab therapy in sarcoidosis patients.
  - **Chapter 7** shows treatment outcomes of the first prospective open-label trial of inflixi mab in sarcoidosis patients with severe and active disease.
- Part III Relapse in sarcoidosis
  - **In chapter 8** we provided evidence for a high relapse rate after discontinuation of infliximab therapy and searched for clinical and biochemical predictors of relapse.
- Part IV Cellular responses in sarcoidosis
  - In **chapter 9** data is presented regarding differential expression of TNFR1 and TNFR2 on subpopulations of human monocytes in sarcoidosis patients and healthy controls.
  - **Chapter 10** shows analysis of monocyte subsets and receptor expression during infliximab therapy to study their role in disease activity and response to therapy.
- Part V Clinical aspects of treatment in complicated sarcoidosis
  - In **Chapter 11.1 and 11.2** two cases are presented; one illustrates a curious disease manifestation refractory to standard treatment, the other illustrates the possible downside of anti-TNF therapy.
- Part VI Summary
  - **Chapter 12** summarizes our results and provides concluding remarks.

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CHAPTER 2

CURRENT THERAPY IN SARCOIDOSIS, THE ROLE OF EXISTING DRUGS AND FUTURE MEDICINE

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ABSTRACT

Sarcoidosis is a systemic, granulomatous disease that can affect multiple organs and has a variable clinical course. Corticosteroids (eg prednisone) remain the mainstay of therapy in sarcoidosis from their first use in this disease in the 1950s. A second-line therapeutic is often added to the treatment regimen in case of intolerable side effects, inefficacy or prolonged use of steroids. Methotrexate is considered by many to be the first choice drug in second-line therapeutics of sarcoidosis. Other often used second-line drugs are azathioprine and leflunomide. No large trials comparing different treatment options have been performed in sarcoidosis. In patients with severe disease who do not respond well to first and second-line therapy, biologicals such as infliximab can be promising. In this review, we provide a complete overview of all currently available therapeutic strategies in sarcoidosis. In addition, the gaps in current literature on sarcoidosis treatment were depicted to underline the importance of research in this mostly empiric field of medicine. Furthermore we highlight future medicine in sarcoidosis with emphasis on the role of personalised medicine.

INTRODUCTION

Sarcoidosis is a systemic, granulomatous disease that can affect multiple organs and has a variable clinical course. Sarcoidal granulomas are often manifested in the lymphatic system and lungs, therefore pulmonary and mediastinal involvement is found in approximately 90% of cases. Yet, because sarcoidal granulomas can form within various organs, different disease phenotypes can occur and organs such as heart, skin, central nervous system or eyes may also be involved. Diagnosis is based on a compatible clinical presentation, supportive histological evidence of noncaseating granulomas and reasonable exclusion of other granulomatous diseases [1-3]. Sarcoidosis can be a self-limiting disease and spontaneous remission occurs within 2-3 year in the majority of patients but it can also have a chronic character in a subgroup of patients [4]. These chronic patients can suffer from a wide variety of symptoms e.g. dyspnoea, pain or fatigue and have endangered organ function. Loss of function is in some cases caused by ongoing inflammation which may lead to severe fibrosis. Pulmonary involvement in sarcoidosis can be categorized using chest radiographs and is classified according to the Scadding criteria. Stage I is bilateral lympheadenopathy, stage II is bilateral hilar lympheadenopathy accompanied by parenchymal infiltration, stage III is only parenchymal infiltration and stage IV is pulmonary fibrosis [1].

In patients with chronic non self-limiting sarcoidosis the disease can be severe and even fatal. The major causes of death in sarcoidosis include respiratory, cardiac, neurologic, and hepatic involvement [5]. Sarcoidosis-associated pulmonary hypertension is correlated with increased death [6,7]. The presence of pulmonary fibrosis is also an important risk factor of mortality in sarcoidosis [5,8]. Because the etiology of sarcoidosis remains unknown, no curative treatment exists. This has given rise to numerous empirical treatments, which mostly have been introduced due to a positive effect in other inflammatory diseases. The aim of treatment is suppressing the inflammatory response and reducing the granuloma burden. As the disease can also be

self-limiting, not all patients receive medical treatment. Main indications for therapeutic intervention in sarcoidosis are danger of organ failure and unacceptable loss of quality of life. The decision to treat is made by both the physician and the patient and depends on the natural history of the disease, expected response to treatment and the potential toxicity of the treatment [9].

The need for systemic therapy in literature ranges from 20-70% of sarcoidosis patients [10-12]. Of those who require systemic therapy, approximately 50% requires therapy for more than two years [13,14]. Patients who do not need systemic therapy in the first six months often have a favourable prognosis with only 10% in need of long-term systemic therapy [13,14]. In most patients stable disease can be maintained on low doses of prednisone or other immunosuppression [15]. Treatment response is also correlated with sarcoidosis phenotype. Extrapulmonary localisations such as Lupus Pernio are known to more regularly require second or third line therapeutics [16], whereas pulmonary sarcoidosis can often be managed with corticosteroids [17]. In a study which surveyed sarcoidosis clinics around the world, the subset of patients that requires long-term aggressive treatment was found to be only 10% of all patients who were still under follow up more than five years after initial diagnosis [18].

Although presently used drugs have an effect on sarcoidosis symptoms, the disease is known to relapse often after discontinuation of treatment [19,20]. Moreover, treatment effect varies widely between different disease manifestations and between individual sarcoidosis patients.

To gain insight into systemic treatment of sarcoidosis, current treatment strategies are reviewed in this article, and a summary of the literature is provided (Table 1). Furthermore, limitations and remaining uncertainties of currently available treatments are discussed while focussing on future goals.

Table 1 Levels of Evidence as stated by the Oxford Centre for Evidence-based

Medicine for each drug and main characteristics of the studies performed

Drug	Author	Year	Study	Level of evidence
Prednisone	Paramothayan	2005	Meta-analysis	1A
	Israel	1973	RCT n=83	
	James	1967	RCT n=51	
	Pietinalho	1999	RCT n=185	
	Roth	1975	RCT n=92	
	Selroos	1979	RCT n=37	
	Zaki	1987	RCT n=134	
Methotrexate	Baughman	2000	RCT n=24	1B
	Lower	1995	Cohort-study n=200	
	Vorselaars	2013	Cohort-study n=150	

Azathioprine	Vorselaars	2013	Cohort-study n=50	2B
	Pachecho	1985	Case-Series n=10	
	Lewis	1999	Case-Series n=9	
	Muller-Quernheim	1999	Case-Series n=11	
Leflunomide	Majithia	2003	Cohort-study n=32	2B
	Sahoo	2011	Cohort-study n=72	
(Hydroxy)chloroquine	Siltzbach	1964	Cohort-study n=43	2B
	Baughman	1999	Cohort-study n= 41	
	Jones	1990	Case-Series n=17	
	Sharma	1998	Case-Series n=12	
Thalidomide	Baughman	2002	Case-Series n=11	4
	Nguyen	2004	Case-Series n=12	
	Fazzi	2012	Case-Series n=19	
Pentoxifylline	Park	2009	RCT n=23	2B
	Zabel	1997	Case-Series n=18	
Apremilast	Baughman	2012	Case-Series n=15	4
Cyclophosfamide	Doty	2003	Case-Series n=7	4
Mycofenolate	Kouba	2003	Case-Series n=5	4
	Brill	2012	Case-Series n=10	
Infliximab	Rossman	2006	RCT n=19	1B
	Baughman	2006	RCT n=148	
	Van Rijswijk	2013	Cohort-study n=48	
	Hostettler	2012	Case-Series n=16	
Adalimumab	Pariser	2013	RCT n=16	2B
	Erckens	2012	Cohort-study n= 26	
			Case-Series n=10	
Rituximab	Lower	2012	Case-Series n=4	4

FIRST LINE THERAPY

Corticosteroids (eg prednisone) remain the mainstay of therapy in sarcoidosis from their first use in this disease in the 1950s. Corticosteroids are very potent inhibitors of inflammation due to their ability to switch off genes that encode pro-inflammatory cytokines and switch on those that encode anti-inflammatory cytokines [21,21]. In sarcoidosis, corticosteroids have been shown to restore the balance between locally produced type-1 and type-2 T-Helper cell cytokines [22].

Most experience with oral steroids in sarcoidosis treatment has been empirical, nevertheless six randomised placebo controlled trials (RCTs) have been performed in this field [23-29]. These studies have shown that oral corticosteroids significantly improve symptoms, lung function and chest X-rays after 3-24 months of treatment compared to placebo. Chest radiography improved most in patients with stage II and III disease. Lung function data of the five most recent RCTs were pooled in a meta-analysis by Paramothayan [30,31]. This meta-analysis revealed a weighed mean

difference (WMD) for vital capacity of 4.2% of predicted (95% Confidence interval (CI) 0.4-7.9) and a WMD for diffusing capacity of 5.7% of predicted (95% CI 1.0-10.5) after treatment with oral steroids (prednisone or methylprednisolone) [31].

The initial recommended dose of prednisone is 20-40 mg/day, which should later be tapered to a dose below 10mg/day under follow up of symptoms and function [32].

Despite the positive effects on lung function and symptoms, downsides of treatment with corticosteroids are the inevitable side effects when administered chronically, such as osteoporosis, diabetes mellitus or obesity. Furthermore, whether corticosteroids provide a beneficial effect in the long run remains uncertain [12,33].

in this disease; extrapulmonary localisations may vary from skin manifestations, to cardiac sarcoidosis, neurosarcoidosis, uveitis and hypercalcaemia [20,21]. The acute form of sarcoidosis, Löfgren's syndrome presents with erythema nodosum, bilateral hilar lymphadenopathy and arthritis. Another typical presentation of the disease is Heertfort's syndrome, which is characterised by uveitis, parotid gland swelling, fever and in some cases facial nerve palsy. In these two scenarios diagnosis relies on the specific presentation of the disease and biopsy is not needed, while other scenarios require a systematic work-up to fulfil the major criteria for diagnosis as mentioned above (Figure 1) [1].

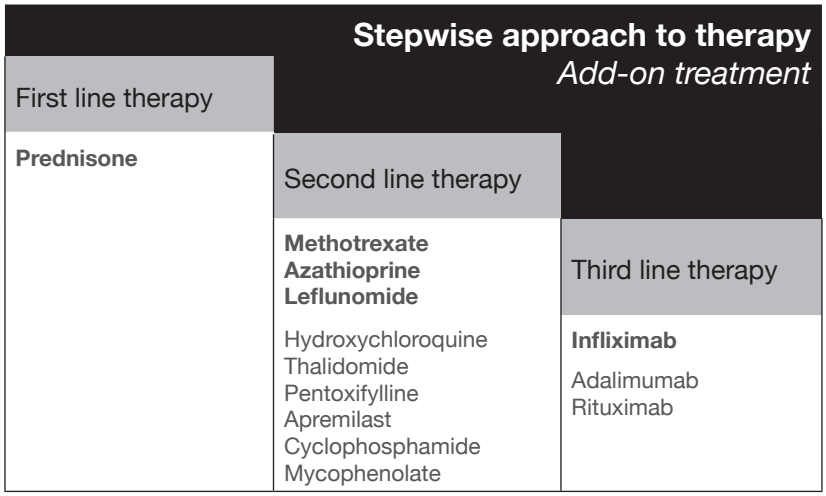

Figure 1: Diagram of a stepwise approach towards systemic therapy in sarcoidosis.
In case of inefficacy or severe side effects to a drug, proceed with another drug of same group or initiate a drug from the next category following an add-on principle

SECOND LINE THERAPY: STEROID SPARING AGENTS

A second-line therapeutic is often added to the treatment regimen in case of intolerable side effects, inefficacy or prolonged use of steroids (Figure 1). As steroids cause irreversible side effects when used chronically, the main reason to initiate second line therapeutics is their steroid sparing capacity. Most second and third-line therapeutics are inserted into the therapeutic regimen in an add-on fashion, e.g. starting methotrexate when the patient is still being treated with prednisone which should later be tapered off.

All proposed second line therapeutics for sarcoidosis have been previously used and studied in other inflammatory diseases such as rheumatoid arthritis, psoriasis and Crohn's disease. However, evidence defining the best second line therapeutic for sarcoidosis is currently unavailable, as different drugs have not been compared in randomised trials.

To underline the importance of research in this field, what is known on the most used steroid sparing agents is discussed in this paragraph.

METHOTREXATE

Methotrexate is a folic acid antagonist capable of affecting the cellular metabolism of actively proliferating cells [34]. Its use in sarcoidosis patients results from positive effects in rheumatology studies. Despite the fact that methotrexate is considered by many to be the first choice drug in second-line therapeutics of sarcoidosis [35-37], evidence is limited.

Methotrexate has been reported effective in small case series [38,39] and a single small randomised controlled trial in 24 patients, which showed a steroid sparing effect in acute sarcoidosis patients [36]. A retrospective cohort study of 105 patients found a significant steroid sparing effect and improvement in lung function or other affected organs in 66% out of 50 sarcoidosis patients completing at least two years of methotrexate therapy [40]. An improvement of unspecified magnitude in forced expiratory volume in one second (FEV1) and diffusion capacity of the lung for carbonmonoxide (DLCO) was found in the majority of 91 sarcoidosis patients while on methotrexate for six months [41].

Most noted side effects of methotrexate are hepatotoxicity, leukopenia and gastrointestinal complaints [40]. Methotrexate is dosed once a week under regular checks of hepatic function and blood count, doses studied vary from 10-15 mg/week. In case of severe gastrointestinal side effects to oral methotrexate, a switch to subcutaneous methotrexate injections can be made.

AZATHIOPRINE

Azathioprine, a thiopurine drug, is another steroid sparing agent in sarcoidosis therapy, which is used widely in inflammatory bowel disease and rheumatoid arthritis. Azathioprine was the second choice in steroid sparing agents in a Delphi consensus study amongst sarcoidosis specialists [37]. To date, no RCTs or large case series are available on azathioprine treatment in sarcoidosis. Azathioprine has been reported effective in smaller case-series and case-reports with a maximum of eleven patients [42-44]. Its efficacy and toxicity profile has recently been compared with

methotrexate in a large retrospective cohort showing a similar efficacy, but with more infections in the patients treated with azathioprine [45]. Baughman and colleagues have described that half of patients who failed to respond to methotrexate had a positive response when they switched to azathioprine [46]. It was not described whether patients were irresponsive to methotrexate or had to quit due to side effects. Improvement of lung function and steroid dose was not quantified in these reports. Moreover, numbers in these studies were small and patients were extremely varied, complicating the clinical implication of these data.

Azathioprine is generally administered orally in a regimen of 50-150 mg/day. Thiopurine Methyltransferase (TPMT) genotyping can be performed at initiation to reveal patients susceptible to thiopurine toxicity and side effects [47]. Azathioprine is often used in cases where methotrexate is contraindicated or failed to induce response. No specific guidelines exist for azathioprine use in sarcoidosis.

LEFLUNOMIDE

Leflunomide is an oral anti-lymphocyte agent which has been used in rheumatoid arthritis because of its ability to repress lymphocyte responses only for actively stimulated lymphocyte clones [48]. In sarcoidosis the first reports of its efficacy appeared in 2003. To date, no RCTs have been conducted investigating leflunomide in sarcoidosis treatment. A retrospective study in 32 sarcoidosis patients with mostly ocular or pulmonary sarcoidosis showed an improvement in 78% of patients who were treated with leflunomide monotherapy or a combination therapy with methotrexate [49]. A more recent report on 76 sarcoidosis patients demonstrated an improvement in lung function, decline of prednisone use and at least a partial response of extra pulmonary organs in 83% of patients [50]. Leflunomide can be administered as monotherapy or combined with methotrexate and appears to have less side effects than methotrexate [49]. Known side effects are gastrointestinal complaints, hepatic enzyme elevation and neuropathy. Leflunomide can be used as second line therapeutic in cases in which methotrexate is not desirable [32].

ANTIMALARIAL AGENTS (HYDROXYCHLOROQUINE AND CHLOROQUINE)

Despite the fact that the antimalarial drug hydroxychloroquine has been used in selected sarcoidosis patients for over 50 years, no RCTs have been conducted in this field of therapy. Use of hydroxychloroquine in treatment of sarcoidosis was first described in a cohort study of 43 patients with intrathoracic and cutaneous sarcoidosis [51]. Another study showed regression of cutaneous sarcoidosis in 12 out of 17 sarcoidosis patients treated with hydroxychloroquine [52]. In neurosarcoidosis, hydroxychloroquine improved or stabilized symptoms in 10 out of 12 patients treated for 6-21 months [53]. A retrospective analysis of 41 sarcoidosis cases with varied symptoms revealed that remission was induced in 27% of patients and another 34% of patients had some benefit of treatment [54]. Hydroxychloroquine is frequently used in cases of sarcoidosis with joint involvement without supporting literature. Hydroxychloroquine is often preferred over chloroquine due to less ocular toxicity.

THALIDOMIDE

Thalidomide reduces Tumor necrosis factor-alpha (TNFa) release from alveolar macrophages, hereby reducing granuloma formation [55]. It has mainly been used to treat cutaneous forms of sarcoidosis. A series of 12 cutaneous sarcoidosis patients treated with thalidomide showed complete responses in four patients, six had partial responses, and two had no regression [56]. A dose escalation trial in fourteen patients demonstrated at least some improvement of skin lesions in all patients [57]. In ten patients with chronic pulmonary sarcoidosis, thalidomide was not found beneficial for pulmonary function or quality of life [58]. However, a recent study in 19 patients revealed an improvement of skin and chest X-ray lesions [59].

The most serious adverse effect has been peripheral neuropathy, which often resolves by reducing the dose or discontinuing the medication. Other common side effects are somnolence, numbness and constipation [57]. In addition, the teratogenic effects of thalidomide are well known and precautious measures are essential.

PHOSPHODIESTERASE INHIBITORS (PENTOXIFYLLINE AND APREMILAST)

Pentoxifylline, a phosphodiesterase type 4 inhibitor, was introduced in treatment of sarcoidosis based on its TNF inhibitory activity. TNF plays a pivotal role in the formation of granulomas. Pentoxifylline was first reported to be effective for the treatment of sarcoidosis in a case series of 18 patients [60]. 61% of patients who completed six months of treatment showed improvement, the other 39% remained stable. Furthermore, pentoxifylline had a significant steroid sparing effect in an RCT in 23 pulmonary sarcoidosis patients, but resulted in severe gastrointestinal complaints at the doses used (1200-2000mg/day) [61].

Apremilast, a new phosphodiesterase type 4 inhibitor that blocks the synthesis of proinflammatory cytokines and chemokines, was found effective in a case series of 15 patients with cutaneous sarcoidosis [62].

CYCLOPHOSPHAMIDE

Cyclophosphamide is a cytostatic agent with DNA alkylating properties which interfere with the cell cycle, hereby inhibiting B and T-cell proliferation. This drug originally roots from oncologic treatment. In addition it is an immunosuppressive agent with a severe side effect profile which has been used to treat inflammatory diseases such as Wegener's granulomatosis and systemic lupus erythematosus. Side effects are mainly bone marrow suppression and infections. Some case reports described its use to treat refractory neurosarcoidosis and myocardial sarcoidosis [63,64]. A case series of seven patients showed positive results and reduction in the dose of corticosteroids [65].

MYCOPHENOLATE MOFETIL

Mycophenolate mofetil is an immunosuppressive drug which has been used extensively in organ transplant recipients. As a reversible inhibitor of inosine monophosphate dehydrogenase it

attenuates lymphocyte proliferation, hence the immunosuppressive effect. Mycophenolate mofetil was first found effective in five patients with refractory mucocutaneous sarcoidoisis [66]. Small series of patients with central nervous system sarcoidosis and sarcoidosis-associated uveitis showed positive results. Recently mycophenolate mofetil was found to significantly reduce the steroid dose while preserving a stable or improved clinical condition in 10 patients with pulmonary sarcoidosis [67]. The toxicity profile of mycophenolate mofetil appears to be mild, with most common side effects being of gastrointestinal nature; however leukopenia and infections can also occur. Thus far, no RCT has investigated the effect of mycophenolate mofetil in sarcoidosis.

BIOLOGICALS

INFLIXIMAB

The chimeric anti-TNF monoclonal antibody infliximab has been subject of investigation in case series and reports of several manifestations of sarcoidosis. Infliximab is a TNFα inhibitor that binds to TNFα, thus preventing it from activating TNFα receptors hereby impairing granuloma formation. Until now, only two RCTs have been conducted investigating infliximab treatment in sarcoidosis [68,69]. A small but significant improvement in lung function was seen in the RCT with 148 patients after six infusions of infliximab [69]. Additional subgroup analysis showed positive effects of infliximab treatment on extra-pulmonary symptoms [70]. A retrospective cohort study by van Rijswijk and colleagues showed that besides improvement of pulmonary function, infliximab also improves quality of life and reduces signs of disease activity measured by 18-FDG-PET and serum biomarkers [71].

Long-term outcome in terms of efficacy and toxicity of this treatment remains largely unknown. Infliximab was beneficial in 14/16 (88%) patients treated for more than 12 months [72]. It has been observed in a series of 14 patients that many patients experienced relapses after discontinuation of therapy with recurrence of symptoms and often function loss [19]. A drawback in this study is the relative high proportion of patients who only had a very short duration of infliximab therapy. Side effects of infliximab therapy constitute of a higher risk of infections and increased cardiac failure in prone patients. Allergic reactions can occur as a result of antibody formation due to the chimeric character of the drug. Because an increased risk of tuberculosis reactivation exists, all patients should be screened prior to initiation of infliximab for current or previous tuberculosis infection. Infliximab is administered intravenously at an interval of once a month. Current research has not provided the optimal dosing regimen and guidelines for patient selection for infliximab therapy in sarcoidosis.

ADALIMUMAB

Another anti-TNF monoclonal antibody used in sarcoidosis treatment is adalimumab. Unlike infliximab, this monoclonal antibody is human and is administered subcutaneously every 7-14 days. Adalimumab was found effective in cases of refractory cutaneous and pulmonary sarcoidosis [73-75]. In a cohort of 26 sarcoidosis patients with uveitis, intraocular inflammatory

signs showed improvement in 22 patients (85%) and stabilization in four patients (15%) [76]. Furthermore, a report of ten sarcoidosis patients treated with adalimumab revealed a significant reduction in 18F-fluorodeoxyglucose positron emission tomography (^{18}F-FDG PET) activity, but no improvement in pulmonary function parameters [77]. Most recently adalimumab was found to improve cutaneous sarcoidosis lesions in a double blind placebo controlled trial in 16 patients. The study did not reveal improvement in pulmonary function or radiology [78].
Although these studies have demonstrated positive results with adalimumab, response rate appears to be lower and delayed compared to infliximab. Increasing dose and intervals between gifts will possibly improve response rate.

RITUXIMAB
Recent findings of B cells as emerging key players in sarcoidosis provide a rationale for a systematic B cell ablative therapy in sarcoidosis patients [79]. Sarcoidosis might become a new indication for existing biologicals such as rituximab, a monoclonal antibody drug that targets the B cell specific protein CD20. Today, literature on rituximab in sarcoidosis is mainly anectodal [80-82]. One small case series of four patients with refractory granulomatous eye disease caused by sarcoidosis described a significant reduction of prednisone use in all four treated patients. Side effects were neutropenia and infection [83].

LIMITATIONS OF CURRENTLY AVAILABLE THERAPIES FOR SARCOIDOSIS

In this review, we discussed what is known on systemic treatment in sarcoidosis. In addition, the gaps in current literature on sarcoidosis treatment were depicted to underline the importance of research in this mostly empiric field of medicine.
The answer to a curative drug for sarcoidosis may lie in a better understanding of the origin of the disease. A shift has occurred in the last few decades from immunosuppressive drugs with a wider spectrum of action, such as corticosteroids, to more specific narrow target drugs, such as infliximab. However, with the cause of sarcoidosis still undiscovered, more specific drug targets and treatment strategies will not easily be uncovered.
Due to the fact that severe sarcoidosis is a rare disease entity, treatment or cure of this disease is not in the scope of pharmaceutical companies that develop new drugs. As a result, all knowledge on treatment of sarcoidosis has been derived from extrapolation of treatment strategies from other inflammatory diseases, such as rheumatoid arthritis and Crohn's disease, to sarcoidosis by physicians seeking the best treatment options for chronically ill patients. The gap of knowledge in the field of second line therapeutics underlines the need for more international cooperation in research. This could enable large retrospective cohort studies for efficacy comparison of different therapeutics.
Although we have summarised positive results of immunosuppressive therapy in the short run, unfortunately, present studies provide no conclusive evidence on the long-term benefit of any form of systemic sarcoidosis treatment. Consequently, the key question remains whether or

not immunosuppressive drugs can prevent fibrosis or damage and ultimately improve survival. New insights in disease aetiology provide new possible drug targets. It is known that sarcoidosis pathogenesis is characterized by peripheral anergy and an exaggerated, pulmonary CD4(+) Th1 response. A recent study showed that reversal of global CD4+ subset dysfunction is associated with spontaneous clinical resolution of pulmonary sarcoidosis. These findings implicate normalized CD4(+) T cell function as a potential therapeutic target for sarcoidosis resolution [84]. Another recent study revealed a potential role for disordered TLR-2 responses in the pathogenesis of pulmonary sarcoidosis [85].

Sarcoidosis treatment might also benefit from new developments in treatment of inflammatory bowel disease, for example with new antibodies against interleukin-12 (IL-12) or interleukin-13(IL-13) [86]. IL-13 expression is increased in bronchoalveolar lavage cells and peripheral blood monocytes of sarcoidosis patients [87] and there is evidence for involvement of the IL-12 pathway in cutaneous sarcoidosis [88]. A focus of recent studies has been the hypothesis of sarcoidosis as a T-helper 17 mediated disease, this current insight might possibly lead to new targets for therapy [88-90].

In present times, where research is focussed mostly on new drug targets and therapies, this review underlines the importance of exploration and confirmation of existing empirical treatment options. Data regarding optimal dosing regimen, treatment duration and timing of corticosteroid and other immunosuppressive therapies are still missing. In addition, due to the heterogeneous character of the disease, patient specific treatment strategies should be the focus of future research. With the variety of steroid sparing agents available today, still no comparative studies of these drugs have been performed in sarcoidosis patients. Moreover, it would be of great importance to predict which phenotype of sarcoidosis patients would benefit most from which drug.

PERSONALISED MEDICINE

As sarcoidosis can have various different disease manifestations, all patients respond differently to therapy. Personalised medicine is a term used to select the most suitable drug for each individual patient regarding maximal effect and minimal side-effects. This selection can be based on phenotype. For instance, it is known that sarcoid uveitis responds well to anti-TNFα drugs and it is known that only a few patients will beneficit from Rituximab therapy [76,83]. The difference in efficacy between patients possibly originates from different mechanisms underlying the disease. Little is known about the specific patient characteristics that predict response to currently available second and third line therapeutics.

Furthermore, the effect of drugs is dependent on a patient's genetic profile. Drug selection and dosing based on a patient's genotype, has the potential to optimise pharmacotherapy of individual patients. The number of subjects in clinical studies on drug treatment of sarcoidosis is very small. This implies that especially data on side effects and toxicity are very limited. Therefore, one should apply the knowledge gained from other diseases in routine patient care of patients with sarcoidosis. One example on genotyping which has found its way into daily clinical sarcoidosis practice in several hospitals is TPMT genotyping which is used in patients treated with azathioprine. The

lower a patient's TPMT activity, the higher the concentration of active thioguanine nucleotide metabolites and the higher the risk of developing toxicity i.e. myelosuppression [91]. Results of clinical studies in other diseases have been translated into dosing and other pharmacotherapeutic recommendations [92-94]. Patients homozygous for a variant TPMT allele which encodes for a nonfunctional TPMT enzyme, so-called poor metabolizers, should receive only 10% of the conventional starting dose of azathioprine three times a week or once daily. In patients with one non functional TPMT allele, the intermediate metabolizer, azathioprine treatment should be initiated at a reduced dose of approximately 50% of the target dose. The pharmacogenetics of other drugs used in sarcoidosis, such as cyclophosphamide, infliximab, mycophenolate mofetil, has been studied in other diseases. Mainly because of limited data or inconclusive results, these have not been implemented in clinical practice yet [95,96]. The association between methotrexate response in terms of efficacy and toxicity, and genetic variants in several genes has extensively been studied in other diseases [97]. Translating these results has potential for personalised medicine in the future of sarcoidosis therapy. Furthermore, large cohort-studies or perhaps international collaboration could aid to knowledge on farmacogenetics in sarcoidosis.

CONCLUSION AND FUTURE DIRECTIONS

As curative treatment does not exist for sarcoidosis, steroids remain the first drug of choice. In case of chronic treatment, steroid sparing agents such as methotrexate are employed. Biologicals such as the anti-TNFα monoclonal antibody infliximab are proven effective in sarcoidosis, however, their place in the therapeutic regimen is still under debate. Due to the high costs of new drugs, they are only available for a restricted group of patients. Future investigations should provide evidence for the role of personalised medicine in sarcoidosis treatment.

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PART I

SECOND LINE THERAPY IN SARCOIDOSIS

CHAPTER 3

METHOTREXATE VERSUS AZATHIOPRINE IN SECOND LINE THERAPY OF SARCOIDOSIS

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ABSTRACT

BACKGROUND

Steroids remain the first choice therapeutic in sarcoidosis, however, chronic use is associated with toxicity. Evidence defining the best second line therapeutic is currently lacking.

The aim was to compare the effect of methotrexate and azathioprine on prednisone tapering, pulmonary function and side effects in second line treatment of sarcoidosis.

METHODS

An international retrospective cohort study was performed, reviewing all sarcoidosis patients who started methotrexate or azathioprine until two years after initiation or until discontinuation. A linear mixed model with FEV1, VC, DLCO and prednisone dose changes over time as endpoints was used. Side effects were compared with $\chi 2$-tests.

RESULTS

200 patients were included, 145 received methotrexate and 55 received azathioprine. Prednisone daily dose decreased with 6.32 mg/year ($p<0.0001$) while on therapy, with a similar steroid sparing capacity for methotrexate and azathioprine. Of all patients completing one year of therapy, 70% had a reduction in daily prednisone dose of at least 10 mg. FEV1 showed a mean increase of 52 ml/year ($p=0.006$) and VC of 95 ml/year ($p=0.001$) in both treatment groups. DLCO (% predicted) increased with a mean of 1.23%/year ($p=0.018$). More patients in the azathioprine group had infections (34.6 vs 18.1% $p=0.01$), but no differences regarding other side effects were found.

CONCLUSIONS

This retrospective study comparing the effect of second line therapy in sarcoidosis shows that both methotrexate and azathioprine have significant steroid sparing potency, a similar positive effect on lung function and comparable side effects except for a higher infection rate in the azathioprine group.

INTRODUCTION

Sarcoidosis is a systemic, granulomatous disease that can affect multiple organs and has a variable clinical course. Although new therapies are slowly emerging, steroids remain the mainstay of therapy [1-2]. Chronic steroid use however, is associated with severe toxicity, e.g weight gain, diabetes and osteoporosis. In case of intolerable side effects, inefficacy or prolonged use of steroids, a second-line therapeutic is added to the treatment regimen. However, evidence defining the best second line therapeutic is currently unavailable, as studies comparing different treatment options are lacking.

Methotrexate is often considered first choice in second-line therapeutics, [3-5] but evidence is limited. Methotrexate has been reported effective in small case series [6-8] and a single small randomised controlled trial in 24 patients that showed a steroid sparing effect in acute sarcoidosis

[9]. A retrospective cohort study in 105 patients found a significant steroid sparing effect and improvement in lung function or other affected organs in 66% out of 50 sarcoidosis patients completing at least two years of methotrexate therapy [10].
Azathioprine is another second-line therapeutic for refractory sarcoidosis [3-4]. To date, no randomised controlled trials or large case series are available. Azathioprine has been reported effective in smaller case-series and case-reports with a maximum of eleven patients [11-13].
New and promising, but expensive treatment options such as biologicals are currently rising [14]. However, in current times of increasing health care costs, the existing and less expensive treatment options for sarcoidosis should be fully explored. The efficacy of methotrexate versus azathioprine as first choice second-line therapeutics in sarcoidosis has not been studied thus far. Therefore, we studied the effect of methotrexate and azathioprine regarding prednisone tapering, pulmonary function tests and side effects.

METHODS

STUDY DESIGN

This is an international retrospective cohort study comparing two different second line therapeutics for refractory sarcoidosis. Patients were recruited from the University Hospitals Leuven, Belgium and St Antonius Hospital Nieuwegein, the Netherlands. Both hospitals serve as tertiary referral centres for interstitial lung diseases in their respective countries. In Belgium, historically, the first drug of choice in second line therapy of sarcoidosis is azathioprine, while in the Netherlands one selects methotrexate.
Medical records were reviewed for demographic data, organ involvement and radiographic Scadding stage. Patients were assigned to a pulmonary and extra-pulmonary treatment subgroup based on reason for initiation of second line therapy. Patients in the extra-pulmonary treatment group could also have mild pulmonary localisations of sarcoidosis. Prednisone dosage and side effects were collected together with the pulmonary function parameters total lung capacity (TLC), forced expiratory volume in one second (FEV1), vital capacity (VC) and diffusing capacity for carbon monoxide (DLCO). Pulmonary function parameters were included from one year before start of therapy until two years after treatment initiation. Follow up of patients ended at discontinuation of second line therapy, at start of third line therapy or after a maximum of two years on second line therapy. Clinical routine and follow-up protocols were comparable in both centres.

PATIENTS

All patients who started methotrexate at St Antonius Hospital Nieuwegein, the Netherlands between June 2004 and September 2011 were selected. Patients who started azathioprine between November 1995 and July 2011 at the University hospitals Leuven were selected. Sarcoidosis was diagnosed according to the ATS/ERS/WASOG statement on sarcoidosis [15].
Patients eligible for second line therapy had persistent active sarcoidosis with impaired organ function or severely diminished quality of life. Furthermore, they were unresponsive to steroids,

showed severe side effects or had other serious contraindications for steroid use. All patients were naïve to second-line therapy.
Patients received methotrexate following a standard protocol starting at 10 mg per week increasing to 15 mg per week with regular checks of hepatic function. Additionally all patients used 5 mg folic acid. Azathioprine was dosed at 2 mg/kg bodyweight with a maximum of 150 mg per day under routine checks of hepatic function and blood counts.
Tapering of prednisone was the physicians decision and not part of a on forehand established tapering schedule.

STATISTICAL ANALYSIS

Students' t-tests were used to compare means of normally distributed variables and Chi-square tests for categorical variables. Treatment effects on pulmonary function and prednisone dosage were calculated with a linear mixed model with FEV1, VC, DLCO and prednisone dose over time as primary endpoints [15]. ERS standard prediction equations for pulmonary function were used in both hospitals. Covariates and factors (eg ethnicity, treatment indication) were inserted into the model to detect confounders. The -2 restricted log likelihood values were used to evaluate whether insertion improved the fit of the model significantly. Pulmonary function tests were corrected for height, sex and age in the linear mixed model The differences in pulmonary function values between the pre-treatment period and during treatment were assessed using the linear mixed model via inserting a pre-post treatment initiation variable which coded 0 for the pre-treatment and 1 for treatment periods. We also inserted a treatment*time interaction to assess whether the lung function continued to increase over time (see Online Repository for a detailed description).
Differences in side effects were estimated using Chi-square tests.
To determine the number of patients responsive to therapy a sub-analysis was performed including all patients completing one year of therapy. Change in pulmonary function parameters and daily prednisone intake were calculated using Chi-square tests.
Statistical analysis was performed using SPSS for windows, version 17.0 and graphs were created using Graph pad Prism 5.0. A P-value of less than 0.05 was considered significant.

RESULTS

PATIENTS

A total of 200 patients were included in this study, 145 patients started methotrexate therapy and 55 patients started azathioprine therapy. Baseline characteristics of both groups are shown in table 1. The methotrexate cohort comprised significantly more non-white patients (p=0.004) and had a higher DLCO at baseline (p=0.01). Extra-pulmonary treatment indications included cardiac sarcoidosis, neurosarcoidosis, joint sarcoidosis, uveitis and extreme disabling fatigue. The group with a multiple organ indication comprised patients with various symptoms of extra-pulmonary origin.
Figure 1 shows the two year follow up of all patients on methotrexate and azathioprine. Out of 37 patients in whom second line therapy was stopped because of side effects 19 patients were not

stable and started another second line therapeutic or third line therapy. Twelve patients remained stable under steroids, which were in some cases increased in dose again. Six patients were lost to follow up.

Table 1 Baseline characteristics of treatment cohort

	All patients* (200)	Methotrexate (145)	Azathioprine (55)	P-value
Mean age at start of therapy in years (SD)	47.4 (10.6)	47.4 (10.1)	47.2 (12.1)	NS
Male n (%)	126 (63%)	90 (62.1%)	36 (65.5%)	NS
Race				
White n (%)	180 (90%)	125 (86.2%)	55 (100%)	0.004
Black n (%)	13 (6.5%)	13 (9.0%)	0 (0%)	
Other n (%)	7 (3.5%)	7 (4.8%)	0 (0%)	
Body mass index (SD)	27.4 (4.4)	27.5 (4.4)	27.1 (4.4)	NS
Smoking n (%)				
Current smoking	15 (8%)	10 (7%)	5 (10%)	NS
Former smoking	72 (36%)	51 (39%)	21 (42%)	
Mean disease duration in months (SD)	59.7 (87.2)	61.1 (85.1)	56.3 (93.4)	NS
Scadding stage n (%)				
0	18 (9%)	16 (11%)	2 (4%)	NS
I	22 (11%)	13 (9%)	9 (17%)	
II	83 (41.5%)	58 (40%)	25 (47%)	
III	45 (22.5%)	31 (22%)	14 (26%)	
IV	29 (14.5%)	26 (18%)	3 (6%)	
Previous therapy n (%)				
Prednisone	173 (87%)	121 (85%)	52 (96%)	NS
Prednisone + plaquenil	11 (6%)	11 (8%)	0 (0%)	
None (contra-indication prednisone)	13 (7%)	11 (8%)	2 (4%)	
Mean prednisone dose in patients using at start (SD)	20.5 (13.2)	20.0(12.6)	21.6(10.2)	NS
PFT % pred (SD)				
TLC	79.1 (17.3)	78.5 (16.7)	80.6 (18.9)	NS
VC	85.1 (20.6)	86.9 (20.3)	80.3 (20.7)	NS
Fev1	75.1 (22.4)	76.1 (22.8)	72.3 (21.1)	NS
DLCO	64.7 (16.8)	66.6 (17.2)	59.7 (14.7)	0.01

Treatment indication n (%)				
Pulmonary	136 (68%)	95 (66%)	41 (74%)	NS
Neurological	15 (7.5%)	10 (6.9%)	5 (9.1%)	
Cardiac	9 (4.5%)	6 (4.1%)	3 (5.5%)	
Uveitis	2 (1%)	2 (1.4%)	0 (0%)	
Joints	10 (5%)	7 (4.8%)	3 (5.5%)	
Fatigue	5 (2.5%)	4 (2.8%)	1 (1.8%)	
Cutaneous	2 (1%)	1 (0.7%)	1 (1.8%)	
Gastro-intestinal	1 (0.5%)	1 (0.7%)	0 (0%)	
Renal	1 (0.5%)	1 (0.7%)	0 (0%)	
Multiple organs	19 (9.5%)	18 (12.4%)	1 (1.8%)	
Tertiary referral n (%)	150 (75%)	113 (79%)	37 (69%)	NS

A subdivision is made between patients treated with methotrexate and azathioprine. P-values are given for significant differences between the two groups. NS=not significant PFT= Pulmonary function test
* Only eight of 145 methotrexate patients were treated in Belgium and only 3 of 55 azathioprine patients were treated in the Netherlands.

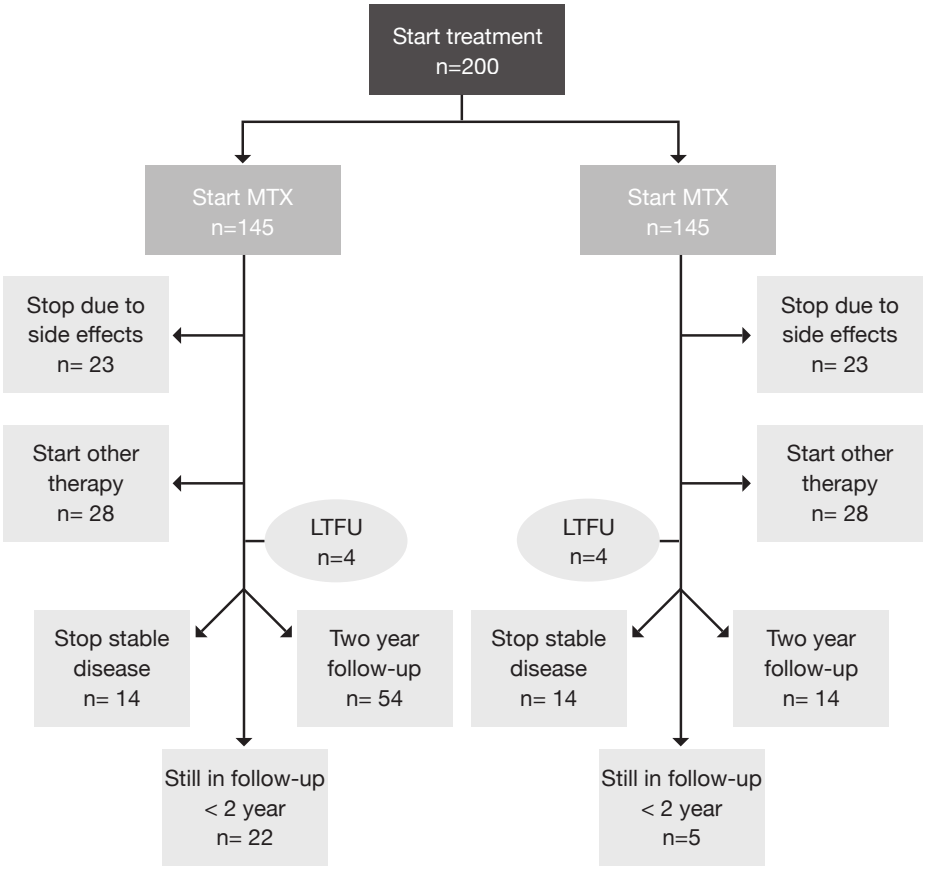

Figure 1: Flowchart of patients on methotrexate (MTX) and azathioprine (AZA) during a 2 year follow-up from start of treatment
All patients were included in analysis whilst on treatment. LTFU= loss to follow-up

PREDNISONE USE

Prednisone daily dosing regimen decreased with 6.32 mg/year (p<0.0001 Standard Error 0.63) while on therapy, with a similar effect on prednisone regimen in both treatment arms (Fig 2). Prednisone tapering in the pulmonary and non-pulmonary subgroups was similar as well.

A sub analysis of all patients completing one year follow-up on treatment who used prednisone at baseline (n=100) showed a decrease in prednisone intake of over 10mg daily in 70 patients (70%) with no significant differences between both drugs. Characteristics of this group are shown in table 2.

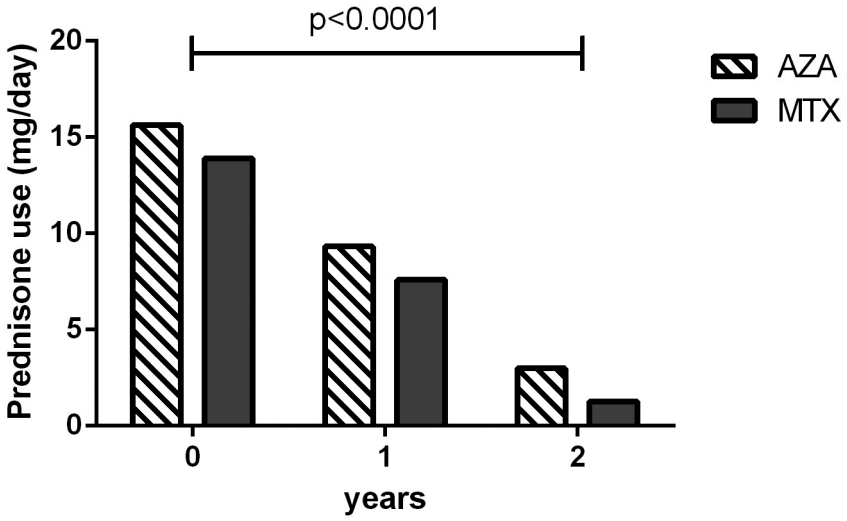

Figure 2: Effect of azathioprine (AZA) and methotrexate (MTX) on daily prednisone dose
Daily prednisone use decreased with 6.32 mg/year (p<0.0001) (Standard Error 0.63). Difference between azathioprine and methotrexate not significant (p=0.139)

Table 2 Subanalysis of 123 patients completing one year of follow-up on second line therapy

	Total group	Methotrexate	Azathioprine	P-value
All patients				
10 mg reduction of prednisone after 1 year (%)*	70 %	65%	80%	NS
Mean increase FEV1 % (SD)	8.3 (14.4)	7.6 (14.4)	10.0 (14.5)	NS
Mean increase VC % (SD)	8.8 (13.5)	9.2 (13.1)	7.8 (14.7)	NS
Mean increase DLCO % (SD)	8.7 (17.8)	6.6 (11.0)	13.4 (27.2)	NS
Increase >10% any PFT	57.0%	56.3%	58.6%	NS
Patients with pulmonary treatment indication				
Mean increase VC % (SD)	10.6 (14.7)	10.6 (14.7)	10.9 (15.6)	NS
VC Increase >10%	45.5%	45.5%	52.4%	NS

* Of patients using prednisone at baseline n=100
PFT: Pulmonary function test

PULMONARY FUNCTION TESTS

We noted significantly higher pulmonary function tests after the start of the treatment compared to the year before treatment initiation. This difference was 81 ml (= 2.94% of predicted) in FEV1 (p=0.001), 97 ml (=2.57% of predicted) in VC (p=0.004) and 0.25 mmol/kPa/s (=1.5% of predicted) in DLCO (p=0.002).

After initiation of treatment, the FEV1 showed a yearly increase during treatment of 52 ml (=2.17% of predicted) (p=0.006), while VC showed a yearly increase of 95 ml(=2.81% of predicted) (p=0.001) The mean increase in FEV1 and VC was similar in both the azathioprine and methotrexate group (Fig 3A-3B). DLCO showed a yearly increase of 0.107 mmol/kPa/s (=1.23% of predicted) per year after treatment initiation (p=0.018).

Mean DLCO was 0.61 mmol/kPa/s (=5.12% of predicted) lower in the azathioprine group with borderline significance (p=0.049), but this difference was stable over time (Fig 3C).

The group with a non-pulmonary treatment indication had an overall 770ml (=23.5% of predicted) higher FEV1, an overall 491 ml (=13.1% of predicted) higher VC and a 13.3% higher DLCO than the pulmonary treatment group (p<0.0001). There was no difference in treatment effect between the group with the pulmonary and the group with the non-pulmonary treatment indication. Ethnicity and DLCO at baseline were not confounding in the analysis of any of the pulmonary function parameters.

A sub analysis of all patients completing one year follow-up on treatment in whom pulmonary function tests were available (n=100) showed that 57 patients (57%) had an improvement of ten

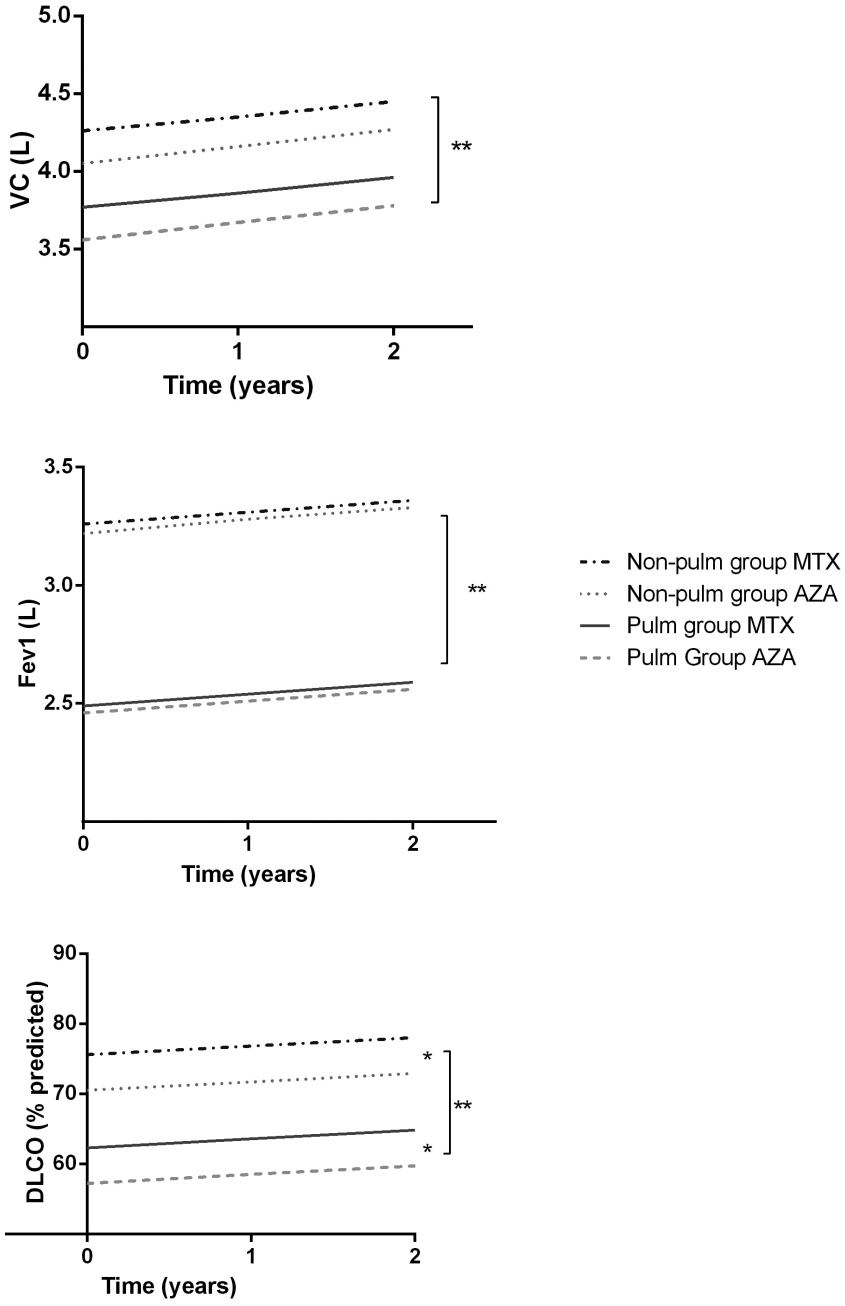

Figure 3A-C: Linear mixed model pulmonary function parameters over time.
Pulmonary function increased significantly over time (FEV1 p=0.006; VC p=0.001; DLCO p=0.018). There was a significant difference between the pulmonary and non-pulmonary treatment indication groups. No difference was found between the azathioprine (AZA) and methotrexate (MTX) treatment effect. * p=0.049 ** p<0.001

percent or more in at least one of the parameters FEV1, VC and DLCO as shown in table 2. For each of these parameters separately, these numbers were 38% of patients for VC, 36% for FEV1 and 37% for DLCO. No significant differences between azathioprine and methotrexate were found for any of these response outcomes.

SIDE EFFECTS

The most frequently reported side effects were infections in a total of 44 patients (22.4%) (Fig 4). All infections requiring antibiotics, hospital admittance or (temporary) discontinuation of treatment were included. There was a significant difference in the amount of patients having infections in the azathioprine group versus the methotrexate group (34.6 vs 18.1% p=0.01). Respiratory infections requiring antibiotics were the majority of all infections (30 patients), four patients experienced varicella zoster (all in MTX group), furthermore one case of empyema and two cases of sepsis were seen (all in AZA group). Gastrointestinal problems were reported by 37 patients (19.0%), this was the most common reason for patients to quit treatment and included, nausea, stomach-ache or diarrhoea. Severe hepatic function decline requiring alteration or discontinuation of treatment was found in 14 patients (7.2%), however, liver function recovered in all patients after discontinuation of treatment. Other repeatedly reported side effects were headache (4.1%) and malaise (7.7%). There were no differences in the occurrence of these side effects between the two treatment groups. A total of 37 patients had to discontinue therapy because of side effects (18.5%), this occurred most often within the first three months. The drop-out rates due to side effects were not significantly different between both treatment groups. Rarely seen side effects (≤ 2 patients)

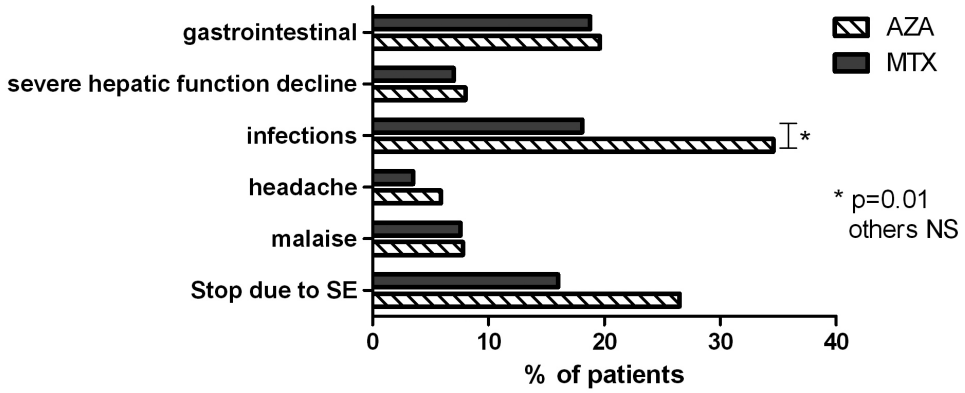

Figure 4: Reported side effects during azathioprine (AZA) and methotrexate (MTX) treatment in percentage of patients.

Patients could have more than one side effect. Severe hepatic function decline= reason to discontinue therapy. There was a significant difference between the amount of patients with infections in both treatment groups (p=0.01). NS= not significant

were hair loss, impotence, gingivitis, decline in renal function, anaemia requiring transfusion and thrombocytopenia. In our cohort, only two patients had leucopenia requiring dose adjustment or discontinuation of the second line drug, both were in the azathioprine group. Two patients in the methotrexate group were hospitalised due to pneumonia, compared to four patients in the azathioprine group. One of these patients was treated for Pneumocystis jiroveci pneumonia.
There were no patients with fatal outcome whilst on second line therapy during two years of follow up.

DISCUSSION

This study shows that both methotrexate and azathioprine have significant steroid sparing potency, a similar positive effect on lung function and comparable side effects except for a higher infection rate in the azathioprine group.
We found a decrease of prednisone daily dosing regimen of 6.32 mg/year while on therapy, with no differences between both drugs. 70% of all patients completing one year follow-up had a decrease of at least 10 mg daily dose. Our results on methotrexate confirm the results of Baughman et al [9], who found a significant reduction of prednisone use after seven months in a randomised controlled trial (RCT) of 24 patients comparing methotrexate to placebo. Lower et al [10] found a reduction of over 10 mg prednisone daily dose in 80% of 30 methotrexate treated patients who were followed up retrospectively for two years, which is in line with our findings. Only three case series [11-13] with a maximum of eleven sarcoidosis patients, describe the effect of azathioprine on lung function or prednisone dose without quantification of effect making comparison with our study difficult.
We found a mean increase in FEV1 of 52 ml/year and in VC of 95 ml/year, with no difference between the two drugs for both. DLCO (% predicted) increased with a mean of 1.23%/year. The only RCT on methotrexate in sarcoidosis did not show an improvement in pulmonary function, which could have been a power problem because the study was conducted in only 24 patients [9]. Lower et al[10] showed that 22 of 50 patients using methotrexate for two years had an improvement of VC >10%, which is in line with 38% in our cohort. Vucivic[8] showed an improvement of unspecified magnitude in FEV1 in 80 percent and DLCO in 65% of 91 sarcoidosis patients while on methotrexate for six months. After one year of treatment we found improvement in FEV1 in 74% of patients and DLCO in 68% of patients. As the size of the improvement was not quantified by Vucivic, the clinical relevance of this improvement remains uncertain so far. Hence, this is the first study describing the treatment effect of both azathioprine and methotrexate on pulmonary function in a large cohort of patients.
With available data from before initiation of methotrexate or azathioprine we were able to detect a significant but small increase in pulmonary function. A yearly drop in lung function would be expected due to natural evolution even in healthy controls, let alone in severe sarcoidosis patients. Therefore, we conclude that therapy caused improvement or at least stabilization of pulmonary function. Unexpected was the equal increase in pulmonary function in both the extra-pulmonary

and pulmonary treatment group. Although pulmonary function was not the main indication for initiating treatment in this group, most patients still had pulmonary localisation (e.g. hilar lymphadenopathy) with or without symptoms.

A total of 37 patients had to discontinue therapy because of side effects (18.5%). This drop-out rate is higher than described by Lower et al [10], where 9.5% of 105 patients quit methotrexate due to side effects or noncompliance. Vucivic [8] found that zero out of 91 patients had to discontinue methotrexate because of side effects in six months of treatment. These lower drop-out rates due to side-effects might be explained by the lower dosing regimen used in these studies (10 versus 15 mg/week). Studies comparing side effects of azathioprine and methotrexate in other diseases included a case control study in over 23000 rheumatoid arthritis patients showing a moderate increased risk of severe infections in patients taking azathioprine [17]. However, a 3-year case control study of 53 rheumatoid arthritis patients revealed more frequent adverse effects in the methotrexate group, but a similar withdrawal rate in both groups [16] and an RCT in 126 ANCA-associated vasculitis patients showed no differences in occurrence of adverse events between both drugs [17].

To date, this is the first study comparing the steroid sparing effect, the effect on pulmonary function and side effects of azathioprine and methotrexate therapy in sarcoidosis patients. In addition, this is the largest cohort of second-line therapy in sarcoidosis patients described thus far. In a review, Baughman et al [20] described that methotrexate gave a response in two thirds of their patients and half of their treated patients had a response to azathioprine. However, the vast majority of patients receiving azathioprine had previously been treated with methotrexate, making a comparison defining the best second line therapeutic impossible. On the other hand, this suggests that patients not responsive to one second-line therapeutic might still benefit from a switch to another second-line therapeutic.

Present day studies in sarcoidosis therapeutics are often initiated by pharmaceutical companies investigating new drugs, while the existing and less expensive options have not been fully explored yet. In the Netherlands and Belgium, a treatment regimen of 100 mg azathioprine daily costs 84-146 euro per patient per year, treatment with 15 mg methotrexate per week represents 58-83 euro per patient per year [21,22]. These costs are insignificant when compared to the high costs of treatment with biologicals (e.g. infliximab, adalimumab) which start at 20,000 euro per patient for six months.

One of the limitations of this study was the retrospective character of the design because non-standardisation of follow-up necessarily occurs in a retrospective study. As a result we might have missed events or side effects, nevertheless through systematic and thorough investigation of patient records we have tried to minimise the loss of follow-up and data. The absence of a placebo group makes it impossible to fully attribute the observed steroid sparing effect to the therapeutic intervention as opposed to resolution of sarcoidosis regardless of drug therapy. However, we feel this is a reflection of daily practice in clinical care, as both hospitals in the Netherlands and Belgium had the same mean prednisone dose at start of second line therapy (20mg for those on prednisone,

15 mg for the total cohort). Furthermore the disease duration before initiating second line therapy was the same in both groups (with a mean of 5 years), indicating that this cohort comprises chronic and often severe sarcoidosis patients in whom second line therapy was certainly not a first refuge. Therefore, we feel it would be too simplistic to attribute the improvement in patients of both centres to the natural history of sarcoidosis.

This large international retrospective cohort study does add important information to the medical literature, as there is a paucity of information concerning this topic, especially concerning azathioprine and its role in sarcoidosis in comparison to methotrexate.

The major strength being that each drug was a first choice second-line drug at a different institution. Therefore, there was no inherent severity selection or symptomatic bias towards using one or the other drug. This study design might have caused bias in the number of patients in whom other agents were initiated (fig 1), as patients in the Netherlands were more likely to receive infliximab, a drug that is not available in Belgium.

We found significantly more patients with infections requiring antibiotics or drug tapering in the azathioprine group (34.6 vs 18.1% p=0.01), with no significant differences regarding other side effects. The observed difference might be a reflection of different antibiotics regime in both countries, with the Netherlands being very cautious with use of antibiotics compared to the global standard [23]. Another option might be the fact that Pneumocystis jiroveci pneumonia prophylaxis was only introduced half way in the inclusion period in the azathioprine group, which is currently routine practice in this group. We did not perform Thiopurine Methyltransferase (TPMT) genotyping [24] on the azathioprine treated patients.

In conclusion, this is the first study comparing the effect of methotrexate and azathioprine in second-line sarcoidosis treatment. Although less is known on azathioprine than methotrexate in sarcoidosis, this study shows that both drugs are equally effective in terms of pulmonary function improvement and have a significant steroid sparing effect. Therefore, both methotrexate and azathioprine are suitable options in second line therapy of chronic sarcoidosis.

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DATA SUPPLEMENT

LINEAR MIXED MODEL ANALYSIS OF PULMONARY FUNCTION AND PREDNISONE DOSE CHANGE DURING TREATMENT

In analysis of retrospective longitudinal data there is a diversity in follow-up time and frequency of measurements between different subjects. To be able to include all patients and measurements in analysis, regardless of follow-up and frequency of measurements, we used a linear mixed model with random slope and random intercept. In this model each individual in the dataset has his own regression line with an individual intercept and slope. The linear mixed model incorporates the existing correlation structure in its mathematics and outcome of the approach is a regression equation with unbiased 95% CI's (p-values) of intercept / slope. The resulting regression equation is interpreted in exactly the same way as standard multiple regression. For each outcome of pulmonary function parameters (FEV1,VC and DLCO) and prednisone daily dose the general form of the regression equation applies $Y = \alpha + (\beta 1*x1) + (\beta 2*x2) + (\beta 3*x3) + \ldots. + (\beta i*xi)$. Each 'x' represents a parameter and 'β' the regression coefficient. For each unit the parameter changes the outcome changes by the magnitude of β.

Possible confounders were inserted in the model, the -2 restricted log likelihood values were used to evaluate if insertion improved the fit of the model significantly. FEV1 and VC were corrected for height and age in this manner. No other parameters (e.g. race, DLCO baseline) were found to be confounders.

In the analytical model we inserted a treatment factor which was coded as 0 in the pretreatment period and as 1 in the treatment period, to estimate the effect on lung function of treatment initiation. A time*treatment interaction was inserted to assess whether lung function continued to rise after treatment initiation.

CHAPTER 4

ACE AND SIL-2R CORRELATE WITH LUNG FUNCTION IMPROVEMENT IN SARCOIDOSIS DURING METHOTREXATE THERAPY

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ABSTRACT

INTRODUCTION

In sarcoidosis, the search for disease activity markers that correlate with treatment response is ongoing. The aim of this study was to investigate the pattern of two proposed markers, serum angiotensin-converting enzyme (ACE) and soluble IL-2 receptor (sIL-2R) during methotrexate (MTX) therapy in sarcoidosis patients.

MATERIALS AND METHODS

We analysed 114 sarcoidosis patients who used MTX for six months, consisting of a subgroup of 76 patients with a pulmonary indication for treatment and a subgroup of 38 patients with an extra-pulmonary indication. ACE and sIL-2R serum levels were measured at baseline and after six months of treatment. Correlation coefficients (R) and odds ratios (ORs) were calculated to study the correlation and predictive effect of serum ACE and sIL-2R levels for pulmonary improvement.

RESULTS

High baseline levels of ACE correlated significantly with lung function improvement after treatment ($R=0.45$, $p<0.0001$; stronger in the pulmonary subgroup R 0.57, $p<0.0001$). ACE baseline levels >90 U/l predicted a 10% improvement in overall lung function (OR 3.55; CI 1.34-9.38), with the highest prediction level for 10% improvement in DLCO (OR 4.63; CI 1.23-17.4).

After six months of MTX, mean ACE decreased with 17.2 U/l ($p<0.0001$) and sIL-2R with 1850 pg/ml ($p<0.0001$). Decreases in both ACE and sIL-2R correlated with an increase in lung function. The strongest correlation was found with change in DLCO in the pulmonary subgroup (ACE $R=0.63$, $P<0.0001$; sIL-2R $R=0.56$, $P<0.0001$).

CONCLUSION

Baseline and serial serum ACE and sIL-2R levels correlate well with lung function improvement during MTX treatment. Serial measurements of these biomarkers are helpful in monitoring treatment effects in sarcoidosis patients.

INTRODUCTION

Sarcoidosis is a systemic, granulomatous disease of unknown origin that has a variable clinical course and affects multiple organs. In sarcoidosis, oral steroids represent the first choice treatment[1,2]. Prolonged steroid use, however, is associated with severe adverse effects, e.g. obesity, diabetes and osteoporosis. Severe toxicity or disease progression often necessitates second line therapy. The goals of second line treatment are the reduction of symptoms, improvement of function and reduction of prednisone use. Methotrexate is often used as the first choice drug in second line treatment of sarcoidosis [3] and was shown to be steroid sparing and reduce symptoms [4-6].

In sarcoidosis treatment, the search for a disease activity marker that correlates with organ function (i.e. pulmonary function) is ongoing. In general, activity markers can correlate with disease

symptoms, prognosis or drug response. Thus far, no disease activity marker is known to correlate with methotrexate response in sarcoidosis.

A proposed marker of disease activity in sarcoidosis is serum angiotensin-converting enzyme (ACE)[7] , although the clinical value of this marker is still under debate as results of different studies are conflicting[8]. ACE levels were found to be elevated in 30-80% of all patients at time of diagnosis, but did not correlate with chest radiographic stages[9]. Nevertheless, a modest degree of correlation was described between serum ACE level and the extent of nodules and consolidation on High Resolution Computed Tomography (HRCT) in another study[10]. High initial serum levels of ACE could predict disease progression after two years[11]. On the other hand, no clear correlation was found between serum ACE levels and response to prednisone treatment, as ACE did decrease during treatment in cases without improvement[12,13]. Furthermore, ACE levels in bronchoalveolar lavage (BAL) fluid were found to correlate better with clinical findings than ACE levels in serum in a cohort of 25 sarcoidosis patients[14].

Another proposed marker of disease activity in sarcoidosis is serum soluble Interleukin-2 receptor (sIL-2R). IL-2 is secreted by T-helper type 1 cells and is thought to play a key role in sarcoidosis as it stimulates T-cell proliferation[15,16]. Its target on activated T-cells IL-2R is partly released as soluble IL-2R and was found to be increased in serum and BAL fluid of patients with sarcoidosis[17-20]. sIL-2R in serum was proposed as marker for disease activity as it was found to correlate with the number of CD4+ T lymphocytes in BAL fluid[21], although another study did not confirm this correlation[22]. Moreover, sIL-2R serum levels were found to correlate with parenchymal infiltration in pulmonary sarcoidosis at disease presentation and might function as prognostic marker at diagnosis[23,24].

Although they are sufficiently promising, the clinical applicability of serum sIL-2R and ACE is still uncertain. After diagnosis, both markers are in general not routinely used in follow up of sarcoidosis patients. Longitudinal data of ACE and sIL-2R levels in sarcoidosis patients are scarce and data on the correlation with pulmonary function in response to methotrexate treatment are lacking.

The aim of this study was to assess the course of serum levels of ACE and sIL-2R during methotrexate therapy in sarcoidosis and to evaluate the predictive value of ACE and sIL-2R in relation to lung function improvement.

MATERIALS AND METHODS

STUDY DESIGN

This observational cohort study includes consecutive sarcoidosis patients treated with methotrexate at St Antonius Hospital Nieuwegein, the Netherlands. This hospital serves as a tertiary referral centre for interstitial lung diseases in the Netherlands and for sarcoidosis in particular. To evaluate baseline characteristics, medical records were reviewed retrospectively for demographic data and smoking status. Also listed were organ involvement from sarcoidosis and radiographic Scadding stage. The effect on serum markers ACE and sIL-2R and pulmonary function tests was evaluated after six months of treatment with methotrexate and collected retrospectively.

Efficacy results after a longer period of time have been described previously comparing the effect of methotrexate and azathioprine on lung function and prednisone use in an international cohort[6]. Authors obtained a declaration of no objection from the local Institutional Review Board with registration number LTME/Z-12.05 and acronym METHVERAZ.

PATIENTS

All patients with sarcoidosis who started methotrexate treatment at St Antonius Hospital Nieuwegein, the Netherlands between June 2004 and September 2011 were selected. These patients had persistent active sarcoidosis with impaired organ function or severely diminished quality of life. Moreover, they were unresponsive to steroids, had severe side effects on steroids or had serious contraindications for steroid use, justifying the initiation of methotrexate therapy .

For the purpose of this study, patients were divided into a pulmonary and an extra-pulmonary treatment group based on main indication for initiation of methotrexate therapy. The indication for therapy was set by the treating physician at time of initiation. A main indication in the pulmonary group required a decreased pulmonary function (of at least <80% on one or more lung function parameters) and most patients had symptoms of dyspnoea. Patients in the pulmonary group could also have some extra-pulmonary symptoms and vice versa. In the extra-pulmonary group, lungs were not the index organ for therapy.

Patients under methotrexate treatment followed a standard treatment protocol starting with 10 mg per week, with an increase to 15 mg per week after four weeks under routine checks of blood cell count and hepatic function. In addition all patients took 5 mg folic acid per week.

SERUM MARKERS

In our hospital, serum ACE and sIL-2R are routinely measured in the follow up of sarcoidosis patients. ACE was measured in lithium heparin plasma using the Bühlmann ACE kinetic test (Siemens Healthcare Diagnostics, Breda, the Netherlands) on a Cobas 6000 platform (Roche Diagnostics) and was quantified in U/l. ACE values of patients who used ACE-inhibitors were excluded from analysis. ACE was also corrected for genotype (I/I, I/D or D/D) and expressed as the Z-score[25] .

SIL-2R levels were quantitatively determined in serum with enzyme immunoassays according to the manufacturer's instructions (until april 2009: Milenia; AMDS Benelux, Malden, the Netherlands, and from April 2009: Diaclone; Sanquin, Amsterdam, the Netherlands). Method comparison was performed to convert the results of the Milenia immunoassay from U/ml to pg/ml.

PULMONARY FUNCTION TESTING

Pulmonary function tests were performed using Master Screen Body (Jaeger ms-pft analyse unit, Würtzburg, Germany). The pulmonary function parameters tested were changes in the percentage of predicted vital capacity (VC), forced expiratory volume in 1 second (FEV1) and diffusing capacity of the lung for carbon monoxide (DLCO) between baseline and six months of treatment with methotrexate.

STATISTICAL ANALYSIS

Variables ACE and sIL-2R before and after six months of treatment were compared with two-tailed paired t-tests. Correlation coefficients (R) between baseline levels of serum markers, change in serum markers and change in lung function were calculated with linear regression. The best cut-offvalues were determined with ROC-curves. Statistical analysis was performed and scatter plots were created using SPSS for Windows, version 22.0. Graphs were created using Graph pad Prism 5.0. A P-value of less than 0.05 was considered significant.

RESULTS

PATIENTS

Methotrexate therapy was initiated in 137 patients, of whom 12 quite methotrexate before completing six months of treatment or started add-on treatment with infliximab. Another 11 patients lacked pre or post values of ACE or sIL-2R at six months, the remaining 114 patients were included in the analysis.

A pulmonary treatment indication was seen in 76 patients and 38 patients had an extrapulmonary treatment indication (table 1). Extra-pulmonary treatment indications included cardiac sarcoidosis, neurosarcoidosis, joint sarcoidosis, uveitis and disabling fatigue. As expected, pulmonary function and Scadding stage were significantly worse in the pulmonary group. Most patients concurrently used prednisone (81%) and ACE at baseline was lower in this group. Mean improvement in pulmonary function was not significantly different between the group with and without prednisone at baseline.

PREDICTIVE VALUE OF ACE AND SIL-2R LEVELS

Mean ACE level at baseline was 76.9 U/l and mean sIL-2R level at baseline was 4854 pg/ml (Table 1). At baseline, elevated levels of above 70 U/l and 3000 pg/ml were seen in 41% and 59% of patients for these markers, respectively.

Correlations between the baseline ACE serum levels and the change in pulmonary function parameters were significant (table 2). The strongest correlation coefficient was found between baseline ACE level and ΔDLCO ($R=0.45$; $p<0.0001$) (Figure 1). Separate analysis of the pulmonary and extra-pulmonary group showed this was due to a high correlation in the pulmonary group ($R=0.57$; $p<0.0001$), while a correlation was absent in the extra-pulmonary group. However, in the extra-pulmonary group, baseline ACE level correlated significantly with ΔFEV1 ($R=0.41$; $p=0.028$) with non significant results for the pulmonary group.

The best cut-off value for ACE, determined by ROC-curves, was 90 U/l. A baseline level of ACE >90 U/l predicted a ≥10% improvement in at least one of the pulmonary function parameters (OR 3.55; CI 1.34-9.38), with this prediction level being even higher for improvement in DLCO of ≥10% (OR 4.63; CI 1.23-17.4). The latter resulted in a low positive predictive value of 30% (CI 12-54%), but a high negative predictive value of 92%.

Table 1 Baseline characteristics of treatment cohort

	All patients	Pulmonary Group	Extra-pulmonary Group	P-value
N	114	76	38	
Mean age at start of therapy in years (SD)	48.1 (10.3)	48.3 (8.7)	47.7 (13.1)	NS
Male n (%)	70 (61)	51 (67)	19 (50)	NS
Race white (n (%))	96 (84)	63 (83)	33 (87)	NS
Histology (n (%))	104 (91)	69 (91)	35 (92)	NS
Previous therapy n (%)				
Prednisone	94 (84)	60 (81)	34 (90)	NS
Prednisone + plaquenil	9 (8)	7 (9.5)	2 (5)	
None (contra-indication prednisone)	9 (8)	7 (9.5)	2 (5)	
Disease Duration at start of therapy in years (SD)	4.0 (7.0)	5.7 (7.2)	3.7 (6.4)	NS
Concurrent prednisone	92 (81)	62 (82)	30 (79)	NS
Scadding stage n (%)				
0	12 (11)	0(0)	12 (32)	p<0.0001
I	11 (10)	4 (5)	7 (18)	
II	46 (40)	35 (46)	11 (29)	
III	23 (20)	17 (22)	6 (16)	
IV	22 (19)	20 (26)	2 (5)	
PFT % pred (SD)				
TLC	79 (17)	75 (15)	87 (19)	P=0.002
VC	91 (21)	85 (19)	101 (21)	p<0.0001
Fev_1	77 (24)	67 (20)	97 (21)	p<0.0001
DLCO	66 (17)	62 (16)	75 (15)	p<0.0001
Serum markers				
ACE U/L (SD)	76.9 (62.1)	82.0 (68.0)	65.5 (44.5)	NS
sIL-2R pg/ml (SD)	4854 (3839)	5019 (3953)	4551 (3651)	NS

A subdivision is made between patients treated with pulmonary and extra-pulmonary treatment indication. P-values are noted for significant differences between the two groups. NS=not significant, PFT= Pulmonary function test, TLC= total lung capacity, VC=vital capacity, FEV1= forced expiratory volume in 1 second, DLCO= diffusing capacity of the lung for carbon monoxide. ACE= Angiotensin Converting enzyme, sIL-2R= soluble interleukin-2 receptor.

Table 2 Correlations between baseline serum values of ACE and sIL-2R and change in lung function parameters (ΔVC, ΔFEV1 and ΔDLCO).

Correlation (R)	All patients	Pulmonary group	Extra-pulmonary group
Baseline ACE – ΔVC	0.36 P=0.003	0.35 P=0.017	0.32 NS
Baseline ACE - ΔFEV_1	0.24 P=0.015	0.19 NS	0.41 P=0.028
Baseline ACE – ΔDLCO	0.45 P<0.0001	0.57 P<0.0001	0.18 NS
Baseline sIL-2R – ΔVC	0.18 NS	0.26 NS	0.07 NS
Baseline sIL-2R - ΔFEV_1	0.08 NS	0.02 NS	0.28 NS
Baseline sIL-2R - ΔDLCO	0.29 P= 0.008	0.39 P<0.0001	0.13 NS

A subdivision is made between patients treated with pulmonary and extra-pulmonary treatment indication.
Δ = delta. R=Correlation Coefficient. NS=not significant

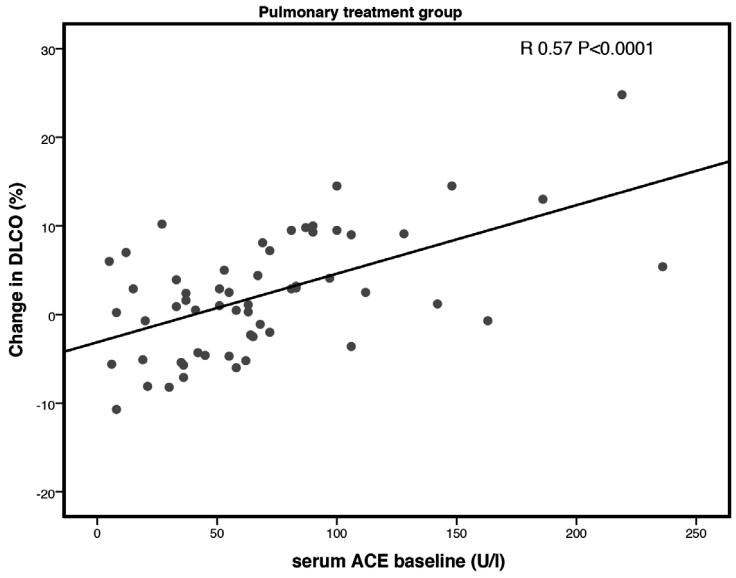

Figure 1: Correlation between baseline serum values of ACE and treatment effect on DLCO.
A positive delta in DLCO represents an increasing lung function during treatment in the group with a pulmonary treatment indication. R=Correlation Coefficient. Filled line=regression line. DLCO= diffusing capacity of the lung for carbon monoxide. ACE= Angiotensin Converting enzyme.

(CI 81-97%) and of even 97% (CI 87-99%) in patients with pulmonary indication. This indicates that in patients with a low ACE at baseline, an improvement of over 10% in DLCO should not be expected and in patients with high ACE levels response is possible.
The correlation coefficient between baseline sIL-2R and ΔDLCO was significant in the pulmonary group with an R of 0.39 ($p<0.0001$).
ACE at baseline was not significantly different between patients of Caucasian and non-Caucasian descent (data not shown). sIL-2R was higher in non-caucasian patients (7948 pg/ml versus 4256 pg/ml; $p=0.015$), but race did not predict pulmonary function increase ($p=0.74$).

ACE AND SIL-2R SERUM LEVELS DECREASE DURING TREATMENT

Serum ACE values before and after treatment were available for 95 patients after excluding patients on ACE-inhibitors. Mean ACE decreased from 71.4 U/l before treatment to 54.2 U/l after six months of treatment ($p<0.0001$).
Before and after treatment values of sIL-2R were available for 104 patients. Mean sIL-2R decreased from 4840 pg/ml to 2990 pg/ml after six months of treatment ($p<0.0001$). The mean baseline values and changes in ACE and in sIL-2R levels were not significantly different between the pulmonary and extra-pulmonary group. Although baseline ACE levels were higher in the small group that used prednisone (100.0 versus 70.9 U/l; $p=0.02$), the change in ACE-levels during therapy was independent of prednisone use.
We found significant correlations between baseline levels of serum ACE and sIL-2R ($R=0.46$; $p<0.0001$) as well as between the change in ACE and change in sIL-2R during treatment ($R=0.63$; $p<0.0001$).

CHANGES IN ACE AND SIL-2R CORRELATE WITH LUNG FUNCTION IMPROVEMENT

We found significant correlations between changes in all lung function parameters and the change in ACE levels after six months of methotrexate therapy (table 3). The highest correlation coefficient was found between ΔACE and ΔDLCO ($R=0.53$; $p<0.0001$). Dividing the patients into a pulmonary and an extra-pulmonary group resulted in a strong correlation between ΔACE and ΔDLCO in the pulmonary group ($R=0.63$; $P<0.0001$).
On the other hand, the extra-pulmonary group showed a correlation between ΔACE and ΔFEV1 ($R=0.56$; $p=0.003$). Patients in the extra-pulmonary group showed high interpatient variability as the mean FEV1 at baseline was 97% of predicted, but still 16% of patients had an FEV1 under 80% predicted. After six months of therapy, mean FEV1 was 101% of predicted (SD 19; p=NS), but in 23% of these patients FEV1 increased over 10% predicted. The correlations between ΔsIL-2R and increases in lung function parameters were significant as well, with the strongest correlation between ΔsIL-2R and ΔDLCO ($R=0.49$; $p<0.0001$) (table 3). Dividing the patients into two groups based on treatment indication resulted in a significant correlation between ΔsIL-2R and ΔFEV1 in the extra-pulmonary group only ($R=0.42$; $p=0.02$). The strongest correlation coefficient was found between ΔsIL-2R and ΔDLCO in the pulmonary group ($R=0.56$; $p<0.0001$).

Table 3 Correlations between differences in before and after serum values of ACE and sIL-2R and treatment effect on lung function parameters (VCmax, FEV1 and DLCO).

Correlation (R)	All patients	Pulmonary group	Extra-pulmonary group
ΔACE – ΔVC	0.43 P=0.001	0.45 P=0.004	0.40 NS
ΔACE - ΔFEV_1	0.401 P<0.0001	0.34 P=0.009	0.56 P=0.003
ΔACE – ΔDLCO	0.53 P<0.0001	0.63 P<0.0001	0.32 NS
ΔsIL-2R – ΔVC	0.36 P=0.003	0.39 P=0.009	0.34 NS
ΔsIL-2R - ΔFEV_1	0.23 P=0.02	0.15 NS	0.42 P=0.02
ΔsIL-2R - ΔDLCO	0.49 P<0.0001	0.56 P<0.0001	0.38 P=0.049

A subdivision is made between patients treated with pulmonary and extra-pulmonary treatment indication.
Δ = delta. R=Correlation Coefficient. NS=not significant

CORRECTING ACE FOR GENOTYPE

In the total cohort, 22 patients had I/I genotype (19.5%), 48 patients had I/D genotype (42.5%) and 43 patients had D/D genotype (38,0%) of the gene encoding ACE. Mean serum ACE levels at baseline were not significantly different between these genotype groups due to high variability between patients. Therefore, the use of Z-scores did not improve correlations with pulmonary treatment effect.

DISCUSSION

In this study not only a decrease in ACE and sIL-2R levels was observed during methotrexate therapy in sarcoidosis patients, but this decrease in ACE and sIL-2R levels was also significantly correlated with improvement of pulmonary function parameters. Furthermore, high baseline levels of ACE at initiation of therapy were predictive for lung functional response after six months of treatment.

Thus far, no studies have focussed on ACE values during methotrexate therapy and its correlation with lung function in sarcoidosis. In a study on the effect of prednisone treatment in sarcoidosis, Baughman and co-workers [12] showed a significant decrease of ACE during prednisone treatment, but the correlation between ACE and lung function was not investigated. Hollinger and colleagues [13]also demonstrated a decline in ACE level during prednisone treatment, but a correlation between pre-treatment serum ACE level and change in FVC was not found in this small

study of 18 patients. In our study the vast majority of patients (81%) used prednisone at baseline and these patients had lower ACE levels at start, but the decrease in ACE as well as the increase in pulmonary function was not different from the small group without prednisone use. Although we used a cut-off for ACE of 90 U/l to determine a predictive Odds-ratio, Figure 1 demonstrates that also patients with ACE >70 U/l are more likely to increase in pulmonary function than patients with low ACE. Patients with increased ACE and sIL-2R values with an indication for treatment should always be treated, whereas patients with low values can be treated according to the judgement of an experienced physician, as improvement is not expected in all cases.

To date, no studies have been performed on serial measurements of sIL-2R levels in sarcoidosis, nor have there been studies on correlation between sIL-2R and pulmonary function during treatment. In a small study with 15 prednisone treated patients, pre-treatment serum sIL-2R did not predict radiologic outcome, but the change in sIL-2R did correlate with the change in chest radiographic stage[21], which can be translated to the findings in our cohort. Because changes in biomarkers during treatment correlated better with treatment outcome than baseline values of biomarkers, one of the main messages of this study is that the value of these sarcoidosis activity markers lies not only in single sample measurement, but especially in serial measurements during patient follow-up.

In our study, we found differences between the pulmonary and extra-pulmonary group. Baseline values of ACE and sIL-2R correlated best with change in DLCO during treatment in the pulmonary group. Remarkably, correlations between FEV1 during treatment and both ACE and sIL-2R values were highest in the extra-pulmonary group. A possible explanation might be that many patients in the extra-pulmonary treatment group had extensive hilar lymfeadenopathy that might have caused low FEV1 values. In contrast, more patients in the pulmonary subgroup suffered from fibrotic disease, in which VC was limited most. Decrease in sIL-2R during treatment did correlate well with improvement in DLCO, which is an important finding with value in the clinical field.

Despite the good correlations, monitoring ACE and sIL-2R will not likely replace pulmonary function tests in clinical practice. Nevertheless, ACE and sIL-2R should be part of the work-up strategy to monitor disease activity during treatment next to function testing and subjective clinical response indicators, because in sarcoidosis still not one single parameter exists that is applicable to all patients. The clinician should base his clinical decision on function, subjective response and biomarkers. In particular in cases where function and subjective response do not correspond, activity markers can have an important supporting role. Furthermore, we found that serum values of ACE and sIL-2R did not significantly differ between the pulmonary and extra-pulmonary treatment group. Therefore, in patients in whom lung function is not an adequate marker of disease severity, for example in neurosarcoidosis, repeated measurements of ACE and sIL-2R levels might guide clinical decision making. Furthermore, as significant lung function change for example after tapering of methotrexate is a often a slow process, increase of biomarkers can provide an earlier signal of relapsing disease.

In our study, ACE correlates better with lung function change during methotrexate than sIL-2R.

During disease, expression of ACE and sIL-2R is influenced by different cellular processes. It has been suggested that ACE reflects total body granuloma burden [26], while sIL-2R is considered a marker of T-cell activation as elevated serum levels of sIL-2R have been found to correlate with the activity of T-cell-mediated diseases [27]. Because both markers have a different origin, they can be used side by side in clinical practice. Furthermore, due to different the mechanism of action in the inflammatory cascade of the various used therapeutics in sarcoidosis, it is possible that both markers will behave differently. However, ACE levels can not be used in the follow-up of patients using ACE-inhibitors.

Correcting ACE values for genotype did not improve the predictive value for lung function improvement because ACE-levels were not different between genotype groups. This is probably due to the fact that current studies investigating ACE-genotyping have mostly been performed in healthy controls [28], whereas in severe sarcoidosis patients ACE-levels depend on more factors such as disease severity and treatment. This indicates that genotyping is mostly useful for diagnosis and not follow-up.

The quest for a sensitive and specific marker for disease activity and treatment response in sarcoidosis has been ongoing for several decades. In the last few years, the ^{18}F-FDG PET scan has earned a firm role in measuring disease activity in sarcoidosis. The ^{18}F-FDG PET scan has much potential in detecting active sarcoidosis and changes in lung function and disease activity during therapy [29-31]. However, ^{18}F-FDG PET is expensive and requires interpretation by a nuclear physician with experience in the field of sarcoidosis. Furthermore, unlike serum measurements of ACE and sIL-2R, the test is invasive and exposes the patient to radiation making it an inappropriate tool for repeated measurements.

In conclusion, our study shows that especially changes in ACE and sIL-2R during treatment correlate well with lung function outcome. Furthermore, high baseline levels of ACE predict lung function improvement upon methotrexate treatment. Therefore, both these serological markers have more clinical value than currently known and serial measurement of these markers helps clinical decision making when monitoring sarcoidosis patients during treatment.

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PART II

THIRD LINE THERAPY IN SARCOIDOSIS

CHAPTER 5

CHANGES IN DISEASE ACTIVITY, LUNG FUNCTION AND QUALITY OF LIFE IN PATIENTS WITH REFRACTORY SARCOIDOSIS AFTER ANTI-TNF TREATMENT

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ABSTRACT

OBJECTIVES

The effect of infliximab therapy in rheumatoid arthritis and Crohn's disease has been well documented. However, literature on infliximab therapy in the rare disease sarcoidosis is scarce. The aim of our study is to evaluate change in disease activity and quality of life upon infliximab treatment in patients with refractory sarcoidosis.

RESEARCH DESIGN AND METHODS

We performed a retrospective cohort study of 48 patients who received infliximab treatment at a national referral centre for sarcoidosis. The clinical effect of infliximab treatment was analyzed in terms of change in disease activity (^{18}F-FDG PET, serum angiotensin-converting-enzyme (ACE), serum soluble interleukin-2 receptor (sIL-2R)), lung function (predicted VC, FEV1 and DLCOc), fatigue severity (CIS) and physical functioning (SF-36). Study parameters were assessed before the first dose (week 0) and after six doses (week 18).

RESULTS

We found significant decreases in the serum markers ACE and sIL-2R and in SUVmax on ^{18}F-FDG PET. Furthermore a significant improvement in fatigue severity and physical functioning scores was observed. In patients with a pulmonary treatment indication we found significant improvements in percentages of predicted VC (7.6%), FEV1 (7.9%) and DLCOc (3.5%).

CONCLUSIONS

Infliximab improves PET-scan, serum markers, lung function and quality of life in patients with refractory sarcoidosis.

INTRODUCTION

Sarcoidosis is a rare systemic inflammatory disorder that is characterized by the development and accumulation of granulomas. Sarcoidal granulomas are often manifested in the lymphatic system and lungs. Yet, other organs such as heart, skin, central nervous system or eyes may also be involved. The cause of sarcoidosis is unknown and no curative medication exists [1-3].

In 80% of cases, sarcoidosis improves or remains stable during two years of follow up without any treatment [4]. However, sarcoidosis may become chronic in a small subgroup of patients. These patients suffer from unremitting disease activity, (risk of) organ failure and symptoms which severely reduce their quality of life. In these cases suppression of symptoms is indicated: corticosteroids are the drug of first choice [5-7]. Even though corticosteroids are generally effective, they may have severe side effects when chronically used. Additional supply of anti-malarial drugs and immunosuppressive drugs like methotrexate (MTX) as steroid sparing drugs are not always effective [5, 8, 9]. Furthermore, these drugs have significant long-term toxicity [10], therefore, new treatment options are needed.

In sarcoidosis, the cytokine tumor necrosis factor-alpha (TNF-α) plays an important role in formation of the granulomas [1]. A chimeric monoclonal antibody directing against TNF-α may therefore be expected to be effective in the treatment of sarcoidosis [11, 12]. Anti-TNF therapy has been proven effective in treatment of rheumatoid arthritis, psoriasis and Crohn's disease [13-15].

The anti-TNF drug Infliximab (Remicade®; Centocor, Inc., Malverna, PA., USA) has been subject of investigation in case series and reports of several manifestations of sarcoidosis. Until now, only two randomized controlled trials (RCTs) have been performed in sarcoidosis [16, 17]. The RCT with 148 patients performed by Baughman et al [16] has revealed a small but significant improvement in lung function after six infusions of infliximab. Additional sub analysis showed positive effects of infliximab treatment on extra-pulmonary symptoms [18]. Furthermore, a case series of 28 sarcoidosis patients demonstrated the long-term effect of infliximab [19]. In general, these studies all focused on lung function improvement. So far, literature on infliximab therapy in sarcoidosis which evaluates also disease activity and health-related quality of life has not been published.

In this study we assessed the change in disease activity and the change in health-related quality of life in addition to changes in lung function in a group of sarcoidosis patients with chronic disease activity who were refractory to corticoid and/or corticoid sparing treatment. We objectified disease activity in terms of uptake of 18F-fluorodeoxyglucose by positron emission tomography (^{18}F-FDG PET) and serum markers as angiotensin converting enzyme (ACE) and soluble interleukin-2 receptor (sIL-2R). Additionally, health-related quality of life was assessed by questionnaires and lung function was determined.

PATIENTS AND METHODS

STUDY POPULATION

At a national tertiary referral centre for sarcoidosis (the centre of Interstitial Lung Diseases of the St. Antonius Hospital Nieuwegein, the Netherlands) 48 sarcoidosis patients were treated with infliximab in the period from 2004 until 2010. In general, only patients who were refractory to regular medication (corticosteroids, anti-malarial drugs, methotrexate) and patients who had severe side effects in response to this medication were allocated to receive infliximab therapy. Furthermore, infliximab therapy was administered in patients with unremitting disease activity, which was objectified by elevated serum markers or increased uptake on the PET-scan. Of note, patients with an active or latent tuberculosis infection were not eligible for treatment.

All patients received infliximab intravenously at a dose of 5mg/kg at weeks 0, 2, 6, 10, 14 and 18. Baseline study parameters were assessed before the first dose at week 0 and efficacy was measured after the last dose at week 18.

In addition, the following data were collected from medical patient records: localization of sarcoidosis (pulmonary and/or extra-pulmonary), main indication for infliximab treatment, previous treatment with immunosuppressive agents, co morbidity of asthma, smoking status and smoking history, sex, age, height and race.

We noted all adverse events and allergic reactions during the induction period of 6 infusions

with infliximab. Furthermore we separately discuss efficacy findings in extrapulmonary treatment indications.
As all measurements were part of routine clinical practice, the authors have obtained a declaration of no objection from the local Institutional Review Board of the St. Antonius Hospital with registration number LTME/Z-12.33 and acronym ORATS.

DISEASE ACTIVITY

Disease activity of sarcoidosis was objectified by ^{18}F-FDG PET and serum parameters. ^{18}F-FDG PET was performed using the Philips Allegro PET system with external 137Cs source for transmission scanning (Philips Medical Systems, Eindhoven, the Netherlands). Uptake of 18F-FDG in the pulmonary parenchyma and the mediastinum was expressed as maximum SUV (SUVmax), which is acknowledged as a sensitive tool to measure disease activity in sarcoidosis [20, 21]. An independent nuclear physician determined SUVmax before and after infliximab treatment. In the St. Antonius Hospital, ^{18}F-FDG PET is part of the routine sarcoidosis activity analysis and treatment evaluation.
Measured serum markers in this study comprised ACE and sIL-2R. These markers of disease activity are both known to be elevated in sarcoidosis patients [22, 23]. ACE was measured in lithium heparin plasma using the Bühlmann ACE kinetic test (Siemens Healthcare Diagnostics, Breda, The Netherlands) on a Cobas 6000 platform (Roche Diagnostics). ACE was corrected for genotype (I/I, I/D or D/D) and expressed as the Z-score [24].
sIL-2R was quantitatively determined in serum using enzyme immunoassays according to the manufacturer's instructions. Either Milenia; AMDS Benelux, Malden, The Netherlands, or (from April 2009) Diaclone, Sanquin, Amsterdam, The Netherlands was used. Within each patient the same method for quantification of sIL-2R was used. Method comparison was performed to convert the results with the immunoassay of Milenia from U/ml to pg/ml.

HEALTH-RELATED QUALITY OF LIFE

Fatigue severity was measured with the subscale 'Fatigue Severity' of the generic fatigue questionnaire Checklist Individual Strength (CIS) [25]. Fatigue severity scores range from 8 (non-fatigued) to 56 (severely fatigued). Physical functioning was assessed by the Medical Outcome Study - 36 Item Short Form Health Survey (SF-36) subscale score 'Physical Functioning'. The SF-36 is a generic questionnaire with 36 items that measure functional health and wellbeing [26, 27]. Scores on physical functioning range from 0 to 100, in which higher scores indicate better physical functioning. Both questionnaires are part of the standard care of sarcoidosis patients treated with infliximab in the St. Antonius Hospital since march 2008.

PULMONARY FUNCTION TESTS

Pulmonary function tests were performed using Master Screen Body (Jaeger ms-pft analyse unit, Würzburg, Germany). Vital capacity (VC), forced expiratory volume in 1 second (FEV1) and diffusing

capacity of the lung for carbon monoxide corrected for haemoglobin concentration (DLCOc) were determined and expressed in respectively ml and ml/min/mmHg, as well as in percentages of predicted.
To evaluate the effect of infliximab on pulmonary function, a subgroup analysis of patients with a pulmonary main indication for initiating treatment was performed.

STATISTICAL ANALYSIS
Change in SUVmax, sIL-2R, ACE, fatigue-severity, physical functioning, percentage of predicted VC, percentage of predicted FEV1 and percentage of predicted DLCOc were calculated by subtracting the score measured after last dose of treatment (at 18 weeks) from the score at baseline before administration of the first dose. Next, Analysis of Covariance (ANCOVA) was used to identify statistical significant changes in response to anti-TNF treatment. The following covariates were added to the model: age, sex, height, number of pack years and co morbidity of asthma. In addition, baseline pulmonary function tests were added to the model and used as correction factor. Data analysis was performed using SPSS version 17.0.

RESULTS

PATIENT CHARACTERISTICS
Forty-eight patients with refractory sarcoidosis were treated with infliximab from 2004 till 2010. Data of three patients were not evaluable after 6 infusions: one patient had an allergic reaction to infliximab after 4 infusions, one patient suffered from progression of dyspnoea after 3 infusions and one patient was hospitalized because of a severe methotrexate hepatitis. In total, data of 45 patients who completed all six cycles of infusions were analyzed. A flow-chart with total number of patients treated and included in analysis is shown in Figure 1.
Prior to infliximab treatment, 30 patients used a combination of prednisone and methotrexate, 12 patients used only prednisone and 1 patient used only methotrexate. Two patients did not receive any previous medication. Both patients had severe SFN, active sarcoidosis, and contraindications for corticosteroid use. All patients were refractory to other immunosuppressive medication or had severe side effects. At the start of infliximab therapy 41 (91.1%) patients were still using concomitant immunosuppressive medication (Table 1). Prednisone dose varied from 5 to 20 mg daily; MTX varied from 5 to 25 mg once a week. The background medication regimen remained stable during the treatment period as clinical evaluation did not give rise to tempering the initial doses. Eight patients (17.8%) who had never received methotrexate before, started with this drug in a dose varying from 7.5-15 mg/week at the start of infliximab treatment as prevention therapy for anti-infliximab antibody formation. One patient used an ACE inhibitor during the therapy, with low levels of ACE before and after the treatment. None of the patients started an ACE inhibitor during the treatment period.

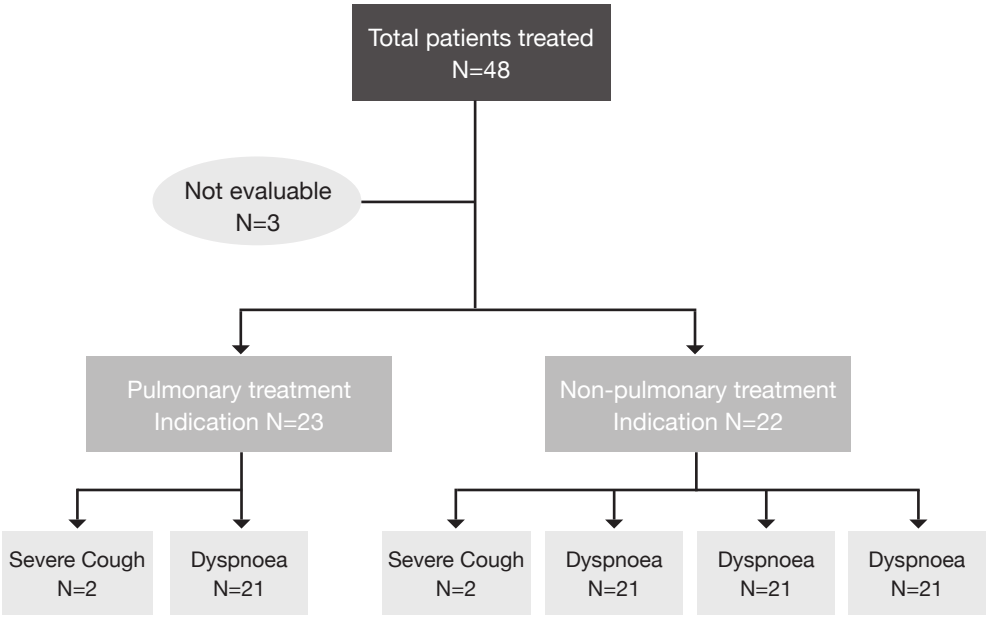

Figure 1: Flow-chart with total number of patients treated and included in analysis.

Table 1 Patient characteristics

	n = 45
Age	48.9 ± 10.1
Male, n (%)	27 (60)
Histologically proven sarcoidosis, n (%)	43 (95.5)
Years since diagnosis	7.6 ± 8.4
Scadding stage, n (%)	
0	5 (11.1)
I	7 (15.6)
II	14 (31.1)
III	5 (11.1)
IV	14 (31.1)
Smoking history, n (%)	
(Former) smoker	16 (35.6%)
Never-smoker	29 (64.4%)
Mean no. of pack years	11 ± 12

Concomitant medication during infliximab treatment, n (%)	
methotrexate	16 (35.6)
prednisone	16 (35.6)
prednisone and methotrexate	8 (17.8)
plaquenil	1 (2.2)
none	3 (6.7)
unknown	1 (2.2)
Baseline ^{18}F-FDG PET	
SUVmax:	
pulmonary parenchyma	4.3 ± 3.6
mediastinum	5.1 ± 3.9
Baseline serum parameters	
Serum ACE Z-score (normal range: - 1.96 – 1.96)	2.6 ± 3.9
Serum sIL-2R (normal range: < 3000 pg/ml)	5001 ± 3919
Baseline health-related quality of life*	
Fatigue Severity (range: 7/no fatigue – 56/ extreme fatigue)	49.4 ± 9.2
Physical Functioning (range: 0/severely impaired – 100/normally functioning)	30.9 ± 22.2
Baseline pulmonary function tests	
VC, % predicted	85.7 ± 19.0
VC, ml	3480 ± 1190
FEV1, % predicted	75.3 ± 22.9
FEV1, ml	2470 ± 950
DLCOc, % predicted	66.7 ± 18.7
DLCOc, ml/min/mmHg	6.50 ± 2.24

Mean ± SD unless specified otherwise

^{18}F-FDG PET scan was available in 40 patients (88.9%)
*Fatigue and health status scores available in 27 patients (60%)

Due to the diverse character of the disease, the cohort comprised sarcoidosis patients with various treatment indications. Twenty-three patients had a pulmonary treatment indication with severe dyspnoea on exertion, or a decline in pulmonary function tests despite treatment with corticosteroids or other immunosuppressive medication. Two of these patients had therapy resistant invalidating cough. The patients with an extra-pulmonary treatment indication (n = 22) suffered from therapy resistant sight threatening uveitis, cardiac sarcoidosis (with reduced exercise capacity or arrhythmias), neurosarcoidosis (i.e. pain, small fibre neuropathy, muscle weakness), or severe and invalidating fatigue. Patients with a pulmonary treatment indication had significantly worse pulmonary function tests and higher Scadding stages. Patients with Scadding stage 0 at infliximab initiation all had an extrapulmonary index organ as main treatment indication, e.g. cardiac sarcoidosis.

DISEASE ACTIVITY

18F-FDG-PET data before and after the infliximab treatment were available in 40 patients (88.9%). The SUVmax measured in the pulmonary parenchyma showed a decrease of 2.7 ($p<0.00005$) and the SUVmax measured in the mediastinum a decrease of 2.3 ($p<0.0005$) (Table 2).
Furthermore, a significant decrease in ACE Z-score (-2.01 U/ml; $p<0.0005$) and in sIL-2R (-2879 pg/ml; $p<0.00001$) was observed (Table 2).

HEALTH-RELATED QUALITY OF LIFE

Fatigue and physical functioning scores were available in patients who started with infliximab treatment after March 2008 (n=27 (60%)). Analysis showed a statistically significant change in fatigue severity scores (-5.3; $p=0.003$) as well as in the physical functioning scores (+ 12.6; $p=0.011$).

LUNG FUNCTION

When evaluating lung function improvement after six doses of infliximab we found an absolute increase in percentage of predicted VC of 5.4% ($p<0.0001$), in percentage of predicted FEV1 of 5.3% ($p<0.001$) and in percentage of predicted DLCOc of 3.1% ($p=0.012$). Subgroup analysis revealed a larger increase in pulmonary function tests in patients who had a pulmonary main indication for initiating treatment (n = 23) (Figure 1). The changes in percentage of predicted VC, percentage of predicted FEV1 and percentage of predicted DLCOc in this subgroup were

Table 2 Mean changes from baseline in pulmonary function tests, SUVmax, ACE Z-score, sIL-2R, CIS fatigue severity score and SF-36 physical functioning score in the total cohort

	n = 45	p-value
^{18}F-FDG PET		
SUVmax:		
pulmonary parenchyma	- 2.7 ± 3.4	< 0.00005
mediastinum	- 2.3 ± 3.4	< 0.0005
Serum parameters		
ACE Z-score	- 2.01 ± 3.31	< 0.0005
sIL-2R (pg/ml)	- 2879 ± 3755	< 0.00001
Health-related quality of life		
Fatigue severity	- 5.3 ± 8.5	0.003
Physical functioning	+ 12.6 ± 23.9	0.011
Pulmonary function tests		
VC, % predicted	+ 5.4 ± 7.6	< 0.0001
FEV1, % predicted	+ 5.3 ± 8.3	< 0.001
DLCOc, % predicted	+ 3.1 ± 7.3	0.012

Mean ± SD unless specified otherwise

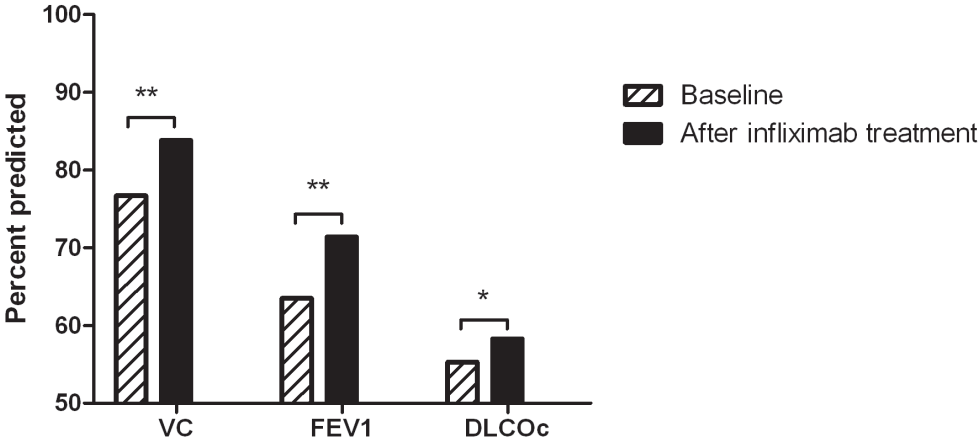

Figure 2: Mean values of VC, FEV1 and DLCOc in the pulmonary subgroup, before and after 6 infusions of infliximab

*p=0.01, **p<0.0001

respectively 7.6% (p<0.0001), 7.9% (p<0.0001) and 3.5% of predicted (p=0.011) (Figure 2). In this pulmonary subgroup, 52% of patients had a relative improvement in VC of 10% or more (table 3). Additional analysis showed that the increase in VC and FEV1 was still significant when we excluded the 8 patients who never used methotrexate before (respectively 7.8 and 8.1%).

Table 3 Relative change in pulmonary functions tests in the pulmonary subgroup during infliximab therapy expressed as % of patients

% change	VC	FEV1	DLCOc
>20%	17.4	30.4	5.3
10-20%	34.8	21.7	31.6
0-10%	30.4	39.1	42.1
decline	17.4	8.7	21.1

EXTRA-PULMONARY EFFICACY

Two patients were treated because of cardiac involvement of sarcoidosis with a decrease in left ventricular ejection fraction (LVEF) and symptoms as arrhythmias, palpitations, dizziness and/or dyspnoea on exertion. Both patients had an improvement of left ventricular function as well as an improved exercise capacity. The first patient had a left ventricular ejection fraction (LVEF) of 37% before and 60% after treatment, measured by a Magnetic Resonance Imaging (MRI)-scan. The other

patient had a LVEF of 35% before and 45-50% after treatment, measured by an echocardiography. In this patient the improvement in cardiac function was related to a decrease of disease activity confirmed by an evident decrease of myocardial uptake on 18F-FDG-PET as well as in ACE Z-score and sIL-2R levels.
All patients with severe uveitis (n=4) showed improvement of vision as validated by ophthalmologist. Furthermore, the patients treated for neurological complications (n=9) and those with severe fatigue (n=7) showed significant improvement in scores of fatigue severity and quality of life.

ADVERSE EVENTS AND ALLERGIC INFUSION REACTIONS
In general the treatment with infliximab was well tolerated. Only a few documented adverse events were found, the majority of which was mild. Just one patient was hospitalized because of pneumonia. Patients treated with TNF- α inhibitors are at higher risk for developing tuberculosis, but no cases of tuberculosis were found in our cohort.
In all patients treated in our hospital from 2004 till 2010 only one case of a severe infusion reaction was seen during cycle 1-6 which forced us to discontinue treatment.

DISCUSSION

This is the first study showing significant change in disease activity and in health-related quality of life in a group of sarcoidosis patients with chronic disease activity who were refractory to corticosteroid and/or corticosteroid sparing treatment. In addition, a significant improvement in pulmonary function was demonstrated upon infliximab treatment.
Significant decreases in the serum markers ACE Z-score and sIL-2R as well as in SUVmax on ^{18}F-FDG PET in relation to infliximab treatment were observed, which indicate a reduction in disease activity in response to infliximab treatment. So far, no studies have included these parameters as study outcome.
In addition to the objective measurement of disease activity, we also studied subjectively reported health-related quality of life. We observed a significant improvement in both fatigue severity and physical functioning. Additionally, we mention that the overall score change of 12.6 on the subscale physical functioning of the SF-36 by far exceeds the minimum score change (2-4) which is considered as clinically important difference [28]. In this light, we may conclude that infliximab treatment improves the health-related quality of life in refractory sarcoidosis.
In this study we have confirmed that infliximab treatment induces positive changes in pulmonary function. In patients with a pulmonary treatment indication we found significant improvements in percentage of predicted VC (7.6%), improvement in percentage of predicted FEV1 (7.9%) and percentage of predicted DLCOc (3.5%). Our results in improvement of lung function exceed the 2.5% improvement of FVC reported in the study of Baughman et al [16]. Our improvement rates on lung function tests are also slightly higher than the results of Hostettler et al [19] and Rossman et al [17] who both describe a 6% improvement of forced vital capacity (FVC) with infliximab treatment in patients with chronic sarcoidosis with pulmonary involvement. An absolute mean improvement

of 7.6% is considered clinically relevant as this mean value is derived from a subset of patients improving over 10%, which is in clinical practice considered the clinically relevant change for an individual patient. The minimal clinically significant change in VC for a sarcoidosis patient is not well established [29].

Hostettler and colleagues found remission of the extra-pulmonary index lesion in 50% of patients, which was a higher success rate than seen in patients with a pulmonary index lesion. In our cohort, patients with extra-pulmonary sarcoidosis did improve more on quality of life questionnaires than their counterparts with pulmonary sarcoidosis.

A limitation of this study is the retrospective character of the design. Even though we have tried to minimise the loss of follow-up and data by systematic and thorough investigation of patient records, we might have missed events. In addition, the absence of a placebo group makes it impossible to fully attribute the observed change in disease activity and health-related quality of life to the infliximab therapy. However, as only two RCTs have been performed, one of which in only 19 patients, our retrospective study of this large cohort does add valuable extra information to the field and is of interest for medical specialists, especially because of new data on disease-activity, PET-scan results, biomarkers and quality of life scores.

Another limitation of this study is that the infusions with infliximab started simultaneously with methotrexate treatment in a few patients (n=8; 17.8%). When we reanalyzed our data excluding these patients, the increases in percentage of predicted VC and percentage of predicted FEV1 were still significant while the increase in percentage of predicted DLCOc was not. The latter might be attributed to a power problem.

This is the first study showing significantly decreased disease activity and significantly improvement of health-related quality of life after six infusions of infliximab in sarcoidosis patients with chronic disease activity who were refractory to corticoid and/or corticoid sparing treatment. To the best of our knowledge, we are also the first to present data on changes in three different markers reflecting disease activity (the serum markers ACE and sIL-2R, as well as ^{18}F-FDG PET) in relation to infliximab treatment in sarcoidosis. This retrospective analysis demonstrates that infliximab treatment is an effective third-line therapy for patients with refractory, active sarcoidosis.

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CHAPTER 6

ANTINUCLEAR ANTIBODIES DO NOT PREDICT ANTI-INFLIXIMAB ANTIBODY INDUCTION IN SARCOIDOSIS

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ABSTRACT

Studies in other immunological diseases have shown that repeated infusion of infliximab can lead to induction of antinuclear antibodies (ANAs) which could predict decreased response and development of antibodies towards infliximab (ATIs). To date, whether ANA formation during infliximab therapy occurs in sarcoidosis patients has not been studied.

The aim of this study was to investigate the significance of ANA development during infliximab therapy in sarcoidosis patients and to determine the possible relation with formation of ATIs.

We performed a retrospective cohort study including 39 sarcoidosis patients treated with infliximab. ANA titres were measured at baseline and on available samples within 6-24 months after start of infliximab therapy. ATI status was assessed in the same monsters 6-24 months after start of infliximab therapy.

Pre-treatment ANA titre was increased in one patient (ANA titre of 1:320) and one other patient had an ANA titre of 1:40. During treatment only one patient developed ANA reactivity (titre 1:320) and three patients had weak ANA reactivity (titre 1:40). ATI tests became positive during treatment in ten patients (28.6%). Only one of these ATI positive patients had ANA reactivity before and during treatment. A correlation between pre-treatment ANA titres and development of ATIs was not observed in our cohort of sarcoidosis patients (Mann Whitney p=0.49).

In conclusion, our study shows that in sarcoidosis patients the presence of antibodies towards infliximab is not reflected by the presence nor development of antinuclear antibody reactivity. This suggests that antinuclear antibody formation during infliximab treatment might be disease specific.

INTRODUCTION

Sarcoidosis is a multisystem granulomatous disorder of unknown aetiology that can present in many clinical phenotypes, ranging from asymptomatic and self-limiting to severe and life threatening disease [1]. Not all sarcoidosis patients need to be treated, as for example the acute form of sarcoidosis often has a favourable prognosis. In case of severe chronic disease with hazard of functional loss, corticosteroids are initiated as first-line systemic therapy [2,3]. Patients with severe side-effects or a contraindication for steroid use continue onto second-line agents with steroid sparing potency such as methotrexate [4,5]. Infliximab, a monoclonal antibody targeted against Tumor Necrosis Factor (TNF), is only reserved for a small portion of patients refractory to standard first and second-line treatment [6,7]. Infliximab is often used in other Immune Mediated Inflammatory Diseases (IMIDs) such as rheumatoid arthritis, Crohn's disease and Psoriasis [8,9]. In the field of these other IMIDs, a considerable amount of research has been performed on the relation between the formation of antibodies towards infliximab (ATIs) and the suggested decreased response to infliximab [8,10-14].

Recently, it has been found that repeated infusion of infliximab can lead to induction of antinuclear antibodies (ANAs) [15-17], although the exact aetiology of ANA formation during infliximab treatment remains unknown. Furthermore, a relation between ANA formation and decreased response

to infliximab was described as ANA formation could predict the development of ATIs [18-21]. However, other studies did not confirm this relation [22,23]. To date, whether ANA formation during infliximab therapy occurs in sarcoidosis patients has not been studied.

The aim of this study was to investigate the significance of ANA development during infliximab therapy in sarcoidosis patients and to determine the possible relation with formation of ATIs.

METHODS

We conducted a retrospective cohort study including all sarcoidosis patients who started infliximab therapy due to refractory disease between March 2005 and May 2010 and of whom baseline serum samples were available. Patients with various and often multiple organ manifestations (e.g. pulmonary, cutaneous, neurological and cardiac) were included. All patients gave written informed consent.

Infliximab was administered intravenously at a dose of 5 mg/kg in a standardised protocol with the first two infusions two weeks apart and the remaining four infusions once a month. ANA titres were measured at baseline and on available samples within 6-24 months after start of infliximab therapy using HEp-2000 Fluorescent ANA-Ro Test System (Immuno Concepts, USA). All slides were evaluated by two technicians. Positive ANA samples were also tested for anti-dsDNA using Varelisa Recombi ANA Screen and the ImmunoCAP (Thermo Fisher Scientific, Sweden). ATI status was assessed at the same monsters 6-24 months after start of infliximab therapy using ELISA (determined by Sanquin, The Netherlands). These samples were all drawn prior to the next infusion with the lowest possible infliximab level present. Analyses were performed using SPSS Statistics version 19.

RESULTS

We included 39 sarcoidosis patients with a mean age of 48.5 years at start of therapy, of whom 56% was male and 74% was of Caucasian origin. In 62% of patients the main treatment indication was pulmonary sarcoidosis, but the vast majority (82%) also had extrapulmonary involvement. The percentage of concomitant methotrexate or prednisone use was 85 % (Table 1).

Pre-treatment ANA titres were increased in one patient (ANA titres of 1:320) and one other patient had an ANA titre of 1:40. None of the 37 other patients showed any pre-treatment ANA reactivity (titres <1:40).

Blood samples during infliximab treatment were available in 35 patients. Only one patient developed ANA reactivity during treatment (titre 1:320) and three patients had weak ANA reactivity (titre 1:40). This includes the two patients with ANA-positivity pre-treatment.

ATI tests became positive during treatment in ten patients (28.6%). Only one of these ATI positive patients had a high pre-treatment ANA titre of 1:320 and an ANA titre during treatment of 1:40. None of the other ATI-positive patients had increased ANA titres at baseline nor during therapy (Table 2). A correlation between pre-treatment ANA titres and development of ATIs was not observed in our cohort of sarcoidosis patients (Mann Whitney p=0.49).

Table 1 Baseline characteristics of the cohort

	Patients N=39
Mean age at start in years (SD)	48.5 (8.8)
Male	21 (60%)
Caucasian ethnicity	29 (74%)
Pulmonary treatment indication	24 (65%)
Extrapulmonary involvement	32 (82%)
Disease duration in years (SD)	5.8 (5.9)
Previous prednisone use	37 (95%)
Concomitant MTX or prednisone use	33 (85%)

MTX=methotrexate

Table 2 ANA titres of patients with and without ATIs

	ATI positive n= 10	ATI negative n= 25
Pre-treatment ANA-titre + (>1:40)	1 (10%)	1 (4 %)
During treatment ANA-titre + (>1:40)*	1 (10%)	3 (12%)
Anti-dsDNA + (> 15 IU/ml)	0 (0%)	0 (0%)

ANA= antinuclear antibodies, ATI= antibody towards infliximab
* Both patients with positive ANA-pre treatment were within the four ANA positive patients during treatment

All positive ANA samples were negative for anti-dsDNA(< 15 IU/ml, IgG ELISA). Sex was no predictor of ATI formation. ATI formation was more common in patients with non-Caucasian ethnicity (6 out of 10 versus 4 out of 25, p=0.016).

DISCUSSION

In this cohort study of 39 refractory sarcoidosis patients receiving infliximab, we found a high number of ATI-positive patients (29%) and no evidence for a relation with ANA reactivity before or during treatment.

As this is the first study investigating ANA formation during infliximab therapy in sarcoidosis patients we can not compare our results to similar studies. In previous sarcoidosis studies it has been suggested that the presence of autoantibodies in general is common in sarcoidosis (29-37%) [24-26]. Although we did not measure ANA in healthy controls, in our cohort we found a low percentage of baseline ANA reactivity.

In other IMIDs, the development of ANA during infliximab therapy has been studied more extensively. Saraceno and colleagues [22] found ANA formation during infliximab treatment in 48% of psoriasis patients receiving infliximab therapy although they did not find a correlation with response to treatment. These finding are in line with results of Arends and colleagues [23] in 60 infliximab treated Bechterew patients. In contrast, two previous articles on psoriasis patients described that pre-treatment ANA titres and ANA development during treatment did correlate with loss of response to treatment [18,20]. Furthermore, a correlation between ANA formation during treatment and decreased responsiveness was also described in 111 patients with rheumatoid arthritis and in 17 patients with Behçet's disease. An increase in ANA titres during infliximab therapy was not seen in our cohort of sarcoidosis patients. The only high ANA titre measured in our study was 1:320, with a median of 0, which is much lower than observed the articles cited above. In the study by Hoffmann for example [18], a median ANA titre of 1:80 pre-treatment was found, which increased to a median ANA titre of 1:640 after 6 infusions and to 1:2560 after 20 infusions of infliximab in 29 psoriasis patients.

Due to the low number of positive ANA titres after treatment in our cohort (4 out of 35), a correlation with response in sarcoidosis patients could not be confirmed.

The percentage of patients developing ATIs in our cohort was 29%. Yet, a correlation between pre-treatment ANA titres and development of ATIs was not observed in our cohort of sarcoidosis patients (Mann Whitney $p=0.49$). The one patient with high ANA-reactivity before treatment did develop ATIs, however, the other nine patients with ATIs remained ANA negative. The percentage of concomitant methotrexate or prednisone use was equal in both ATI positive and negative groups. It has been suggested that repeated administration of protein agents such as infliximab may cause a systemic immune response, leading to the formation of ANAs and ATIs [27]. From this hypothesis a role for ANA formation as predictor for ATIs could be derived. Our study, however, shows that in sarcoidosis patients the presence of ATIs is not reflected by the presence nor development of ANA reactivity. This suggests that ANA formation during infliximab treatment might be disease dependent. Since sarcoidosis is primarily a T-cell mediated inflammatory disease in which autoantibody formation in general has no major role [28], this might explain why ANA formation is less common and does not correlate with the induction of ATIs.

Although our study only comprised 39 patients, using the results of the study by Hoffmann and colleagues [18] as parameter values in the power calculation, we had 80% power to detect a significant difference ($p<0.05$) between the baseline ANA values of the ATI positive and negative patients. However, we did not find these differences nor any trend supporting the notion that a larger cohort size would result in a significant correlation between ANA and the formation of ATIs.

In conclusion, our study shows that in sarcoidosis patients the presence of antibodies towards infliximab is not reflected by the presence nor development of antinuclear antibody reactivity. This suggests that antinuclear antibody formation during infliximab treatment might be disease specific.

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CHAPTER 7

EFFECTIVENESS OF INFLIXIMAB IN REFRACTORY FDG-PET POSITIVE SARCOIDOSIS

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ABSTRACT

Inconclusive evidence for the efficacy of infliximab in sarcoidosis hinders the global use of this potentially beneficial drug. To study infliximab efficacy in a clinical setting, we performed a prospective open-label trial in patients refractory to conventional treatment.

Patients (n=56) received eight infusions of 5 mg/kg infliximab. Pulmonary function, disease activity measured by 18F-fluorodeoxyglucose by positron emission tomography (^{18}F-FDG PET) and quality of life were part of the clinical work-up. Infliximab levels were measured before every infusion.

After 26 weeks of infliximab, mean improvement in forced vital capacity (FVC) was 6.6% predicted (p=0.0007), whereas six months before start lung function decreased. Maximum Standardised Uptake Value (SUVmax) of pulmonary parenchyma on ^{18}F-FDG PET decreased by 3.93 (p<0.0001). High SUVmax of pulmonary parenchyma at baseline predicted FVC improvement (R=0.62; p=0.0004). In total79% of patients were classified as having an overall excellent or good response and 17% of patients were classified as partial responsers. No correlation between infliximab trough level (mean 18.0 µg/ml) and initial response was found.

In conclusion, infliximab causes significant improvement in FVC in refractory sarcoidosis. Especially in pulmonary disease, high ^{18}F-FDG PET SUVmax values at treatment initiation predict clinically relevant lung function improvement. These results suggest that inclusion of ^{18}F-FDG PET is useful in therapeutic decision-making in complex sarcoidosis.

INTRODUCTION

Sarcoidosis is a systemic disease with a wide variety of symptoms and is histologically characterized by the formation of non-caseating granulomas [1]. Although the disease is often self-limiting, it can also follow a chronic course in a subgroup of patients [2,3]. Self-limiting disease does not necessitate treatment, but severe disease with organ failure or unacceptable loss of quality of life requires therapeutic intervention. When immunosuppressive treatment is indicated, corticosteroids remain first choice therapy [4,5]. Even though corticosteroids are generally effective, continued use is known to have severe side effects such as diabetes mellitus, osteoporosis or obesity [6]. Therefore, second line therapy usually involves agents with steroid-sparing capacity, such as methotrexate or azathioprine [7-10].

Because some patients are refractory to these agents or develop considerable side effects, biologicals targeted against tumor necrosis factor (TNF) have been introduced as a third-line treatment option [11]. The chimeric monoclonal anti-TNF drug infliximab (Remicade; Centocor, Inc., Malverna, PA., USA) has been extensively investigated and is widely used for treatment of immune-mediated inflammatory diseases (IMIDs) such as Crohn's disease, rheumatoid arthritis and psoriasis [12-14], but a knowledge gap remains in the field of sarcoidosis treatment. Current recommendations are mostly derived from extrapolations from other chronic inflammatory diseases or based on experience and eminence-based medicine. In sarcoidosis, infliximab has shown positive results in retrospective series of several manifestations of sarcoidosis [15,16], but the one large randomized controlled trial (RCT) investigating infliximab treatment in sarcoidosis

only revealed a small improvement in pulmonary function and extrapulmonary symptoms after 24 weeks of treatment [17,18]. Critics doubt whether this small improvement of 2.5% in FVC is clinically relevant [19,20]. Because this is the only prospective study on infliximab in sarcoidosis, more evidence is needed to determine efficacy and assess which patients will benefit most. Unfortunately, pharmaceutical companies are hesitant to further invest in the field of orphan diseases, especially when a drug has already been approved for other treatment indications [21]. Furthermore, as positive effects have been described, it is considered unethical to perform another RCT in this category of patients with severe disease and organ failure. In this unfortunate situation, in absence of phase III RCT trials, the use of infliximab in refractory sarcoidosis is still not endorsed by health care insurance companies in many countries and remains off-label globally [22].

A key question remains: how to select the patients who will benefit most from this expensive therapy. Patients with high activity on 18F-fluorodeoxyglucose by positron emission tomography (^{18}F-FDG PET) were often shown to relapse after infliximab therapy was discontinued [23], possibly suggesting a good initial response in those patients.

Besides patient selection, knowledge on interindividual variance in response is derived from the use of infliximab in rheumatic diseases and gastroenterology. In these diseases it is known that formation of antibodies towards infliximab (ATIs) can result in decreased levels of infliximab and diminished drug efficacy. Additionally, ATIs can sometimes cause allergic reactions [12,24-26]. It is not known whether formation of ATIs and associated low trough levels play a role in treatment effect in the case of sarcoidosis.

The aim of this trial was to for the first time study the effect of infliximab in a prospective clinical setting, and to investigate whether sarcoidosis phenotype, inflammatory activity, infliximab trough levels or formation of antibodies towards infliximab are related to the initial response rate after 26 weeks.

MATERIAL AND METHODS

STUDY SUBJECTS

All sarcoidosis patients in whom infliximab therapy was initiated at St. Antonius Hospital Nieuwegein, the Netherlands, between January 2011 and April 2013, were invited to participate in this prospective, open-label cohort study. St. Antonius Hospital Nieuwegein is a national tertiary referral centre for sarcoidosis. Only patients with severe sarcoidosis, unresponsive to first- and second-line treatment, or who have experienced severe side effects from these agents (e.g. worsening diabetes, psychological deterioration or liver function disorders) were eligible for inclusion in the study. The diagnosis of sarcoidosis was made according to ATS/ERS criteria [1]. The treating physician judged disease severity at the moment of initiation based on loss of function (e.g. lung function, cardiac function), impaired quality of life and disease activity on ^{18}F-FDG PET. Exclusion criteria were vaccination with live or bacterial vaccines or with the last dose within the previous three months, active or untreated latent tuberculosis, serious infections in the last two months, serious right ventricular heart failure, active hepatitis, history of allergic reactions to

monoclonal antibodies or their fragments, opportunistic infections within the last six months, HIV, transplantation, known malignancy, pregnancy or breastfeeding.
The institutional review board and the ethics committee approved the study and patients gave written informed consent.

TREATMENT

Patients received infliximab intravenously following a standard protocol starting with 5 mg/kg bodyweight at weeks 0 and 2 and then every 4 weeks during a period of six months. Dosing of prednisone could be tapered according to judgement of the treating physician.

FUNCTIONAL RESPONSE

Organ function was assessed by functional evaluation of the index organ (e.g. for patients with pulmonary sarcoidosis we used forced vital capacity (FVC), forced expiratory volume in one second (FEV1) or diffusing capacity of the lung for carbon monoxide corrected for haemoglobin (DLCOc)). Small fibre neuropathy was tested with the the Small Fibre Neuropathy Screeningslist (SFNSL) and clinical judgement by the treating neurologist [27]. As the minimal important difference for change in FVC has not been elucidated in sarcoidosis, we also reported mean change in percent predicted and percentage of patients with an increase of at least 5 and 10% [28].

INFLAMMATORY RESPONSE

Parameters in the inflammatory activity dimension included biomarkers soluble interleukin-2 receptor (sIL-2R), angiotensin converting enzyme (ACE) and ^{18}F-FDG PET maximum standardised uptake value (SUVmax) of the pulmonary parenchyma and, if applicable, other index localization of sarcoidosis. ^{18}F-FDG PET Imaging was performed using a Philips Gemini TF-64 combined PET/CT device (Philips, Medical systems, Eindhoven, the Netherlands). The SUVmax was calculated in the mediastinal/hilar region, in the lung parenchyma and in the target organ when appropriate. Regions of interest (ROI) were drawn over the visually affected part of the organ to measure the SUVmax. ROI at baseline and follow-up scan was drawn at the same lesion/area. ROI drawing was performed using the automatic ROI drawing program provided by Hermes Diagnostics (Hermes Medical Solutions, Stockholm, Sweden). Blood glucose was measured before injecting FDG in all patients.

QUALITY OF LIFE

Finally, quality of life was measured with the help of two questionnaires: a patient Global Assessment (PGA) score, ranging from 0 (best imaginable health status) to 100 (worst imaginable health status) on a visual analogue scale and Physical functioning on Short Form-36 (SF-36). An improvement of 10 points was considered clinically relevant.

COMPOSITE OVERALL RESPONSE

Additionally we classified response rate as a composite of three dimensions: organ function, inflammatory activity and quality of life. Improvement in a dimension was scored only when one of the parameters improved significantly without deterioration of the others. For the functional response we used an improvement of at least 5% in FVC. A decrease in biomarkers or SUVmax greater than 40% of baseline was considered a relevant improvement. Changes within the normal range of sIL-2R, ACE and SUVmax on FDG PET were not taken into account when gauging response. Change in SUVmax of target organ was regarded as superior to change in inflammatory biomarkers. Clinically relevant improvement on two or three dimensions was classified as good or excellent response, on one dimension as partial response and on none of the dimensions as nonresponse. All parameters were also evaluated individually.

INFLIXIMAB TROUGH LEVELS AND ANTIBODIES TOWARDS INFLIXIMAB

Infliximab trough levels were measured using an enzyme-linked immunosorbent assay (ELISA) developed by Sanquin, the Netherlands [29]. This ELISA only detects infliximab that is able to bind TNF. It does not bind immune complexes consisting of infliximab and TNF or infliximab bound to neutralizing ATIs. The presence of ATIs was determined using radioimmunoassay [30].

ANALYSIS

Changes of values before and after six months of treatment were compared with two-tailed paired t-tests. Pearson's correlation coefficients (R) between parameters were calculated with linear regression. Statistical analysis was performed using SPSS for Windows, version 22.0. Graphs were created using Graph pad Prism 5.0. A P-value of less than 0.05 was considered significant.

RESULTS

STUDY SUBJECTS

Between January 2011 and April 2013, infliximab was initiated in a total of 58 active refractory sarcoidosis patients, two of whom were not included in this study (Fig 1). A total of 56 patients were included in the study, 64.3% of whom were male and 87.5% Caucasian (Table 1). The most common treatment indication was pulmonary sarcoidosis (60.7%), other common indications were cutaneous sarcoidosis and small fibre neuropathy. The vast majority of patients suffered from chronic sarcoidosis with a mean disease duration at initiation of 6.8 years and the use of at least two immunosuppressant drugs prior to infliximab initiation in 92.9% of patients. Furthermore, patients had signs of high disease activity with a mean SUVmax of 6.6 on ^{18}F-FDG PET in the pulmonary parenchyma, a high sIL-2R of 8824 pg/mL (normal value <3000 pg/mL) and a high ACE of 89.7 U/L (normal value < 68 U/L) at start of therapy. Baseline SUVmax on ^{18}F-FDG PET did not correlate with Scadding stage 0-III vs Scadding stage IV (6.3 vs 6.8, p=0.76).

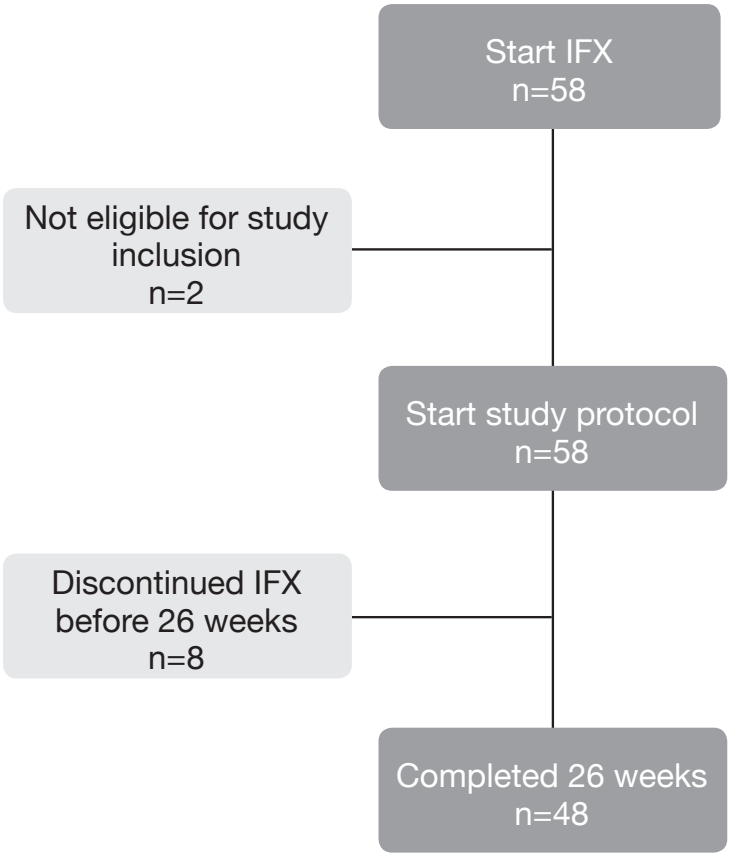


Figure 1: Flowchart of patient inclusion
Two patients were excluded from the study protocol, one due to incapability to give informed consent based on mental retardation and one because his cold agglutinin disease would not permit routine sampling at every visit.

Table 1 Baseline characteristics n=56

Gender, n (%)	
Male	36 (64.3%)
Ethnicity, n (%)	
Caucasian	49 (87.5%)
Age at initiation of infliximab therapy, y	48.7 (±10.1)
Disease duration at initiation of infliximab therapy, y	6.8 (±7.1)

Diagnosis of sarcoidosis	
Biopsy	52 (92.9%)
BAL	3 (5.4%)
Clinical	1 (1.8%)
Smoking status, (%)	
Never smokers	27 (48.2%)
Current smokers	4 (7.1%)
Former smokers	25 (44.6%)
Scadding stage, n (%)	
Stage 0	5 (8.9%)
Stage I	6 (10.7%)
Stage II	16 (28.6%)
Stage III	14 (25.0%)
Stage IV	15 (26.8%)
Main treatment indication, n (%)	
Pulmonary	34 (60.7%)
Cardiac	2 (3.6%)
Small fibre neuropathy	8 (14.3%)
Cutaneous	4 (7.2%)
Central nervous system	3 (5.4%)
Sinus	1 (1.8%)
Myositis	1 (1.8%)
Vocal cord paralysis	1 (1.8%)
Ossal	1 (1.8%)
Hypercalcemia	1 (1.8%)
Tertiary referral, n (%)	52 (92.9%)
Medication use prior to initiation of infliximab, n (%)	
Corticosteroids	54 (96.4%)
Methotrexate	51 (91.1%)
Azathioprine	6 (10.7%)
Leflunomide	1 (1.8%)
Plaquenil	8 (14.3%)
Anti-TNF-treatment	13 (23.2%)
None	0 (0%)
Use of ≥ 2 different drugs prior to infliximab, n (%)	52 (92.2%)
Medication use prior to initiation of infliximab, n (%)	
Corticosteroids	54 (96.4%)
Methotrexate	51 (91.1%)
Azathioprine	6 (10.7%)
Leflunomide	1 (1.8%)
Plaquenil	8 (14.3%)
Anti-TNF-treatment	13 (23.2%)
None	0 (0%)
Use of ≥ 2 different drugs prior to infliximab, n (%)	52 (92.2%)
Concomitant medication, n (%)	
Corticosteroids	24 (42.9%)
Methotrexate	46 (82.1%)
Azathioprine	4 (7.1%)
Leflunomide	1 (1.8%)
None	0 (0%)

Pulmonary function parameters	
Forced vital capacity, L (% predicted)	3.32 (78.8%)
Forced expiratory volume in one second, L (% predicted)	2.30 (66.8%)
Diffusing capacity for carbon monoxide corrected for haemoglobin, L (% predicted)	6.01 (59.8%)
6-minute walking distance, m (% predicted)	460.4 (62.2%)
Disease activity and severity measurements	
SUVmax lung parenchyma	6.6 (±5.3)
SUVmax mediastinum	5.7 (±3.2)
SUVmax total (including index localization)	9.0 (±5.2)
Angiotensin converting enzyme (U/L)	89.73 (±49.7)
Angiotensin converting enzyme Z-score	4.26 (±4.8)
Soluble interleukin 2-receptor (pg/mL)	8824 (±8503)

ORGAN FUNCTION

Of the total study population, 52 patients (93%) had decreased PFT (one or more PFT parameters <80%). In patients with a pulmonary treatment indication, FVC increased with 6.64% predicted (p=0.0007), FEV1 increased with 5.80% predicted (p<0.0001) and DLCOc increased with 4.12% predicted (p=0.001) after 6 months of infliximab treatment. An improvement of at least 5% predicted FVC and FEV1 was seen in 71% and 64% of patients, respectively. In 46% of patients this increase even exceeded 10% predicted (Fig 2A).

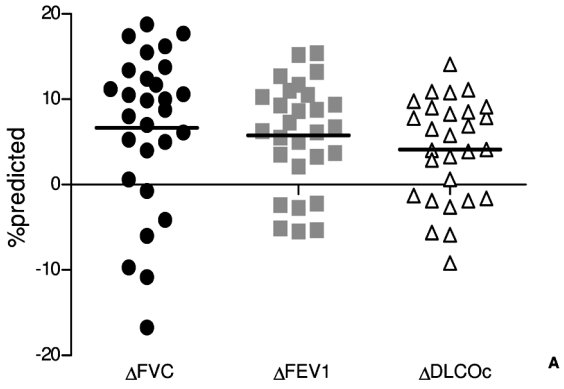
Notably, even in patients with an extrapulmonary treatment indication mean FVC and FEV1 increased significantly with 3.88 (p=0.027) and 3.54% predicted (p=0.034), respectively. Moreover, an increase

Table 2 Mean baseline disease parameters and change after 26 weeks of infliximab treatment in patients with pulmonary treatment indication n=28

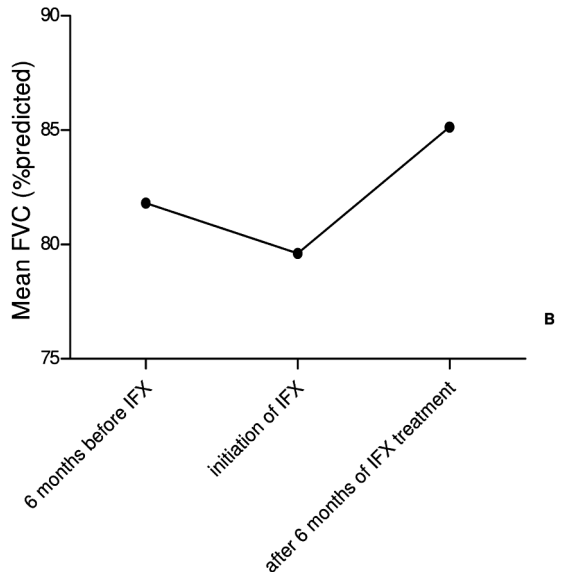
	Baseline	Change after infliximab treatment
Pulmonary function parameters		
Forced vital capacity, % predicted	73.6%	+6.6%
Forced expiratory volume in one second, % predicted	55.8%	+5.8%
Diffusing capacity for carbon monoxide corrected for haemoglobin, % predicted	56.6%	+4.1%
6-minute-walk distance, % predicted	61.0%	+4.2%
Disease activity and severity measurements		
SUVmax lung parenchyma	9.0 (±5.0)	-5.3 (5.6)
SUVmax mediastinum	5.9 (±3.3)	-2.7 (3.8)
SUVmax index localization	9.8 (±5.3)	-5.5 (5.6)
Angiotensin converting enzyme (U/L)	86.2 (±46.3)	-21.8 (43.3)
Angiotensin converting enzyme Z-score	3.7 (±3.9)	-1.78 (3.33)
Soluble interleukin 2-receptor (pg/mL)	7631 (±4259)	-3955 (3883)

Values are mean ± SD unless noted otherwise

Figure 2: Pulmonary response in sarcoidosis



A Change in pulmonary function after 26 weeks of infliximab therapy in patients with a pulmonary treatment indication (change in percent predicted)



B Mean forced vital capacity six months before initiation and during 26 weeks of infliximab therapy in the total cohort (percent predicted). Bars represent Standard error of the mean.
IFX: infliximab; FVC: forced vital capacity; FEV1: forced expiratory volume in one second; DLCOc: Diffusion capacity of the lung for carbon monoxide corrected for haemoglobin.

in FVC and FEV1 of at least 5% predicted was seen in 37% of these patients for both parameters. Prior to initiation of infliximab, stable or deteriorating pulmonary function was seen in the total cohort (Fig 2B). Using repeated measurement ANOVA, FVC after 26 weeks of treatment was found to be significantly higher than at initiation of treatment and than at 6 months before treatment ($p<0.0001$ and $p=0.007$, respectively). Baseline pulmonary function tests did not predict outcome (data not shown).

All four patients with cutaneous sarcoidosis had marked improvement or total resolution of skin lesions confirmed by photograph and clinical comparison. Patients with small fibre neuropathy had subjective improvement of symptoms.

INFLAMMATORY ACTIVITY

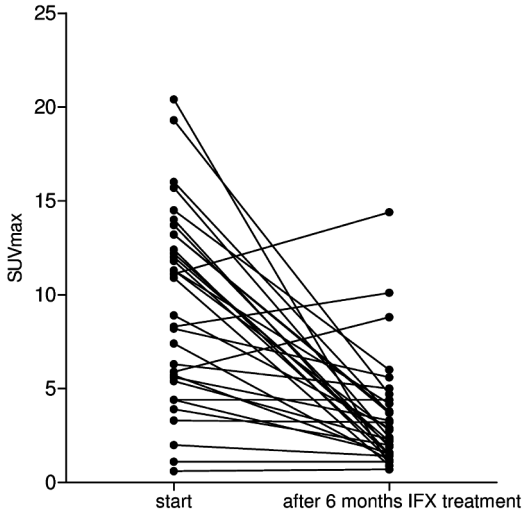
The number of patients in whom serum sIL-2R exceeded the upper limit of normal (3000 pg/ml) was 47 (84%). Due to use of ACE-inhibitors, ACE levels were only available in 49 patients, 30 (61%) of whom had levels exceeding 70 U/l. When measuring disease activity by means of ^{18}F-FDG PET scan before and after 26 weeks of treatment we found a mean decrease in SUVmax of mediastinum and lung parenchyma of 2.97 ($p<0.0001$) and 3.93 ($p<0.0001$), respectively. Moreover, the SUVmax of lungs and index localization (e.g. heart) decreased significantly by 5.76 ($p<0.0001$) (Fig 3).

Both serum markers ACE and sIL-2R decreased significantly by 28.2 U/L ($p=0.0003$) and 4269.4 pg/mL ($p<0.0001$), respectively. Interestingly, ACE was higher in patients with an extrapulmonary treatment indication (97.8 and 86.2 U/ml, respectively).

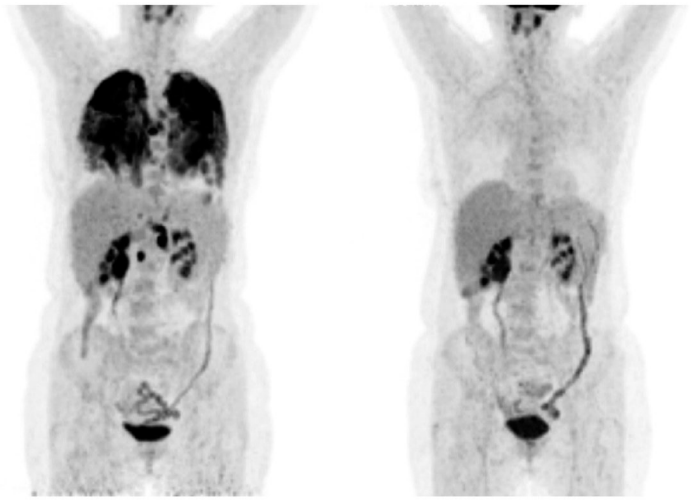
Furthermore, we found significant correlations between the change in pulmonary function and level of disease activity, indicating that pulmonary function improved for the majority of patients with the highest disease activity. In patients with a pulmonary treatment indication, improvement in FVC following treatment correlated best with SUVmax of the pulmonary parenchyma before treatment initiation, having a correlation coefficient (R) of 0.62 ($p=0.0004$) (Fig 4). Linear regression analysis, including parameters SUVmax of the parenchyma and FVC at treatment initiation, predicts that FVC will improve by 1.1% per unit SUVmax of the parenchyma at start of therapy. Consequently, a SUVmax of 10 in the parenchyma at initiation predicts an FVC increase of 11% predicted. Baseline sIL-2R correlated with improvement in DLCOc (R 0.50, $p=0.007$), while ACE at baseline correlated with improvement in FEV1 in patients with an extrapulmonary treatment indication in particular (R 0.51 ($p=0.04$)). When comparing patients with deteriotation of any lung function parameter (Fig 2A) to the other patients, SUVmax of the pulmonary parenchyma and sIL-2R at baseline were found to be significantly different. No significant correlation between ACE, PFTs, age, sex, Scadding stage, ethnicity or disease duration was found.

Prednisone was used concurrently in 19 patients at start of infliximab therapy. The mean daily dose decreased by 8.8 mg after 26 weeks of therapy ($p=0.001$). In none of the patients the dose of concomitant immunosuppressive drugs was increased.

Figure 3: ^{18}F-FDG-PET activity during infliximab treatment



A Maximum Standardised Uptake Value (SUVmax) on ^{18}F-FDG PET of the target organ at initiation and after 26 weeks of infliximab treatment.



B Example of ^{18}F-FDG PET in a patient with pulmonary sarcoidosis before (left) and after 26 weeks of infliximab treatment (right).

QUALITY OF LIFE

Mean PGA score on a visual analogue scale at treatment initiation was 61.0 out of 100 (being worst imaginary health status) and showed clinically significant decrease of -14.6 after 26 weeks of treatment ($p<0.0001$). The mean Physical functioning score on the SF-36 was 40.6 out of 100% and increased by 8.2 ($p=0.009$).

COMPOSITE OVERALL RESPONSE RATE

When evaluating response after 26 weeks of treatment as a composite of three dimensions (organ function, inflammatory activity and quality of life) we found a high response rate (fig 5). Classification of excellent or good response was seen in 79% of patients, indicating clinically relevant improvement in at least two out of three dimensions. Seventeen percent classified as partial responders indicating improvement in one dimension. Four percent of patients did not respond. When dividing response into the three dimensions, we found that 69% responded on the functional dimension, 79% on the inflammatory dimension and 67% on the quality of life dimension. Patient characteristics such as age, race, gender and disease duration did not predict overall response.

INFLIXIMAB TROUGH LEVELS AND ATIS

Infliximab trough levels showed high interindividual variation, but intraindividual variation was low throughout the 26 weeks o f treatment. Trough levels were high: the mean trough level was 18.0 µg/ml. No significant correlation between trough level and response was found. Patients with excellent or good response had a mean trough level of 18.5 µg/ml, partial responders a mean trough level of 17.4 µg/ml and nonresponders a mean trough level of 27.5 µg/ml.

Three patients showed continuously undetectable trough levels of infliximab, even though the interval between infusions was not prolonged. Two of these patients had an allergic reaction within 26 weeks of treatment. The other patient developed an allergic reaction after one year of treatment. Corresponding with low levels of infliximab, high levels of ATIs were present in all three patients. All of these patients received concomitant immunosuppressive therapy during infliximab treatment: one patient received prednisone 20 mg/day, one patient methotrexate 7.5 mg/week and one patient was on prednisone 10 mg/day and methotrexate 7.5mg/week.

SIDE EFFECTS AND DISCONTINUATION OF THERAPY

Severe side effects were pneumonia, requiring hospitalization and discontinuation of therapy in three patients, one of whom was hospitalized in ICU after two infusions and one other who was hospitalized after three infusions and eventually passed away of respiratory failure. One patient was hospitalized with severe progressive disease after three infusions. Therapy was then discontinued and the patient passed away several months later at home of respiratory failure. Another patient, known to have peritoneal dialysis, initially responded well, but developed peritonitis, requiring discontinuation of treatment. In another patient, therapy was discontinued due to severe

gastrointestinal complaints. Allergic reactions along with antibody formation occurred in two patients within 26 weeks of treatment, one of whom discontinued infliximab treatment within 26 weeks. Both patients eventually successfully switched to adalimumab.
One patient did not want to continue infliximab therapy for undisclosed reasons.
Five patients had mild infections of the upper or lower respiratory system that did not require hospitalization. Other side effects were mild; such as headache in two patients, dizziness in one patient, oedema in three patients and joint pain in two patients. The majority of patients had no side effects (n=34).

DISCUSSION

In this prospective open-label trial of infliximab in active sarcoidosis patients refractory to conventional treatment, and including ^{18}F-FDG PET in the clinical work-up, we found a very high overall response rate and a mean improvement of 6.6% predicted in FVC.
The only large RCT performed in this field only showed a small improvement of 2.5% predicted in FVC, and no treatment benefit on other major secondary clinical endpoints [17]. Importantly, only patients with stable disease were eligible for participation in the RCT. In contrast, the high response rate in our study might be attributable to the high disease activity measured by ^{18}F-FDG PET in this cohort. This could be explained by the fact that infliximab, being an anti-TNF drug, likely finds more 'anti-TNF target' in sarcoidosis patients with higher inflammatory activity compared to those with lower or no sign of inflammatory activity on ^{18}F-FDG PET.
Besides high activity on ^{18}F-FDG PET, patients included in our study also had high serum levels of disease activity markers ACE and sIL-2R. At initiation of infliximab, mean levels of ACE and sIL-2R were 89.7 U/l and 8824 pg/ml (with upper limit of normal for reference values 68 U/l and 3000 pg/ml, respectively). These values are clearly higher than ACE at initiation of the large RCT of 47.4 U/l [18], or described in a recent retrospective cohort of 20.7 U/l (upper limit of normal 25 U/l in this study) [31]. In the latter study, mean sIL-2R at start of therapy was 3073 pg/ml. The serum biomarkers ACE and sIL-2R were also found to decrease dramatically after 26 weeks of treatment and correlated with improvement in pulmonary function. Although the findings in our study might suggest that they could serve as less expensive surrogates for ^{18}F-FDG PET, the value of these markers in sarcoidosis is still under debate [32]. Interpretation based on one measurement is more difficult due to high interpatient variability. Moreover, ACE and sIL-2R are less sensitive for detecting activity and most importantly reflect systemic activity whereas ^{18}F-FDG PET can reveal specific organ involvement such as cardiac sarcoidosis [33,34]. In our view, ACE and sIL-2R are especially valuable for follow-up in individual patients.
Another explanation for the high response rate found in our study could be that levels of infliximab found using the current dosing regimen were much higher than those reported in other IMIDs [35-37]. Consequently, it will be of interest to study whether a lower dose would achieve the same results in patients with active disease. The only RCT on infliximab in sarcoidosis did not show a difference between the groups treated with 3 and 5 mg/kg [17], however, overall improvement

was much lower in this RCT than in our study. A different dosing regimen could possibly further minimize toxicity and could, moreover, serve to reduce the unfortunately considerable costs of treatment with biologicals such as infliximab. We only found low trough levels in three patients during the first 26 weeks. It is possible that low trough levels due to presence of ATIs occur more frequently after long-term treatment, or that concurrent use of methotrexate prevented ATI formation.

In our clinic it is standard procedure to screen patients awaiting infliximab therapy for disease activity by measuring serum activity markers ACE and sIL-2R and performing ^{18}F-FDG PET. Though ^{18}F-FDG PET may be considered an expensive diagnostic tool, the value of being able to identify these patients with severe active sarcoidosis outweighs the costs, as treatment with the biological drug infliximab is over ten times more expensive than performing ^{18}F-FDG PET. The use of ^{18}F-FDG PET is a valuable tool in identifying those patients for whom treatment with infliximab is expected to have beneficial effect.

A limitation of this study is the absence of a control group. Because of the large number of infliximab-treated patients described in case reports and case series, anti-TNF agents are incorporated into reviews guiding treatment of sarcoidosis [6,19,28,38]. Therefore, it is regarded unethical to perform an RCT whereby infliximab treatment is withheld from patients who are appointed to the placebo arm. To compare treatment results with conventional therapy, data from the time period prior to initiation of infliximab treatment have been used in data analysis as the best alternative to a placebo-controlled trial in evaluation of treatment for rare diseases [39].

Previous studies have focused mainly on response on functional parameters rather than parameters regarding inflammatory activity or quality of life [17,31]. Measurement of the inflammatory activity might be indicative of future organ damage, as suggested by the predictive value of FDG-PET and sIL-2R regarding therapeutic response. Quality of life is an increasingly important, but under-reported dimension in sarcoidosis [40]. The combination of function, inflammation and quality of life (on a visual analogue schale) resembles the three dimensions also used in rheumatology in the DAS28-score [41,42]. However, a limitation of our composite overall response score, is that deterioration in one dimension, when another dimension is improving, is not taken into account. Therefore, this composite overall response should be interpreted with care. Furthermore, it has not yet been validated and future studies should reveal its value in clinical research.

Another possible limitation of the study is the high activity on FDG-PET and biomarkers in most patients in the study. Hereby we were able to show better results compared to the large RCT (Baughman), however the observed correlation between FDG-PET and pulmonary improvement could hypothetically be even stronger when more patients without disease activity would have been included.

The pharmaceutical industry has shown no interest in obtaining registration of approval by Food and Drug Administration and European Medicines Agency for infliximab in sarcoidosis. This leaves physicians unable to prescribe the drug, unless this is done off-label based on evidence for efficacy mainly from observational data with as additional consequence low pharmacovigilance

[21]. Furthermore, global financial endorsement by health insurance companies is unlikely to be granted without substantial evidence of effect. Our selection criteria and findings may convince health insurance companies to endorse infliximab therapy as treatment option for sarcoidosis. As trials investigating newer biologicals in sarcoidosis were unsuccessful [43], infliximab remains the best option for the group with severe refractory sarcoidosis.

In conclusion, infliximab therapy is very effective in selected patients with refractory disease and evidence of persistent disease activity. Patient selection for this indication should therefore ideally be based on both disease severity and inflammatory activity on ^{18}F-FDG PET. In addition we have found that with current fixed dosing regimen, levels of infliximab are high, suggesting room for dose and associated cost reduction, e.g. by an infliximab level based flexible dosing regimen.

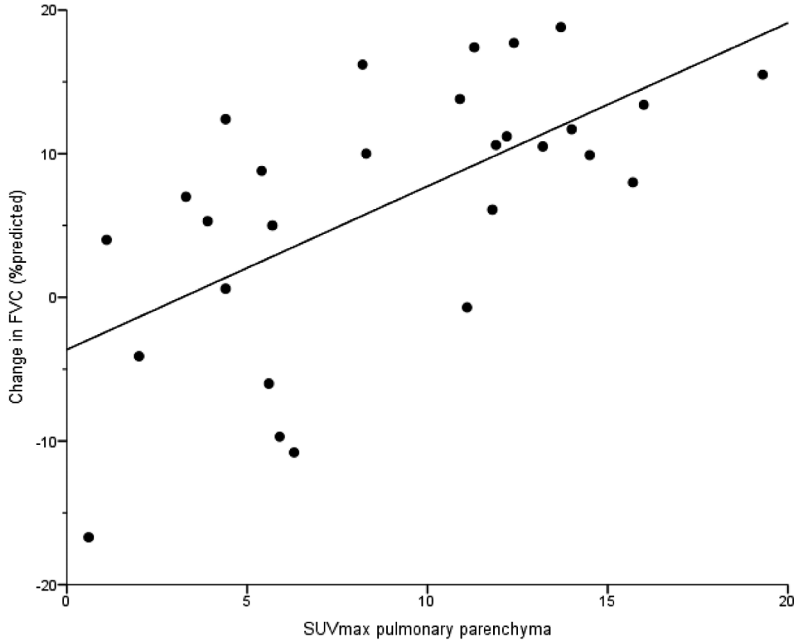


Figure 4: ^{18}F-FDG PET activity and pulmonary function improvement
Correlation between high activity of pulmonary parenchyma on ^{18}F-fluorodeoxyglucose by positron emission tomography (^{18}F-FDG PET) (SUVmax) at baseline and improvement in forced vital capacity (FVC) in patients with a pulmonary treatment indication (R=0.62 (p=0.0004)).

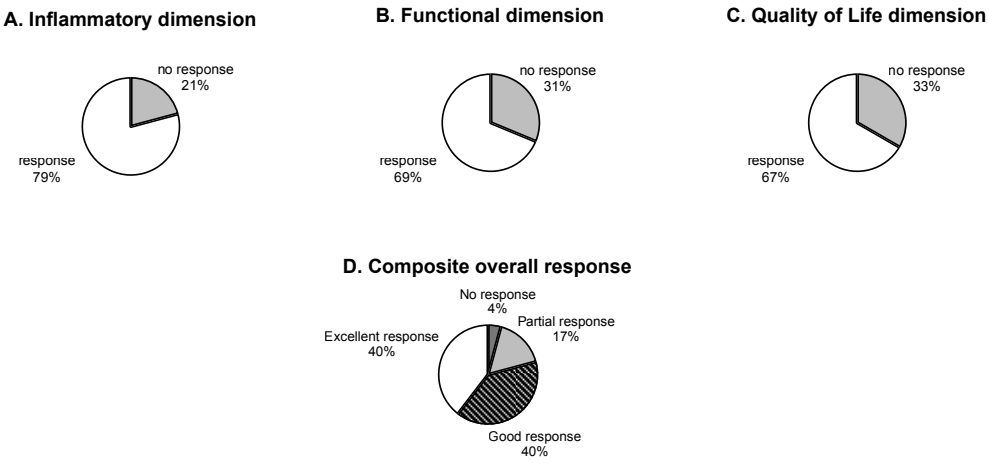


Figure 5: Response after 26 weeks of infliximab therapy.
Response was measured on three dimensions: organ function, disease activity and quality of life. Excellent responders showed marked improvement in all three categories, good responders in two categories and partial responders in one category. Nonresponders showed no marked improvement on any of the three dimensions.

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PART III

RELAPSE IN SARCOIDOSIS

CHAPTER 8

PREDICTION OF RELAPSE AFTER DISCONTINUATION OF INFLIXIMAB THERAPY IN SEVERE SARCOIDOSIS

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ABSTRACT

Infliximab is effective as third-line therapeutic for severe sarcoidosis, however, long-term efficacy is unknown. The aim of this study was to assess the relapse rate after discontinuation of infliximab in sarcoidosis patients and predict relapse by analysis of activity markers soluble interleukin-2 receptor (sIL-2R) and maximum standardised uptake value (SUVmax) of ^{18}F-fluorodeoxyglucose positron emission tomography (^{18}F-FDG PET).

In this retrospective cohort study, the proportion of relapse was analysed using Kaplan-Meier and predicting factors were studied with Cox regression.

47 sarcoidosis patients who started infliximab therapy were included in risk analysis. Kaplan-Meier analysis revealed a median time to relapse of 11.1 months and showed that 25% of the cohort relapsed within 4 months. Both mediastinal SUVmax ≥ 6.0 on ^{18}F-FDG PET (HR 3.77, P<.001) and serum sIL-2R ≥ 4,000 pg/mL (HR 2.24, P =.033) at start of therapy predicted relapse. In multivariate analysis, a mediastinal SUVmax ≥ 6.0 at initiation was an independent predictor of relapse (HR 4.33, P<.001).

In conclusion, the majority of patients that discontinued infliximab therapy relapsed. High serum sIL-2R and high SUVmax on ^{18}F-FDG PET at initiation of therapy were significant predictors of relapse. These results suggest close monitoring of patients in this category when they discontinue infliximab treatment.

INTRODUCTION

Sarcoidosis is a systemic, granulomatous disease that has variable clinical course and can affect multiple organs. This disease can be self-limiting, but can also follow a chronic course in a subgroup of patients [1-3]. When immunosuppressive treatment is indicated, corticosteroids are the first choice drugs [4;5]. Even though corticosteroids are generally effective, continued use may cause severe side effects. Second line therapy therefore usually involves agents with steroid-sparing effects such as azathioprine or methotrexate [6-11]. However, some patients are resistant to these types of treatment or develop considerable side effects. Therefore, biologicals have been introduced as new treatment options.

The biological anti-TNF drug infliximab (Remicade; Centocor, Inc., Malverna, PA., USA) has been used widely for treatment of inflammatory diseases such as rheumatoid arthritis, Crohn's disease and psoriasis [12-14]. Furthermore it has been subject of investigation in case series and reports of several manifestations of sarcoidosis. Two randomized controlled trials (RCTs) have investigated short term infliximab treatment in sarcoidosis and revealed improvement in lung function after 14 weeks of infliximab treatment [15;16]. Post-hoc analysis showed positive effects of infliximab treatment on extra-pulmonary symptoms [17].

Due to the fact that these studies have focused on treatment outcomes after an induction phase of a maximum of six months, long-term outcome after discontinuation of treatment remains largely unknown. Because the optimal treatment duration or best moment to discontinue infliximab treatment have not been studied, in clinical practice, treatment duration is based on the physicians'

opinion. However, like most biologicals, infliximab is an expensive drug, which makes excessive or redundant use not desirable in times of rising health care costs. On the other hand, re-initiation after discontinuation might contribute to anti-infliximab antibody formation resulting in decreased drug efficacy and sometimes allergic reactions [18].

The fact that relapse of symptoms can occur after discontinuation of infliximab in sarcoidosis, was shown in a small series of 14 patients [19]. Prediction of relapse is important to identify patients with a low risk of relapse and possibly shorten therapy duration because this will reduce the burden of therapy for the patient (long-term safety and efficacy) and lower health care costs. Moreover, identifying patients with a high relapse risk after discontinuation would be useful to adjust or even prolong treatment with infliximab in case of objectified response.

In Crohn's disease, leukocyte count and C-reactive protein (CRP) levels were found to be predictors of relapse after discontinuation of infliximab therapy [20]. As leukocyte count and CRP are generally within normal limits in sarcoidosis patients, other markers of disease activity in sarcoidosis were also studied: angiotensin converting enzyme (ACE), soluble interleukin-2 receptor (sIL-2R) and uptake of 18F-fluorodeoxyglucose by positron emission tomography (^{18}F-FDG PET) [21-28].

The aim of this study was to investigate the long-term outcome of infliximab treatment in patients with severe sarcoidosis and to predict which patients will relapse after discontinuation of infliximab therapy.

MATERIAL AND METHODS

STUDY SUBJECTS

Sarcoidosis patients in whom infliximab therapy was initiated at St. Antonius Hospital Nieuwegein, the Netherlands, between August 2004 and October 2010 were included in this retrospective study. St Antonius Hospital Nieuwegein serves as a national tertiary referral centre for sarcoidosis. All patients were naïve to infliximab or other anti-TNF therapy. All patients had severe and chronic sarcoidosis. Disease severity was assessed by the treating physician at moment of initiation based on clinically significant loss of function (e.g. lung function, cardiac function) and severe impairment of quality of life. To be eligible for infliximab therapy, patients needed to be unresponsive to first- and second-line treatment, or experience severe side effects from these agents or have contraindications (e.g. worsening diabetes, psychological deterioration or liver function disorders). Patients received infliximab intravenously following a standard protocol starting with 5 mg/kg bodyweight at weeks 0 and 2 and then every 4 weeks during a period of six months. Duration of infliximab therapy after the induction phase of six months was based on response of symptoms and disease activity by the treating physician. Patients were considered clinically stable at time of treatment cessation. Relapse was defined as the necessity for retreatment due to worsening of symptoms and function in combination with renewed signs of disease activity. Relapse could be of different extent and severity between different patients or organ systems involved. The moment of initiation of retreatment in previously stable patients was defined as the time point of relapse.

STUDY PARAMETERS

Medical records were reviewed retrospectively for relevant demographic data, disease and treatment characteristics and disease activity parameters (ACE, sIL-2R, CRP and leukocyte count). In the same manner, ^{18}F-FDG PET results by maximum Standardised Uptake Value (SUVmax) and details of pulmonary function tests were collected. These parameters were recorded at several time points during the treatment period: before infliximab initiation, after six months of treatment and when treatment was discontinued in case of prolonged treatment. For relapsing patients, the clinical and laboratory parameters were again collected, in addition to the type of retreatment and outcome.

Patients were excluded from relapse risk analysis if they had not completed the induction phase of six months, because a possible deterioration of symptoms might be due to under-treatment of these patients in stead of relapse. All clinical and laboratory tests were performed as part of a standardised protocol and part of clinical practice. The study was approved by the investigational review board of our hospital.

ANALYSIS

The proportion of patients who relapsed after discontinuation of therapy was analyzed using Kaplan-Meier.

Factors associated with time to relapse were studied using a Cox proportional hazards model. Initially, a univariate analysis was performed, selecting variables with a P-value of less than 0.20 for multivariate analysis. Appropriate cut-off points were determined using scatter plots.

Statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) for Windows, version 19.0. A P-value of less than 0.05 was considered significant.

RESULTS

STUDY SUBJECTS

Between August 2004 and October 2010, 56 patients started with infliximab therapy at St. Antonius Hospital, Nieuwegein, the Netherlands. Four pa¬tients did not complete the induction phase of six months, three of which developed an aller¬gic reaction and one passed away due to co-morbidity. Three patients were still on anti-TNF-alpha treatment at the moment of inclusion and two patients were lost to follow-up. A total of 47 patients were therefore included in risk analysis (Figure 1).

Demographics, clinical characteristics and disease activity parameters at baseline are summarised in Table 1. The most common reason for initiating treatment was a pulmonary treatment indication (30 patients; 64%), however, of the total cohort, 41 patients (87%) suffered from extrapulmonary manifestations of sarcoidosis. Most patients (46 out of 47) were treated with prednisone and/or methotrexate before infliximab was initiated. In the 4 patients who did not use prednisone, the most common contra-indications were obesity and diabetes. The majority of patients received additional immunosuppressive or immunomodulatory agents whilst being treated with infliximab to prevent formation of anti-infliximab antibodies; only three patients were not on any

type of concomitant medication due to intolerance. The mean treatment duration was 8.5 (SD ± 5.8 months. After treatment discontinuation, the mean overall follow-up time was 36.6 months (SD ± 22.6).

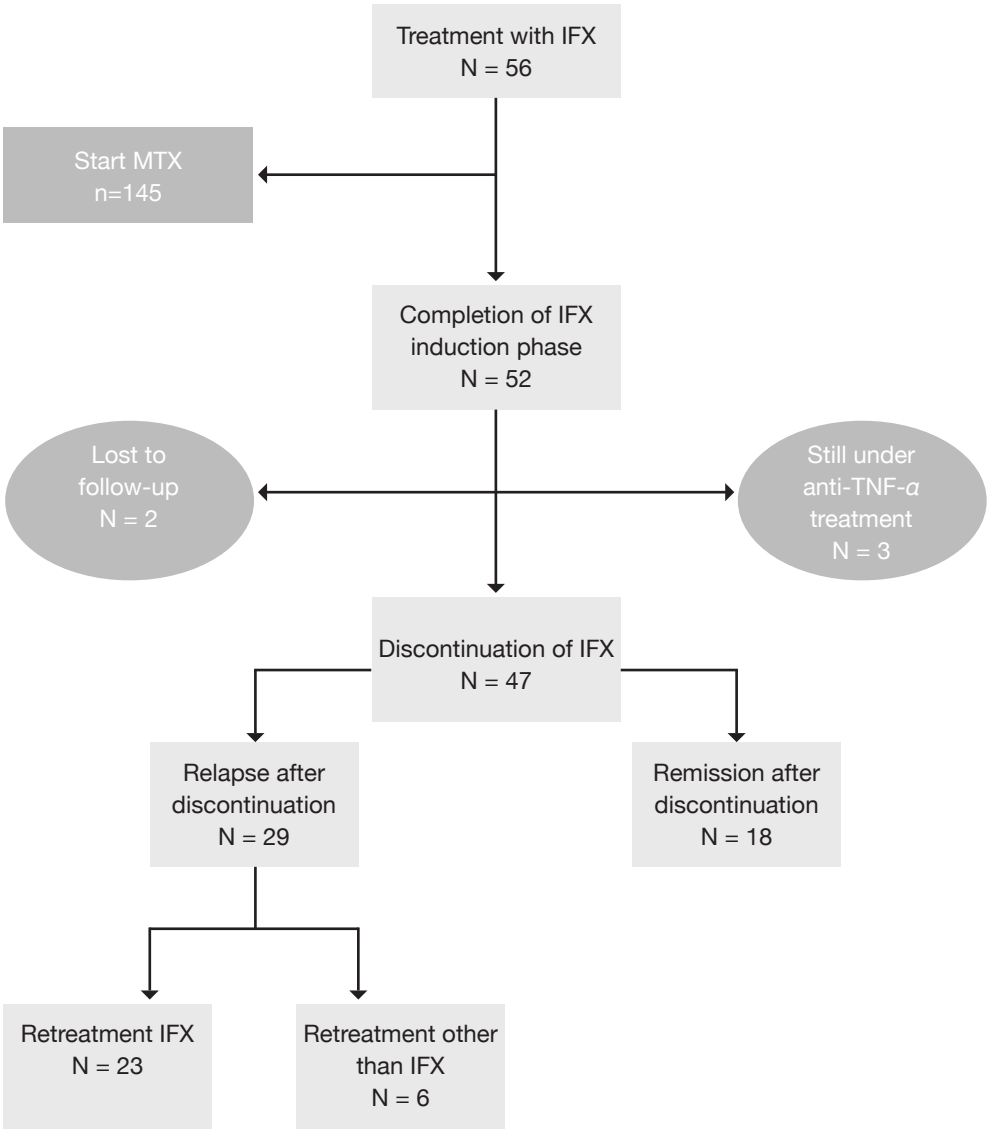

Figure 1. Flow diagram of all patients included in the study.
Grey boxes indicate exclusion from risk analysis; the induction phase was set at six months. IFX = infliximab; TNF = tumour necrosis factor.

Table 1 Baseline characteristics of the included patient population at initiation of infliximab therapy

Gender, n (%)	
Male	26 (55)
Ethnicity, n (%)	
Caucasian	36 (77)
Age at initiation of infliximab therapy, mean (range) y	48 (28-71)
Disease duration at initiation of infliximab therapy, y	6.3 ± 7.8
Smoking status, (n=43) (%)	
Never smokers	25 (58)
Current smokers	7 (15)
Former smokers	11 (26)
Scadding stage, n (%)	47
Stage 0	3 (6)
Stage I	5 (11)
Stage II	16 (34)
Stage III	10 (21)
Stage IV	13 (28)
Extrapulmonary involvement, n (%)	41 (87)
Main treatment indication, n (%)	
Lungs	30 (64)
Heart	2 (4)
Eye	5 (11)
Central nervous system	3 (6)
Small fibre neuropathy	5 (11)
Spleen	1 (2)
Ear	1 (2)
Medication use prior to initiation of IFX, n (%)	
Corticosteroids	10 (21)
Immunomodulation	3 (6)
Corticosteroids & immunomodulation	33 (70)
None	1 (2)
Concomitant medication (n = 46), n (%)	
None	3 (7)
Corticosteroids	13 (28)
Immunomodulation	19 (41)
Corticosteroid & immunomodulation	11 (24)
Duration of infliximab treatment, months	8.5 ± 5.8
Disease activity measurements	

SUVmax lung parenchyma (n = 42)	4.8 ± 4.1
SUVmax mediastinum (n = 42)	5.5 ± 4.3
Angiotensin converting enzyme (U/L)	72.2 ± 42.4
Soluble interleukin 2-receptor (pg/mL)	5649 ± 5020
Vital capacity (%)	84.7 ± 19.1
Forced expiratory volume in 1 second (%)	74.8 ± 22.2
Diffusing capacity for CO (%) (n = 41)	67.1 ± 17.1

N = 47 unless noted otherwise; values are mean ± SD unless noted otherwise
SUVmax= Maximum Standardised Uptake Value

RELAPSE

Kaplan-Meier analysis revealed a median time to relapse of 11.1 (± SE 2.57) months after discontinuation of infliximab treatment and showed that 25% of the total cohort relapsed within 4 months (Figure 2). Of 47 included patients, 29 experienced relapse (62%) after a mean time of 7.8 (± SD 7.7) months. Relapse occurred within the first 10 months in 20 out of 29 relapsing patients. Moreover, only 2 out of 29 relapsing patients did so after a period of 20 months. Relapse in most patients was a severe deterioration of symptoms requiring retreatment, with some patients being as ill as they were before.

The majority of patients who experienced relapse were retreated with infliximab (23 out of 29 patients). Three of those 23 patients developed an allergic response to infliximab and switched to alternative medication. Six patients were retreated with other agents than infliximab due to varying reasons, including pregnancy wishes, previous adverse events during infliximab treatment and the degree of relapse severity. Of these six patients, one was treated with prednisone, two were treated with azathioprine and another two were treated with hydroxychloroquine. One patient was enrolled in a clinical trial for an experimental drug.

PREDICTIVE FACTORS OF RELAPSE

The results of univariate analysis for predictive factors of relapse are shown in Table 2. At time of initiation of infliximab therapy, two factors were found to be predictors of relapse. Patients with mediastinal SUVmax scores ≥ 6.0 have a significantly higher chance of relapse than patients with SUVmax scores < 6.0 (P<.001; HR 3.77). Moreover, a serum sIL-2R concentration ≥ 4,000 pg/mL at the start of therapy was found to predict relapse (P =.033; HR 2.24). None of the factors which were analysed at the time of treatment discontinuation were found to predict relapse.

In multivariate analysis, mediastinal SUVmax scores ≥ 6.0 at initiation were found to be an independent predictor of relapse (P<.001; HR 4.33 corrected for ethnicity). It must be noted that levels of sIL-2R and mediastinal SUVmax scores correlate significantly (P=.005; Pearson's R 0.446). Duration of treatment as such was not found a predictor for relapse.

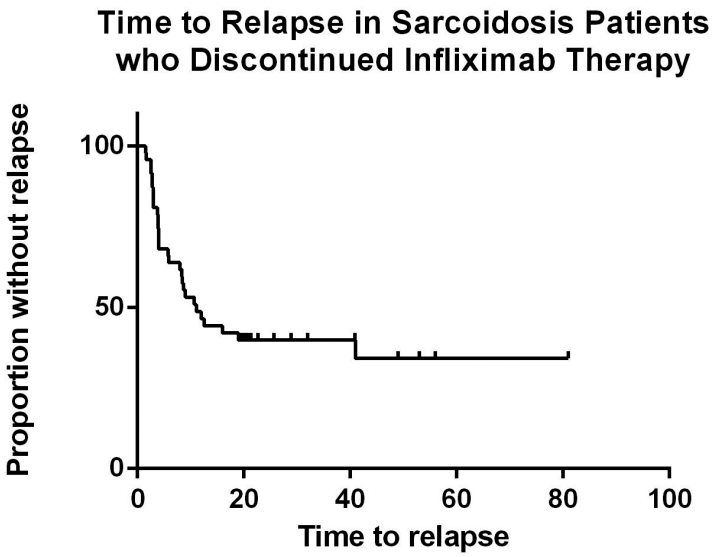

Figure 2: Kaplan-Meier analysis of time to relapse after discontinuation of infliximab therapy.
The median time to relapse in the total cohort was 11 months. In total, 29 out of 47 patients (62%) experienced relapse.

Table 2 All factors investigated in primary univariate analysis.

Risk factor	P-value	Hazard ratio estimate	95% CI
Treatment duration	0.670	1.01	0.95-1.08
Age (at treatment initiation)	0.361	0.98	0.94-1.02
Sex (female)	0.875	0.94	0.45-1.98
Ethnicity (non-caucasian)	0.090*	2.03	0.90-4.60
Extrapulmonary involvement	0.964	1.03	0.90-4.60
Biomarkers start of therapy:			
SUVmax total	0.033*	1.10	1.00-1.20
SUVmax lungs	0.703	1.02	0.93-1.11
SUVmax mediastinum	0.001*	1.16	1.06-1.26
ACE	0.925	1.00	0.99-1.01
CRP	0.712	1.17	0.52-2.63
sIL-2R	0.103*	1.00	1.00-1.00
VC (%)	0.410	0.99	0.97-1.01
FVC (%)	0.367	0.99	0.97-1.01
FEV1 (%)	0.648	1.00	0.98-1.02
Tiffeneau index	0.967	1.00	0.97-1.03
DLCO (%)	0.255	0.99	0.96-1.01

Biomarkers after 6 months of treatment:			
SUVmax total	0.312	1.12	0.90-1.41
SUVmax lungs	0.758	1.08	0.66-1.78
SUVmax mediastinum	0.226	1.14	0.92-1.41
ACE	0.816	1.00	0.99-1.02
CRP	0.637	1.25	0.50-3.09
sIL-2R	0.957	1.00	1.00-1.00
Leukocytes	0.941	0.99	0.84-1.12
Biomarkers end of therapy:			
ACE	0.917	1.00	0.98-1.02
CRP	0.343	1.50	0.65-3.43
sIL-2R	0.841	1.00	1.00-1.00
Leukocytes	0.750	1.03	0.86-1.24
VC (%)	0.406	0.99	0.96-1.01
FEV_1 (%)	0.839	1.00	0.98-1.02
Tiffeneau index	0.762	1.01	0.98-1.04
DLCO (%)	0.542	0.99	0.97-1.02
Δ ACE (%)	0.671	0.67	0.99-1.00
Δ sIL-2R (%)	0.374	1.00	0.99-1.00
Δ VC (%)	0.815	1.00	0.95-1.04
Δ FEV_1 (%)	0.649	1.01	0.97-1.05

DISCUSSION

In this study we found a high number of relapse (62%) after discontinuation of infliximab therapy in sarcoidosis patients, after a mean time of eight months. These relapse episodes were characterised by recurrence of sarcoidosis symptoms with need for therapeutic intervention. Both a high mediastinal SUVmax score on ^{18}F-FDG PET and a high serum sIL-2R concentration at the initiation of therapy were found to significantly predict the chance of relapse in sarcoidosis patients.

In a study of 14 sarcoidosis patients by Panselinas and colleagues, relapse of the disease after infliximab discontinuation in sarcoidosis patients was first noted [19]. The relapse rate in this cohort was even higher (86%) than in our cohort. Based on the natural history of disease, patients with a longer duration of disease could be more prone to relapse after discontinuation of infliximab. However, as the time from diagnosis until infliximab initiation was longer in our cohort (6.3 vs 4.7 years), this is unlikely to be the cause of the lower relapse incidence found in our cohort. Patients in our cohort were treated for a longer period of time, in the study by Panselinas 9 out of 14 patients had been treated with less than 6 infusions. It is not known whether a more intensive infliximab treatment regimen can prevent relapse or change the course of the disease.

No studies have focussed on predicting relapse after discontinuation of infliximab therapy in sarcoidosis patients. In Crohn's disease, leukocyte count and CRP levels were found to be predictors of relapse after discontinuation of infliximab therapy [20]. However, CRP levels and leukocyte count were no predictors of relapse in our sarcoidosis cohort as these levels are mostly within the normal range in this disease.

In this study, mediastinal SUVmax score on ^{18}F-FDG PET and a high serum sIL-2R concentration at the start of therapy were identified as predictors of relapse. ^{18}F-FDG PET has previously demonstrated to be a predictor for decline in lung function in untreated patients and is more sensitive than 67Ga imaging in the assessment of sarcoidosis activity in mediastinal and pulmonary areas [26;27]. In previous studies, serum sIL-2R levels at disease presentation were correlated with parenchymal infiltration in pulmonary sarcoidosis and were possible prognostic markers at diagnosis [29;30].

In our cohort, patients with SUVmax scores equal or higher than 6.0 were found to be four times more likely to experience relapse than patients with SUVmax below 6.0. In addition, we showed that patients with serum levels of sIL-2R equal or higher than 4000 pg/mL were more than twice as likely to relapse than those with lower serum sIL-2R levels. However, this does not mean that patients with normal sIL-2R at start of therapy can not have relapse.

^{18}F-FDG PET results were found to be more adequate predictors of relapse than serum levels of sIL-2R, although it must be acknowledged that ^{18}F-FDG PET should not be performed repetitively, because of radiation exposure and associated health care costs. Furthermore, not all treatment centres may have access to an ^{18}F-FDG PET and qualified nuclear physicians with specific knowledge in the field of sarcoidosis. Therefore, serum levels of sIL-2R, when measured in a standardised fashion, can still serve as indicators of relapse risk.

Although in our opinion sIL-2R is a parameter suited to guide infliximab therapy, and a normal sIL-2R level might be required before infliximab therapy is tapered off, we could not prove this in our study. In our cohort, infliximab treatment was sometimes prolonged when clinically indicated. These patients could have elevated sIL-2R levels. In fact, at the moment of discontinuation only 7 patients had elevated sIL-2R levels (>4000 pg/ml). Our study might therefore underestimate the guiding effect of sIL-2R in tapering of infliximab.

Besides the finding that the incidence of relapse was high in our cohort, the median time to relapse in the total cohort was 11 months. Interestingly, 93 percent of relapsing patients did so within the first 20 months, making relapse unlikely in patients who are still in remission after this period of time. In clinical practice, follow-up of these patients can probably be performed at longer time intervals.

A limitation of this study is the retrospective nature of the design, because non-standardisation of follow-up necessarily occurs in a retrospective study. For instance, patients differed in terms of treatment indications and conditions, especially regarding the type and dose of concomitant and consolidation treatment used. While this may be considered a limitation, this variation does reflect clinical practice.

From this study, it appears that patients with high disease activity at the start of therapy, reflected by high sIL-2R or mediastinal SUVmax on ^{18}F-FDG PET, have an increased chance of relapse after discontinuation of treatment. A possible explanation could be that these patients, suffering from severe active disease, need a more intensive treatment regimen, either in dose or duration. No study has focussed on the optimal duration of infliximab therapy in sarcoidosis after the six-month

induction phase. It might be worthwhile to investigate whether the risk of relapse would be lower if infliximab administration was continued for a longer period or at a higher dosage schedule than the standard 5 mg/kg in selected patients. For now, close monitoring of patients with high SUVmax scores and serum sIL-2R levels at the start of treatment is warranted.

The high relapse rate of 55% one year after discontinuation of infliximab therapy found in this study underlines the importance of additional research in this field and the need for new therapeutic approaches. The relapse rate in sarcoidosis after discontinuation of infliximab appears to be higher than described in inflammatory bowel disease with a relapse rate of 25% after one year for colitis ulcerosa and of 39%-44% for Crohn's disease [20;31]. Even though most sarcoidosis patients benefit from infliximab therapy initially, infliximab nor any of the currently available drugs can actually cure the disease.

In conclusion, this study provides evidence for a high relapse rate after discontinuation of infliximab therapy in sarcoidosis patients. For the first time it was shown that both high serum sIL-2R level and high SUVmax on ^{18}F-FDG PET at initiation of therapy are valuable predictors of relapse after infliximab discontinuation. Close monitoring of patients in this category who discontinue treatment is therefore indicated. In addition, the results suggest that longer duration of infliximab treatment might be needed in these patients, but prospective trials are needed to prove the clinical benefit of such a practice.

Table 3 Factors Associated with Time to Relapse in Univariate and Multivariate Analysis
Association with Time to Relapse in Univariate Analysis

Association with Time to Relapse in Univariate Analysis

Risk factor	P-value	Hazard ratio estimate	95% CI
Non-caucasian ethnicity	0.090	2.03	0.95-1.08
SUVmax mediastinum (start of therapy) ≥ 6.0	**0.001***	**3.77**	1.71-8.33
SUVmax total (start of therapy) ≥ 6.0	0.076	2.06	0.93-4.57
sIL-2R (start of therapy) ≥ 4000 pg/mL	**0.033***	**2.24**	1.07-4.68

Association with Time to Relapse in Multivariate Analysis

Risk factor	P-value	Hazard ratio estimate	95% CI
SUVmax mediastinum (start of therapy) ≥ 6.0	**<0.001***	**4.33**	1.92-9.81
Corrected for ethnicity			

* values considered significant
SUVmax total is the maximum score of mediastinum and lung parenchyma

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PART IV

CELLULAR RESPONSES IN SARCOIDOSIS

CHAPTER 9

DIFFERENTIAL EXPRESSION OF TNFR1 (CD120A) AND TNFR2 (CD120B) ON SUBPOPULATIONS OF HUMAN MONOCYTES

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ABSTRACT

BACKGROUND

Three subpopulations of monocytes can be distinguished in human blood: classical (CD14++CD16-), intermediate (CD14++CD16+), and nonclassical (CD14+CD16++) monocytes. CD16 expressing monocytes are expanded in patients with sarcoidosis and in various other inflammatory diseases. In sarcoidosis, it is unclear whether either intermediate, nonclassical or both CD16 expressing monocytes are responsible for this increase. Data relating to the monocyte subpopulations is receiving increasing attention, but the expression of TNF receptors on these subpopulations has not been studied thus far. The aim of this study was to determine frequencies of monocyte subpopulations and their expression of TNFR1 and TNFR2 in both sarcoidosis patients and healthy controls.

METHODS

Peripheral blood cells of sarcoidosis patients and healthy controls were stained for the markers HLA-DR, CD14, CD16, CD120a and CD120b. Cells were measured on a FACSCalibur and analyzed with FlowJo. We used Student's t-test and a parametric One-way ANOVA for statistical analysis.

RESULTS

Sarcoidosis patients had a significant higher frequency of intermediate monocytes than healthy controls. Significant differences in TNF receptor expression were found between the monocyte subpopulations, both in sarcoidosis patients as well as in healthy controls: intermediates expressed more TNFR1 than classicals and nonclassicals and nonclassicals expressed more TNFR2 than intermediates, whereas intermediates showed higher expression than classicals.

CONCLUSIONS

In both sarcoidosis patients and healthy controls, intermediate monocytes show the highest expression level of TNFR1 among monocyte subpopulations and nonclassical monocytes show the highest expression level of TNFR2. These findings, as wells as the higher frequency of intermediate monocytes in sarcoidosis patients, provide evidence for the existence of two functionally-distinct CD16 expressing monocyte subpopulations and could be related to therapy response.

BACKGROUND

Monocytes originate from myeloid progenitors in the bone marrow, circulate in the blood for up to three days, and then enter peripheral tissues where they differentiate into macrophages or dendritic cells [1].

Human monocytes can be divided into three subpopulations: classical, intermediate, and nonclassical monocytes. This subdivision is based on the expression levels of the lipopolysaccharide (LPS) co-receptor CD14 and the Fcγ receptor III CD16. Classical monocytes, the major population of monocytes, are CD14++CD16-. The minor population of CD16 expressing monocytes are

further subdivided in intermediate monocytes (CD14++CD16+) and nonclassical monocytes (CD14+CD16++) [2]. Recently, monocyte subpopulations have been extensively investigated. In addition to CD14 and CD16, also other markers, such as HLA-DR, CCR2 and CCR5, can distinguish the three human monocyte subpopulations [3,4].

An imbalance in the relative proportion of CD16 expressing monocytes has been found in a variety of immune mediated diseases such as rheumatoid arthritis, diabetes, atherosclerosis, bacterial and HIV infections (reviewed by Ziegler-Heitbrock [5]). An increase in the relative number of CD16 expressing monocytes was found in newly diagnosed sarcoidosis patients, suggesting an activated state of the monocytes. This activated state might be an indicator for disease activity [6,7]. These investigations have not discriminated between the two CD16 expressing monocyte subpopulations. Recently, it has been shown in patients with rheumatoid arthritis [8] and severe asthma [9] that intermediate monocytes are responsible for the increase in CD16 expressing monocytes. So far, this has not been investigated in patients with sarcoidosis.

Sarcoidosis is a systemic, granulomatous disease of unknown origin that primarily affects the lungs. It is a disease of all races and ethnic groups with varying incidence throughout the world [10-12]. The highest incidence of sarcoidosis in Europe has been reported from Sweden: 24 cases per 100,000 [13]. In The Netherlands, the incidence of sarcoidosis is estimated to be 20 cases per 100,000 [14]. In the United States, the incidence rate in black people is 36 cases per 100,000 compared to 11 cases per 100,000 in white people [15]. The most important feature of sarcoidosis is the formation of non-caseating granulomas [10-12].

In the human lung, tumor necrosis factor (TNF) is mainly produced by alveolar macrophages and thought to play a major role in the development of the granulomatous inflammation. The release of TNF is increased in the lung of patients with pulmonary sarcoidosis [16,17]. Therefore TNF is an attractive target for immunotherapy in sarcoidosis.

TNF exerts its function by binding to and signaling via two different receptors. TNFR1 (CD120a) is constitutively, but in low levels, expressed on nearly all nucleated cell types. TNFR2 (CD120b) is inducible and expressed by cells of myeloid lineage, peripheral T cells and alveolar lymphocytes and macrophages [18-23]. TNFR1 activates the caspase family, which induce cell death. TNFR2 signalling leads to NF-κB transcription and can induce proliferation, differentiation, cytokine production and even apoptosis. When TNFR1 and TNFR2 are co-expressed on the same cells, intracellular cross-talk between the receptors may occur [24]. Both TNF receptors can be enzymatically cleaved from the cell surface and form soluble TNF receptors. Soluble TNF receptors can neutralize TNF and clear it from the circulation and act as a TNF antagonist, but can also prolong its bioactivity by binding TNF and thus stabilize the trimeric structure [22]. Membrane-bound TNF binds more strongly to TNFR2 than soluble TNF does. It may be that TNFR2 only becomes fully activated with membrane-bound TNF [21].

There are a few reports of TNFR1 and TNFR2 expression on peripheral blood cells. Approximately 25% of the peripheral blood lymphocytes in healthy subjects is CD4+TNFR2+. Interestingly, in sarcoidosis the percentage of CD4+TNFR2+ cells is increased, especially in patients in remission

or with stable disease (respectively 35% and 33%), which may indicate a role in down regulation of a cell-mediated immune response [23]. FoxP3+ T reg cells expressed the highest levels of TNFR2 among subsets of human peripheral blood CD4+ T cells and were able to shed large amounts of sTNFR2, suggesting an important role for T reg cells in suppressing TNF [25]. It is attractive to speculate about a similar role for one of the subpopulations of human monocytes. However, the expression of TNFR1 and TNFR2 on monocyte subpopulations has not been studied thus far.
The aim of the present study was to determine frequencies of the three monocyte subpopulations in peripheral blood of patients with sarcoidosis compared to healthy controls and the relative expression of TNFR1 and TNFR2 on these subpopulations.

METHODS

PATIENTS AND HEALTHY CONTROLS

Thirty-eight patients (11 female, 27 male; median age 48 years, range 22 – 70 years) with proven sarcoidosis were included in the present study. For this study, all sarcoidosis patients were grouped as one, independent of severity of the disease and independent of use of medication. Thirteen healthy volunteers (4 female, 9 male; median age 50 years, range 27-58 years) were used as controls. Peripheral blood was collected in sodium heparin tubes. The study was approved by the medical ethical evaluation committee of the St Antonius Hospital in Nieuwegein, The Netherlands.

ANTIBODIES AND FLOW CYTOMETRY

Anti-HLA-DR FITC and anti-CD16 PE were obtained from BD Biosciences (San Diego, CA, USA). Anti-CD14 PerCP-eFluor710 was from eBioscience (San Diego, CA, USA). Anti-CD120a APC (TNFR1) and anti-CD120b APC (TNFR2) were from R&D Systems (Minneapolis, MN, USA). After staining, flow cytometry data were acquired on a FACSCalibur (BD Biosciences) and analyzed using FlowJo software (Tree Star, Ashland, OR, USA).
Monocytes were gated based on FSC x SSC and SSC x HLA-DR+ expression. Subsequently, monocytes were subdivided in three populations based on CD14 and CD16 expression pattern. We used fluorescence minus one (FMO) staining as control for the TNF receptor staining by substracting the MFI of the FMO control of the MFI of the TNF receptor.

STATISTICAL ANALYSIS

Data are expressed as mean ± SEM. Comparison of two groups of data (patients versus controls) was performed by a two-tailed Student's t-test and comparison of three groups of data (subpopulations of monocytes) was performed by a parametric One-way ANOVA, both by using GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, CA, USA). Differences were considered statistically significant at P values of 0.05 or less.

RESULTS AND DISCUSSION

MONOCYTES SUBPOPULATIONS

The frequency of classical, intermediate, and nonclassical monocyte subpopulations was analyzed in 38 sarcoidosis patients and 13 healthy controls, based on HLA-DR, CD14 and CD16 expression pattern. In this study, classical monocytes accounted for 60-90% of SSC x HLA-DR+ gated monocytes, intermediate monocytes for about 2-5% and nonclassical monocytes for 5-25%, both in sarcoidosis patients as well as in healthy controls. Others (reviewed by Ziegler-Heitbrock [5]) have found increased or decreased relative numbers of CD16 expressing monocytes in a variety of inflammatory and/or infectious diseases in man. In most of these studies nonclassical and intermediate monocytes were grouped together as one subpopulation. Rossol et al reported that increased frequencies of CD16 expressing monocytes in rheumatoid arthritis were due to an increase in intermediates [8]. Moniuszko et al reported similar findings for severe asthma [9].We also found a higher frequency of intermediate monocytes in sarcoidosis patients (3.08% ± 0.25%) than in healthy controls (1.98% ± 0.22%; P=0.017; Figure 1), while nonclassical monocytes were not increased (data not shown). It should be stressed that CD16 expressing monocytes should be considered as two different populations.

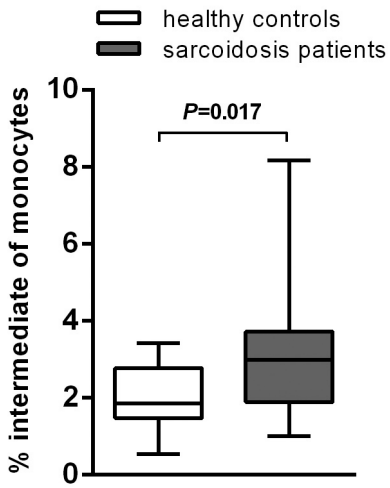

Figure 1: Sarcoidosis patients show a higher frequency of intermediate monocytes than healthy controls.

Percentage of intermediates within the monocyte population of patients with sarcoidosis (n=38) and healthy controls (n=13). Data are presented as box plots, where the boxes represent the 25th to 75th percentiles, the lines within the boxes represent the median, and the lines outside the boxes represent the 10th and 90th percentiles.

All three monocyte subpopulations differed significantly in HLA-DR expression, both in sarcoidosis patients as well as in healthy controls. Intermediates showed the highest expression of HLA-DR, followed by nonclassicals (P<0.0001; Figure 2A). This differential expression of HLA-DR on monocyte subpopulations previously has been described for patients with rheumatoid arthritis [8] and for healthy controls [3].

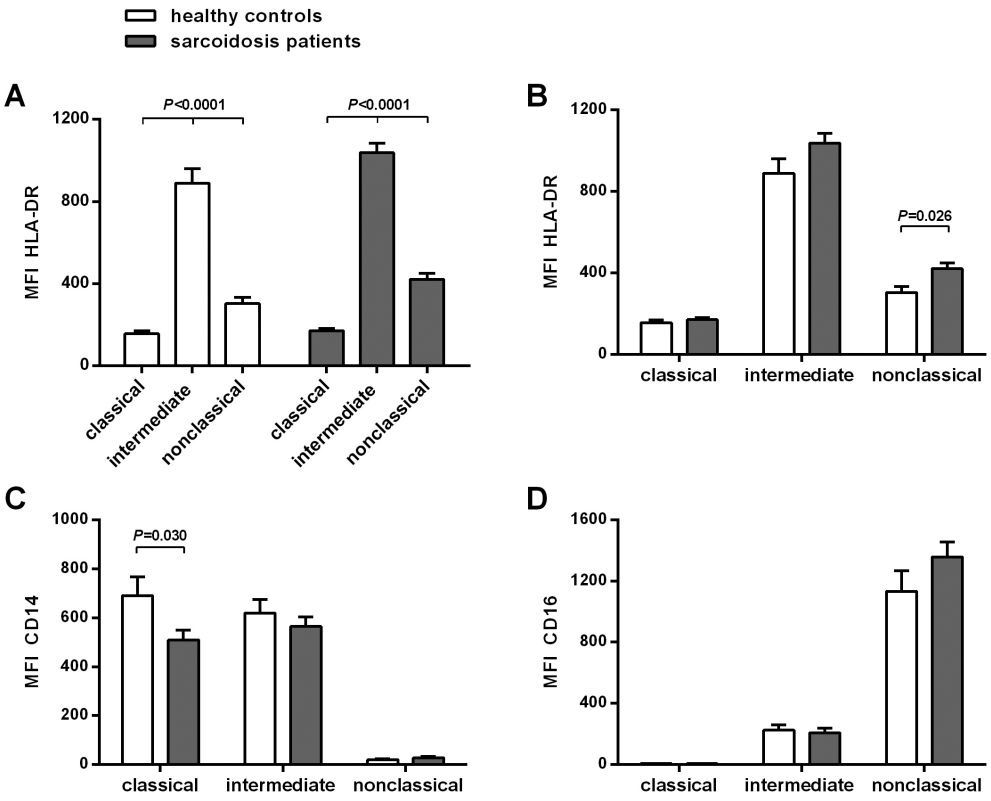

Figure 2: Differential expression of HLA-DR, CD14, CD16 on monocyte subpopulations in sarcoidosis patients and healthy controls.

Median fluorescence intensity (MFI) of HLA-DR (A,B), CD14 (C) and CD16 (D) on classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$) and nonclassical ($CD14^{+}CD16^{++}$) monocytes in sarcoidosis patients (n=38) and in healthy controls (n=13). Please note that, for sake of clarity, the data in panel A are regrouped in panel B to allow better comparison between the monocyte subpopulations. Values are expressed as mean ± SEM. All three markers were significantly different between the three monocyte subpopulations. Between sarcoidosis patients and healthy controls, nonclassical monocytes showed significant different levels of HLA-DR expression and classical monocytes showed significant different levels of CD14 expression. Comparison of three groups of data (subpopulations of monocytes) was performed by a parametric One-way ANOVA and comparison of two groups of data (patients versus controls) was performed by a two-tailed Student's t-test.

We found significant differences in HLA-DR and CD14 expression between patients with sarcoidosis and healthy controls. Nonclassical monocytes of sarcoidosis patients expressed a higher level of HLA-DR than nonclassicals of controls (P=0.026; Figure 2B). Classical monocytes of sarcoidosis patients expressed a lower level of CD14 than classicals of healthy controls (P=0.030; Figure 2C). Intermediate monocytes of sarcoidosis patients tended to have a higher level of HLA-DR and a lower level of CD14 than intermediates of healthy controls (Figures 2B and 2C). Nonclassical and intermediate monocytes of sarcoidosis patients tended to have a higher level of CD16 than nonclassicals and intermediates of controls (Figure 2D).

TNF RECEPTORS

TNF is a major cytokine regulating the activity of monocytes and therefore we have extended the phenotypic characterization of monocyte subpopulations for expression of TNF receptors. Although no differences were found between patients and controls, analysis of TNFR1 and TNFR2 revealed differential expression on the monocyte subpopulations, both in sarcoidosis patients as well as in healthy controls. All monocytes expressed TNFR1, but intermediates showed a higher expression

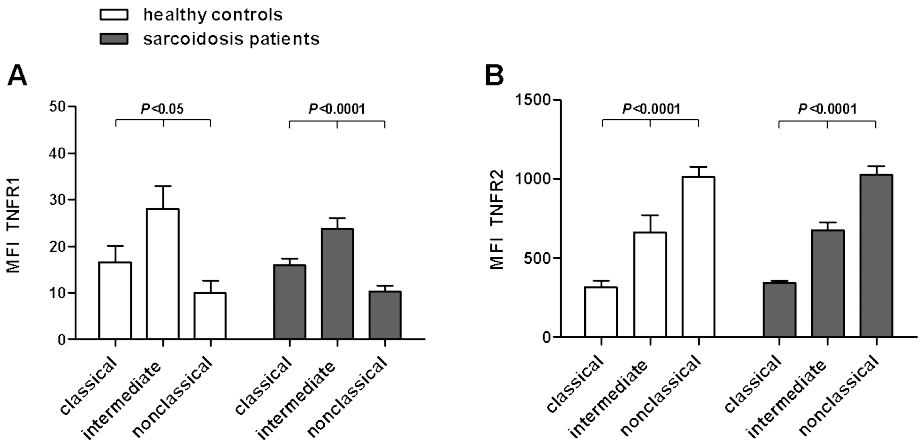

Figure 3: Differential expression of TNFR1 and TNFR2 on monocyte subpopulations in sarcoidosis patients and healthy controls.

Median fluorescence intensity (MFI) of TNFR1 (A) and TNFR2 (B) on classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$) and nonclassical ($CD14^{+}CD16^{++}$) monocytes in sarcoidosis patients (TNFR1 n=18, TNFR2 n=38) and in healthy controls (TNFR1 n=5, TNFR2 n=13) minus the MFI of the fluorescence minus one (FMO) control. Values are expressed as mean ± SEM. Both markers were significantly different between the three monocyte subpopulations. All monocytes expressed TNFR1, but intermediates had a higher expression of TNFR1 than classicals and nonclassicals. All monocytes also expressed TNFR2, but nonclassicals had a higher expression of TNFR2 than intermediates and a much higher expression than classicals. Comparison of the three subpopulations of monocytes was performed by a parametric One-way ANOVA.

of TNFR1 than classicals and nonclassicals ($P<0.05$; Figure 3A). All monocytes also expressed TNFR2, but the three subpopulations showed major differences in TNFR2 expression ($P<0.0001$; Figure 3B). Nonclassical monocytes expressed the highest levels of TNFR2. Intermediates expressed less TNFR2 than nonclassicals, but more than classicals. Although classical monocytes expressed the lowest levels of TNFR2, values were still largely positive compared to a fluorescence minus one (FMO) control.

CONCLUSIONS

Subpopulations of monocytes not only express different levels of CD14, CD16 and HLA-DR, but also different levels of TNFR1 and TNFR2. This finding is new and emphasizes the diversity and potential different functions of the three monocyte subpopulations. Frequencies of intermediate monocytes are increased in sarcoidosis patients, suggesting an activated state of the monocytes in this disease. This finding may be of especial relevance because intermediate monocytes express the highest level of HLA-DR among the monocyte subpopulations. This activated state might be a novel indicator for disease activity. Intermediate monocytes also express the highest level of TNFR1 among the monocyte subpopulations, while nonclassical monocytes, the other CD16 expressing monocyte subpopulation, express the highest level of TNFR2. This data supports the evidence of two functionally-distinct CD16 expressing monocyte subpopulations.

HLA-DR may be a valuable marker to monitor the disease course of sarcoidosis patients. Intermediate and nonclassical monocytes plus their TNF receptor expression pattern might be relevant to monitor for sarcoidosis patients who are going to be treated with anti-TNF (Remicade), as well as for other patients on anti-TNF therapy, such as those suffering from rheumatoid arthritis, psoriasis or Crohn's disease. Prospective monitoring of these patients should demonstrate whether particular monocyte subpopulations display differential sensitivity to this form of treatment and whether this correlates with the clinical response.

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CHAPTER 10

SIGNIFICANT CHANGES IN MONOCYTE SUBSETS DURING INFLIXIMAB THERAPY IN SEVERE SARCOIDOSIS

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ABSTRACT

RATIONALE

Blockade of TNF by infliximab can be effective as third-line treatment for severe sarcoidosis. Monocyte subsets have differential expression of TNF-receptors 1 and 2 (TNFR1 and TNFR2). The effect of infliximab on monocytes is unknown, but may reveal further insight into the pathogenesis of sarcoidosis.

METHODS

In this prospective study we collected serum of 38 patients with severe and active sarcoidosis during monthly treatment with infliximab and 18 healthy controls. Peripheral blood cells were stained for expression of HLA-DR, CD14, CD16, CCR2, CCR5, TNFR1 and TNFR2 and analyzed by multiparameter flow cytometry.

RESULTS

The percentage of monocytes at baseline correlated positively with disease activity as measured by soluble interleukin-2 receptor (sIL-2R) at baseline (R=0.47; p=0.003), angiotensin converting enzyme (ACE) at baseline (R=0.43; p=0.014) and negatively with change in ACE during therapy (R=0.56; p=0.001).

Monocytes were classified as classical, intermediate or non-classical based on CD14 and CD16 expression. The percentage of intermediate monocytes at baseline was significantly higher in patients than in controls (3.2% vs 1.8%; p<0.0001) and increased significantly after 14 weeks of therapy (3.2% vs 4.1%; p=0.010), but fell back to baseline at week 26. During therapy, the percentage of non-classical monocytes decreased from 15.6% to 12.3% (p=0.0063). Furthermore, the percentage of TNFR1 expressing classical monocytes decreased significantly from 59.8% to 43.6% (p=0.0065), while TNFR2 expression remained stable. Mean CCR5 expression increased significantly on intermediate monocytes from 40.3% to 46.4% (p=0.03), and on non-classical monocytes from 7.5% to 11.6% (p=0.001). Both the change in expression of CCR5 and CCR2 on non-classicals correlated with overall response to treatment (R=0.36; p=0.049 and R=0.37; p=0.03).

CONCLUSIONS

During infliximab therapy, the percentage of non-classical monocytes decreases significantly. Furthermore, expression of TNFR1 on classical monocytes decreases, and CCR5 expression on intermediates and non-classicals increases. Our findings suggest a differential role for monocyte subsets in sarcoidosis. Further investigations are needed to clarify their role in disease pathogenesis, and also their potential as biomarker for therapeutic decision making.

INTRODUCTION

Sarcoidosis is a systemic granulomatous disease of unknown origin that can cause a wide variety of symptoms, but most often affects the lungs [1]. Because sarcoidosis is self-limiting in the majority of patients, not all patients require systemic therapy [2]. In case of severe sarcoidosis, therapy consists of prednisone as first-line therapeutic and methotrexate or azathioprine as most often used second-line options [3,4]. Infliximab, a monoclonal anti-TNF drug, can be effective as third-line therapy for severe sarcoidosis [5].

Sarcoid granulomas can appear in virtually all organs and can cause significantly impaired organ function [2]. The exact nature and order of immunological events that is associated with formation of these sarcoid granulomas remains obscure. However, granuloma formation may be initiated following interaction between mononuclear derived macrophages and lymphocyte subsets [6]. Macrophages of sarcoidosis patients are known to produce excessive amounts of TNF compared to healthy controls [7,8]. The important role of TNF in granuloma formation is also supported by mouse studies [9,10] and is the rationale behind infliximab therapy in sarcoidosis [11].

In blood, monocytes are the precursors of tissue macrophages. Monocytes are characterized by forward scatter (FSC) and side scatter (SSC) as well as the expression of the MHC class II receptor HLA-DR, the LPS co-receptor CD14 and the Fc-gamma receptor III CD16 [12]. Based on the relative expression of CD14 and CD16, monocytes can be divided into three subsets. Classical monocytes represent the majority of monocytes and are CD14++CD16-. The minor population of CD16-expressing monocytes is subdivided into non-classical monocytes (CD14+CD16++) and intermediate monocytes (CD14++CD16+) [12-14]. CD16-expressing monocytes are expanded in various immune mediated diseases such as rheumatoid arthritis, diabetes, atherosclerosis, bacterial and HIV infections [15]. Furthermore, high percentages of CD16 expressing monocytes were found in sarcoidosis patients when compared to controls [16,17]. CD16+ monocytes, and intermediate monocytes in particular, may play a pro-inflammatory role as high producers of TNF as well as other cytokines [18,19].

The differences between the three subsets of monocytes are also reflected by their relative expression of HLA-DR, TNF-receptors 1 and 2 (TNFR1 and TNFR2) and chemokine receptors 2 and 5 (CCR2 and CCR5). Classical monocytes show highest expression of CCR2, intermediate monocytes show highest expression of HLA-DR, TNFR1 and CCR5, and non-classical monocytes of TNFR2 [13,14]. TNF signals through soluble and membrane bound TNF binding to TNFR1 or TNFR2, with most of biological activity signaling through TNFR1 [20]. Chemokine receptors and ligands are important for recruitment of monocytes from bone marrow to blood and to sites of inflammation [21,22] and elevations of their ligands CCL2, CCL5, MIP1-α and MIP1-β in bronchoalveolar lavage (BAL) fluid have been associated with pulmonary sarcoidosis [23-25].

Monocyte subsets and their surface expression of TNF and chemokine receptors have never been studied during infliximab therapy in sarcoidosis. Analysis of these cells may reveal novel insight in their role in disease pathogenesis and prediction of response to systemic therapy.

METHODS

PATIENTS

All sarcoidosis patients in whom infliximab therapy was initiated in our hospital between January 2011 and April 2013, were invited to participate in this prospective open-label cohort study. St. Antonius Hospital Nieuwegein serves as a national tertiary referral centre for sarcoidosis in the Netherlands. Only patients with severe sarcoidosis, which was organ and/or life threatening, were eligible. Furthermore, patients had to be unresponsive to standard first and second line therapy.

Patients received infliximab intravenously in a standard protocol starting with 5 mg/kg bodyweight at weeks 0 and 2 and then every 4 weeks during a period of 26 weeks (6 months). After the induction period of 26 weeks, treatment effect was measured based on response in three dimensions: (1) organ function (pulmonary function tests), (2) inflammatory disease activity measured by surrogate markers 18F-fluorodeoxyglucose by positron emission tomography (^{18}F-FDG PET), soluble interleukin-2 receptor (sIL-2R) and angiotensin converting enzyme (ACE) and (3) the response in quality of life measured by two questionnaires: a Patient Global Assessment (PGA) score on a visual analogue scale and physical functioning on Short Form-36 (SF-36). The total composite overall response score could vary from 0 (no response in any category) to 3 (excellent response in all categories). The exact method of gauging of response was described in chapter 7. All outcome parameters were also evaluated individually.

In addition to infliximab treated patients, blood was collected from healthy controls.

The institutional review board and the ethics committee approved the study and all patients gave written informed consent.

CHARACTERISATION OF PERIPHERAL BLOOD MONOCYTES BY FLOW CYTOMETRY

Peripheral blood was collected in sodium heparin tubes at baseline, after 14 weeks and after 26 weeks of therapy and stained within 3 hours after sampling. In a four-colour set-up, cells were stained for HLA-DR, CD14, CD16, and either TNFR1, TNFR2, CCR2, CCR5 or buffer (used as a FMO control). Live cells were gated based on FSC x SSC and subsequently monocytes were gated based on SSC x HLA-DR+ expression. Monocytes were subdivided into three subsets based on their relative CD14 and CD16 expression. A representative image of this gating strategy is shown in Figure 1.

Anti-HLA-DR FITC was obtained from BD Biosciences (San Diego, CA, USA). Anti-CD14 PerCP-eFluor710 and anti-CD16 PE were obtained from eBioscience (San Diego, CA, USA). Anti-CD120a APC (TNFR1), anti-CD120b APC (TNFR2), anti-CD192 APC (CCR2), anti-CD195 APC (CCR5) were from R&D Systems (Minneapolis, MN, USA).

After staining, flow cytometry data were acquired on a FACS-Calibur (BD Biosciences) and analyzed using FlowJo software (Tree Star, Ashland, OR, USA).

Figure 1: Gating strategy of monocyte subsets.

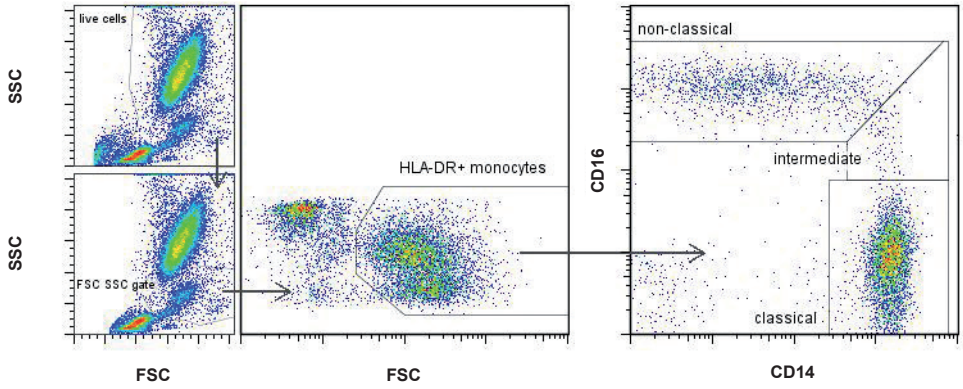

Figure 1: Four colour set-up determining monocyte subsets
In a 4 colour set-up, cells were stained for HLA-DR, CD14, CD16, and either TNFR1, TNFR2, CCR2, CCR5 or buffer (used as a FMO control). Live cells were gated based on FSC x SSC and then monocytes on SSC x HLA-DR^{+} expression. Subsequently, monocytes were subdivided into three subsets based on their relative expression of CD14 and CD16. The FMO control was used to determine the expression of the TNFRs and the CCRs.

STATISTICAL ANALYSIS

The statistical evaluation of the data was performed using SPSS for Windows, version 22.0 (IBM, New York, USA). The statistical significance of observed differences was calculated by Student's t-test. Non-parametric tests were used when appropriate. Pearson's Correlation coefficients (R) were calculated with linear regression. Graphs were created using GraphPad Prism 5.0 for Windows (GraphPad Software, San Diego, USA). A p-value of less than 0.05 was considered significant.

RESULTS

CHARACTERISTICS OF PATIENTS AND CONTROLS

Complete sets of data at baseline and after 26 weeks of infliximab therapy were available for 38 patients. Patients treated with infliximab therapy had a mean age of 48.9 years at the start of treatment and 63% was of male sex. Severe pulmonary sarcoidosis was the main treatment indication in 53% of cases (Table 1). According to the three dimensions of response, 15 patients had an excellent response (40%), another 15 patients had a good response (40%), 7 patients had partial response (17%) and only 1 patient did not respond to infliximab (3%). Eighteen healthy controls were included with a mean age of 47.8 years and 67% was of male sex.

One patient with an extremely high percentage of non-classical monocytes at baseline (65%), which decreased significantly during therapy, was excluded from analysis of monocyte subsets.

Table 1 Baseline characteristics of patients and healthy controls.

	Patients (n=38)	*Healthy controls (n=18)*	
Male gender	24 (63%)	12 (67%)	NS
Age at start of study in years (SD)	48.9 (10.1)	47.8 (10.1)	NS
Treatment indication:			
Pulmonary	20 (53%)		
Extrapulmonary	18 (47%)		
Lung function parameters:			
FVC % predicted (SD)	80.6 (15.8)		
FEV1 % predicted (SD)	68.7 (23.3)		
DLCO % predicted (SD)	62.3 (17.1)		
Biomarkers:			
ACE U/l (SD)	84.5 (46.5)		
sIL-2R pg/ml (SD)	7886 (5638)		

Abreviations:
FVC= forced vital capacity, FEV1= forced expiratory volume in one second, DLCO= diffusion capacity of the lung for carbonmonoxide, ACE=angiotensin converting enzyme, sIL-2R= soluble interleukin-2 receptor.

MONOCYTE SUBSETS AT BASELINE AND RELATION TO DISEASE ACTIVITY MARKERS

First, we compared monocytes between sarcoidosis patients and controls. Baseline counts and percentages of monocytes of sarcoidosis patients were within normal range (Figure 2A). Monocytes were further classified as classical, intermediate or non-classical based on CD14 and CD16 expression as shown in Figure 1. In patients, the relative numbers of classicals, intermediates and non-classicals at start of therapy was 77.6%, 3.2% and 15.6%, respectively. The percentage of intermediates at baseline was significantly higher in patients than in controls (3.2% vs 1.8%; $p<0.0001$) (Figure 2B).

Elevated levels of sIL-2R and ACE are characteristic for active disease in sarcoidosis [26-28]. The percentage of HLA-DR+ monocytes correlated positively with level of sIL-2R at baseline $R=0.47$; $p=0.003$, ACE at baseline $R=0.43$; $p=0.014$ and negatively with change in ACE during therapy $R=0.56$; $p=0.001$ (Figure 2C).

The absolute cell count of classical monocytes at baseline correlated negatively with sIL-2R levels at baseline ($R=0.40$; $p=0.014$).

Figure 2A-E: Changes in monocyte subsets during infliximab therapy.

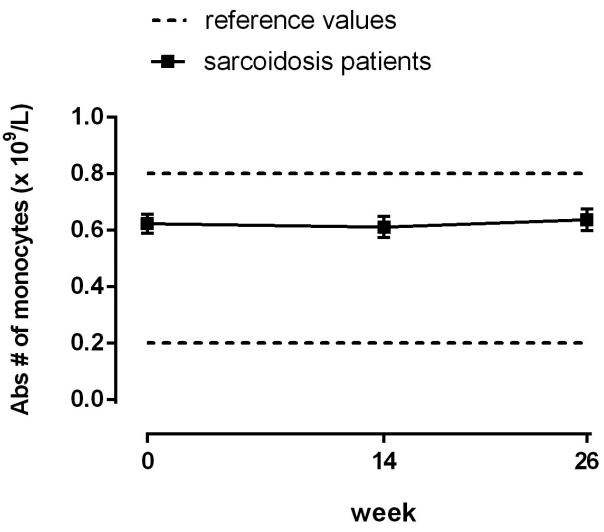

Figure 2A: Absolute number of monocytes during infliximab therapy.

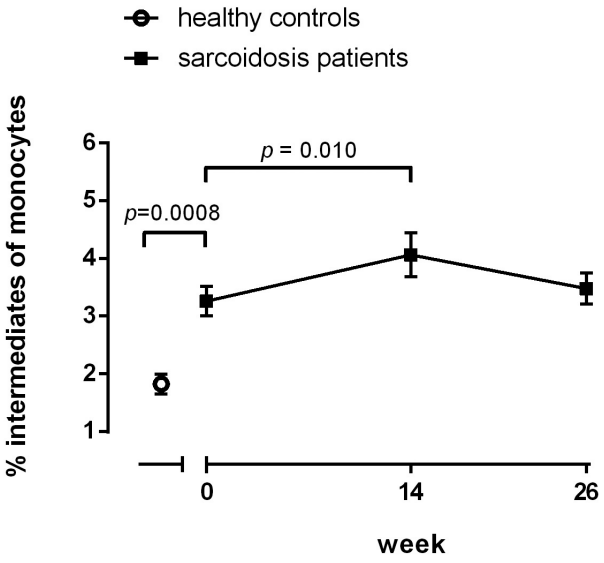

Figure 2B: Percentage of intermediate monocytes during infliximab therapy.

TNF-RECEPTOR AND CHEMOKINE-RECEPTOR EXPRESSION AT BASELINE

Monocytes were further characterized for surface-expression of the main receptors for TNF, TNFR1 and TNFR2, and chemokine receptors CCR2 and CCR5.

TNFR1, TNFR2, CCR2 and CCR5 expression varied significantly between the three monocyte subsets, but no differences were found between patients and controls (data not shown).

In patients, percentage of TNFR1 expressing classical monocytes at baseline correlated significantly with ACE at baseline (R=0.44; p=0.03) and negatively with change in ACE during treatment (R=0.42; p=0.035). Furthermore, percentage of CCR2 expressing non-classical monocytes at baseline did correlate negatively with ACE at baseline (R=0.42; p=0.02).

No noteworthy relation was observed between monocyte subsets and organ function and quality of life parameters.

MONOCYTE SUBSETS DURING THERAPY AND RELATION WITH DISEASE ACTIVITY MARKERS

To test how monocytes respond to infliximab, monocytes and its subsets were measured after 14 and 26 weeks of therapy. The mean absolute cell count of monocytes was 0.6x109/L at week 0 (within normal range) and remained stable during 26 weeks of therapy (Figure 2B). The mean

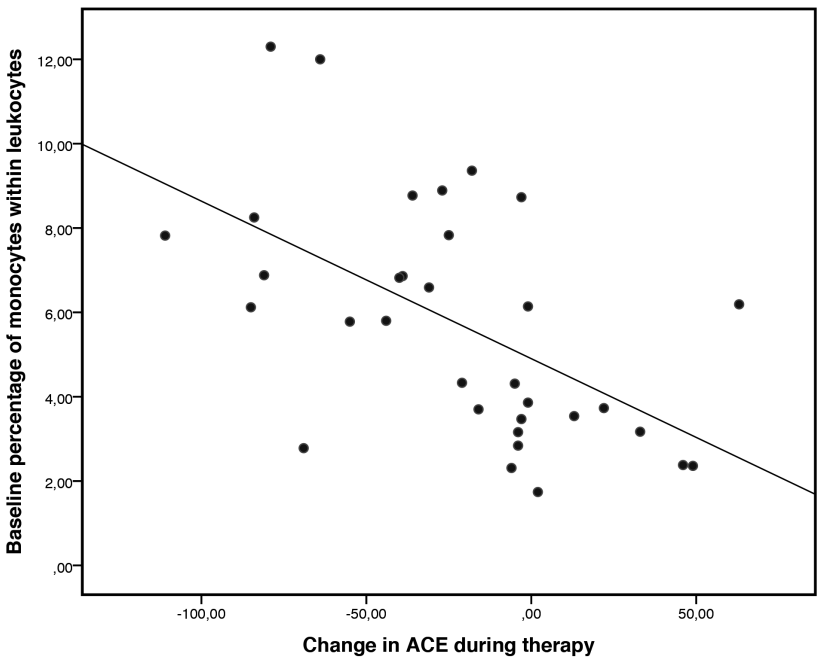

Figure 2C: Baseline HLA-DR positive monocytes in correlation to change in ACE during therapy (R=0.56; p=0.001).

percentage of HLA-DR+ monocytes within leukocytes also remained stable.

After 14 weeks of therapy the frequency of intermediate monocytes increased from 3.2% to 4.1% (p=0.010), but fell back to baseline after 26 weeks (Figure 2C). During 26 weeks of therapy,

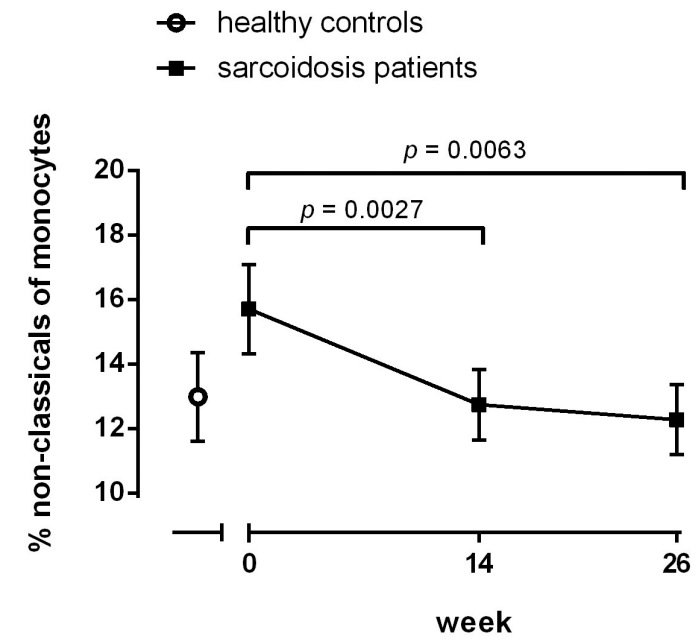

Figure 2D: Percentage of non-classical monocytes decreases during infliximab therapy.

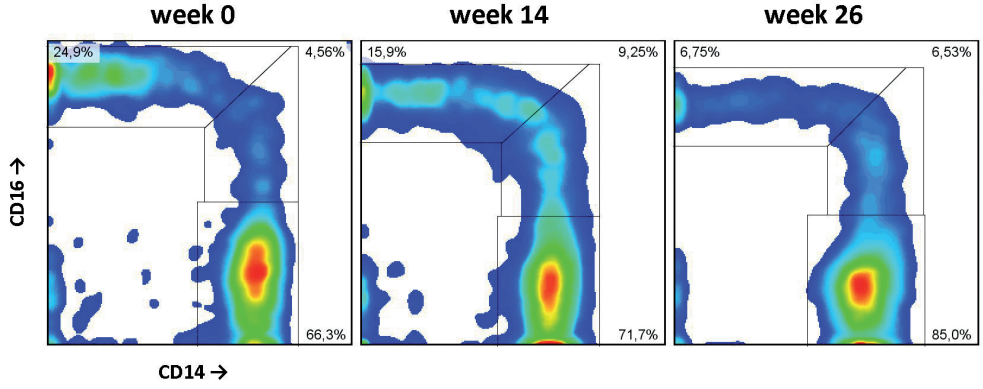

Figure 2E: Change in CD16 expression during infliximab therapy in a representative patient.

the percentage of non-classicals decreased from 15.6% to 12.3% (p=0.0063; Figure 2D). Flowcytometric data of a representative patient are shown in Figure 2E. During treatment, the percentage of classical monocytes showed a trend towards increase (data not shown).

TNF-RECEPTOR AND CHEMOKINE-RECEPTOR EXPRESSION DURING THERAPY

To investigate the effect of infliximab therapy on TNF-receptor expression, we studied the expression after 14 and 26 weeks of therapy. In our four-colour set-up, we found that the percentage of classical monocytes expressing TNFR1 decreased significantly from 59.8% to 43.6% (p=0.0065; Figure 3A). The change in percentage of non-classical monocytes expressing TNFR1 correlated with change in the lung function marker forced vital capacity (FVC) during therapy (R0.44; p=0.02). TNFR2 expression exceeded 90% on all three subsets and remained stable throughout therapy (data not shown).

We next investigated whether chemokine receptor expression changed after 26 weeks of therapy. The mean percentage of monocytes expressing CCR5 increased significantly in both intermediate monocytes from 40.3% to 46.4% (p=0.03) and non-classical monocytes from 7.5% to 11.6% (p=0.001) (Figure 3B). The change in percentage of CCR5-positive non-classical monocytes correlated with change in SUVmax of lung parenchyma on ^{18}F-FDG PET (R=0.39; p=0.03) and

Figure 3A-B: TNFR1 and CCR5 expression during therapy.

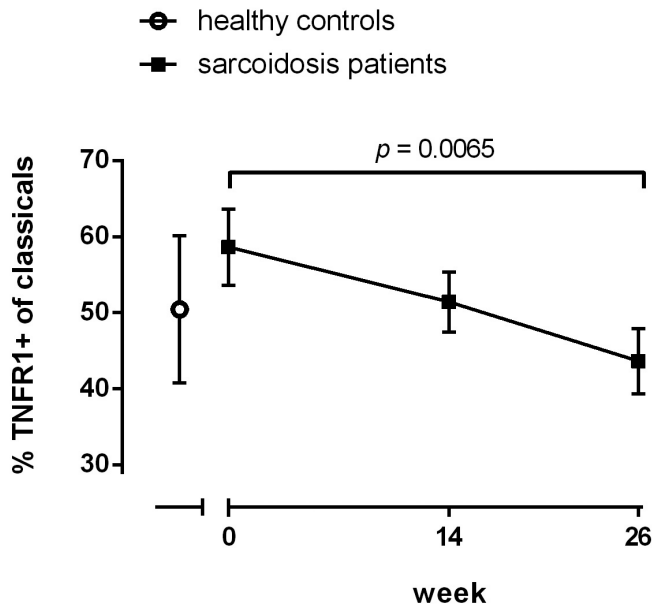

Figure 3A: Percentage of classical monocytes expressing TNFR1 decreases during therapy

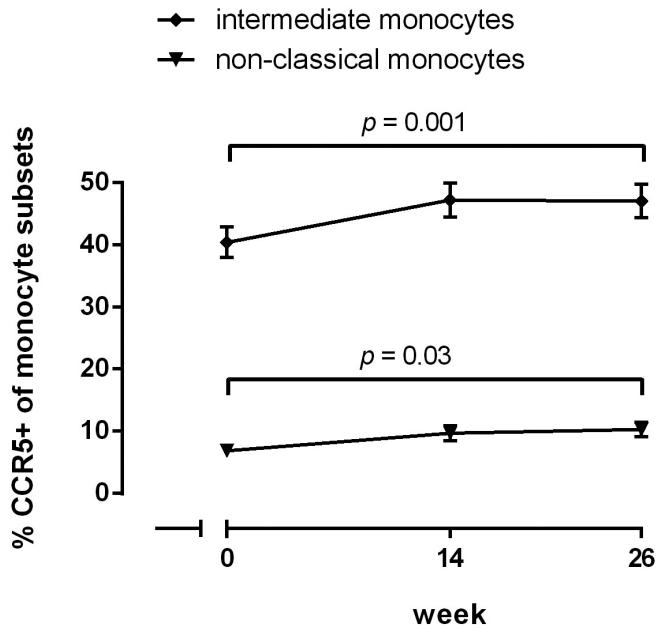

Figure 3B: Percentage of non-classical and intermediate monocytes expressing CCR5 increases during therapy.

negatively with overall clinical response (R=0.36; p=0.049).

Mean CCR2 expression on all three monocyte subsets remained stable during therapy. Interestingly, a negative correlation was found between change in CCR2 expression on non-classical monocytes and the composite overall response to treatment (R=0.37; p=0.03).

DISCUSSION

By investigating monocyte subset changes during 26 weeks of treatment with infliximab, we found a striking decrease in the percentage of non-classical monocytes and an increase in the percentage of classical monocytes during therapy, while the absolute number of monocytes remained unchanged. Furthermore, expression of receptor TNFR1 on classical monocytes decreased, while receptor CCR5 on intermediate and non-classical monocytes increased significantly during therapy. Moreover, changes in CCR2 and CCR5 expression correlated with the overall clinical response.

A decrease in percentage of non-classical monocytes during infliximab therapy has been described before in a case report of a sarcoidosis patient in which a decrease of CD16+ cells paralleled a good response to infliximab after two infusions of 3 mg/kg. However, no subdivision was made between intermediate and non-classical CD16+ monocytes in this report [29]. The change in

non-classical monocytes during infliximab therapy in our cohort did not correlate with the degree of clinical response to treatment. However, it should be mentioned that the vast majority of our cohort comprised responders and it is possible that the decrease of pro-inflammatory non-classical monocytes found in our cohort does in fact reflect response to therapy.

The fraction of intermediate monocytes was increased in our sarcoidosis cohort as described before [13] and responded dynamically to therapy, with an initial increase in percentage after 14 weeks (4 infusions), followed by a return to baseline after 26 weeks (8 infusions). A similar initial increase of intermediate monocytes following infliximab therapy was seen in patients with Crohn's disease after 3 infusions [19]. The authors of that work proposed that this could be a compensatory response of the host to TNF depletion, as intermediate monocytes are high TNF producers [18,19]. Long-term effects were not studied by Nazareth and colleagues. In our cohort of sarcoidosis patients, after 26 weeks (8 infusions) of infliximab this increase had disappeared. Using a different anti-TNF antibody, adalimumab, in treatment of psoriasis patients, Brunner and colleagues showed a lower percentage of intermediate monocytes in adalimumab treated patients compared to untreated patients [30]. Overall, it appears that an initial compensatory increase in percentage intermediate monocytes is seen in response to depletion of TNF, which later stabilizes. This indicates that 26 weeks of infliximab treatment does influence intermediate monocytes, without normalization to levels found in healthy controls.

When depleting TNF, it is of interest how this influences expression of its receptors on monocytes. We previously described differential expression of TNF-receptors 1 and 2 on the various monocyte subsets in patients and healthy controls [13]. When treated for 26 weeks with infliximab therapy, TNFR1 on classical monocytes decreased significantly whereas expression of TNFR2 did not change. This might be explained by the notion that most of the biological activities of TNF are mediated through TNFR1 [31]. Our results support the idea that depletion of TNF by infliximab directly affects TNFR expression by monocytes.

Chemokines and their cognate receptors are important for recruitment of monocytes, particularly to inflammatory sites [21,22]. Elevations of CCL2 and CCL5 concentration in BAL fluid have been associated with pulmonary sarcoidosis [23]. Changes of chemokine receptors on monocytes during anti-TNF treatment have not been described before in sarcoidosis or other diseases. In our cohort of severe sarcoidosis patients, we found that CCR5 expression on intermediate and non-classical monocytes increased after 26 weeks of infliximab therapy. Furthermore, change in CCR2 and CCR5 expression on non-classicals correlated inversely with treatment response, indicating that chemokine expression increased most in patients with the lowest response. As chemokine receptors, and CCR2 in particular, are important for monocyte recruitment to inflammatory sites, this indicates that in patients with the lowest response, recruitment of monocytes to sites of inflammation is still ongoing. One study investigating chemokine receptor expression on T-cells in rheumatoid arthritis found decreased CCR2 expression on T-cells during infliximab therapy [32].

In order to interpret our findings it is important to determine whether and how monocyte subsets relate to each other, with respect to sarcoidosis. Recently, a possible modulatory effect of

non-classical monocytes on classical monocytes was found in systemic lupus erythematosus [33]. This indicates that the various subsets of monocytes have the ability to interact. Whether this influences the way the various subsets behave after treatment with infliximab in sarcoidosis remains to be determined.

We found correlations between ACE level and percentage of monocytes and ratio of monocyte subsets. ACE is normally produced by pulmonary epithelioid cells and elevated in sarcoidosis due to overproduction in sarcoid granulomas [34]. In mice, the ACE – Angiotensin II pathway has a driving effect on the production of monocytes/macrophages and is thought to sustain inflammation [35,36]. Furthermore, studies in humans have shown immunomodulating effects of ACE-inhibitors [37,38]. Whether and how ACE influences monocyte subsets in sarcoidosis through a yet undefined mechanism or that ACE increase is merely a reflection of disease activity correlating with monocyte subsets in sarcoidosis, cannot be concluded from our data, but has to be investigated further.

A fascinating new therapeutic strategy for sarcoidosis recently described is the combined monocyte and granulocyte apheresis [39]. Although only seven patients were included in a pilot study, the positive effects on dyspnoea score along with the decrease in the percentage of lymphocytes underline the importance of monocytes in the sarcoidosis disease process.

During infliximab therapy, we revealed phenotypic changes in the ratio of monocyte subsets and receptor expression. In future experiments it will be of interest to perform functional tests on monocytes and/or macrophages of sarcoidosis patients to study whether the phenotypic change in monocytes influences the in vitro excretion levels of cytokines pre- and post-infliximab treatment. Furthermore, recently described differences in transcription factor profiles of the three subsets of monocytes also indicate functional differences between the subsets [40]. Other studies favour a linear differentiation model in which non-classicals represent the monocyte subset with most mature differentiation [41,42]. The decrease in the, more mature, non-classical monocytes observed in our cohort could be due to a left shift after increased monocyte apoptosis after infliximab therapy. Monocyte apoptosis after anti-TNF therapy has been described in in vitro experiments in rheumatoid arthritis and Crohn's disease [43,44]. Transcriptome analysis of sarcoid monocyte subsets, in comparison with subsets from healthy donors, will be of particular interest to better understand the processes of cellular differentiation, inflammation and ultimately response to therapy.

In conclusion, during infliximab therapy, the percentage of non-classical monocytes decreases significantly paralleled by an increase of CCR5 expression on non-classicals and intermediates and a decrease of TNFR1 on classical monocytes. Our findings show that the ratio of monocyte subsets reverts to normal during infliximab therapy, and suggest that each subset has a different role in sarcoidosis. Further investigation of their function may help us to understand the disease process and predict response to therapy.

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PART V

CLINICAL ASPECTS OF TREATMENT IN COMPLICATED SARCOIDOSIS

CHAPTER 11.1

EARLOBE SARCOIDOSIS

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Sarcoidosis Vasc Diffuse Lung Dis. 2012;29:55-57

ABSTRACT

BACKGROUND

Infliximab, a TNF-α blocking agent, is an upcoming therapeutic option for cases of refractory sarcoidosis. In pulmonary sarcoidosis, changes imaged by ^{18}F-FDG PET during infliximab treatment in sarcoidosis patients correlate with signs of clinical improvement.

DESIGN

Case-report

RESULTS AND CONCLUSIONS

A patient with severe earlobe sarcoidosis, treated with infliximab, is presented. This case shows that even relatively small extrapulmonary localisations of sarcoidosis can be visualised by ^{18}F-FDG PET, and that a decrease of FDG-uptake correlates well with clinical improvement on infliximab treatment.

INTRODUCTION

Infliximab, a TNF-α blocking agent, is an upcoming therapeutic option for cases of refractory sarcoidosis [1;2]. In pulmonary sarcoidosis, changes imaged by ^{18}F-FDG PET during infliximab treatment in sarcoidosis patients correlate with signs of clinical improvement [3].

CASE REPORT

A 56-year old male Caucasian patient was referred to our out-patient clinic because of complex sarcoidosis. Alongside fatigue and dyspnoea, he suffered from severe and incapacitating pain and swelling of the right earlobe, histologically proven to be sarcoidosis (Fig. 1). Previously, he was treated with high dose prednisone, methotrexate, plaquenil and local corticosteroid injections without sufficient response. Since his quality of life was severely impaired, we decided to treat him with infliximab therapy. After six intravenous gifts of 5 mg/kg bodyweight, the pain and swelling of his earlobe reduced significantly. Evaluation of disease activity using ^{18}F-FDG PET showed a marked improvement of his pulmonary sarcoidosis and normalisation of the previously active right earlobe with a decrease in SUVmax from 4.5 to 0.6 (Fig. 2).

CONCLUSION

This case shows that response to infliximab can be measured by ^{18}F-FDG PET, even in cases of relatively small extrapulmonary lesions, such as earlobe sarcoidosis. A decrease of FDG-uptake correlates well with clinical improvement on infliximab treatment.

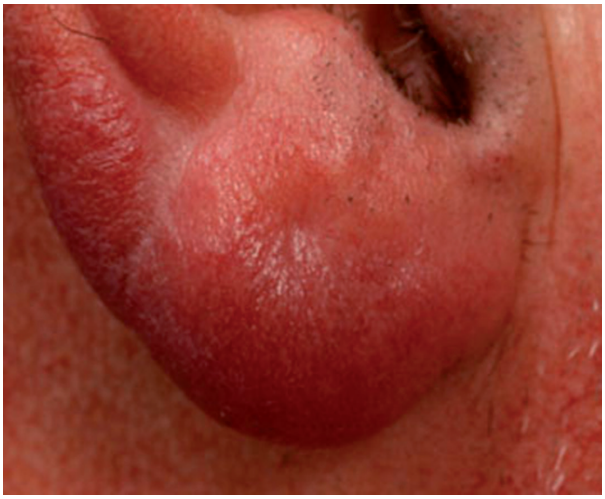

Figure 1: Right earlobe with painful swelling

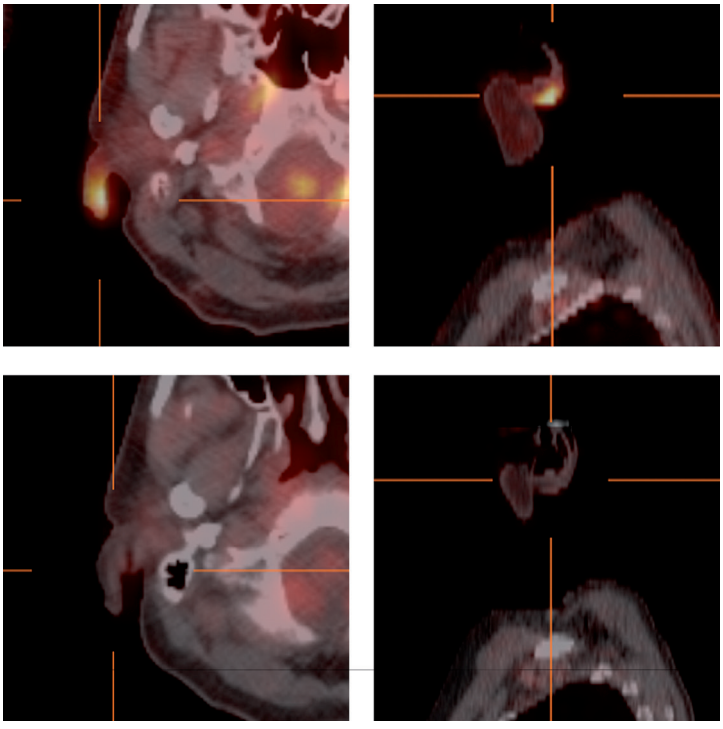

Figure 2: ^{18}F-FDG-PET scanning with focus on right earlobe before (first row) and after (second row) treatment with 6 doses of infliximab

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CHAPTER 11.2

BILATERAL VOCAL CORD CARCINOMA IN A SARCOIDOSIS PATIENT DURING INFLIXIMAB THERAPY

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Elisabeth V Sjögren
Coline HM van Moorsel
Jan C Grutters

Case Case Rep Pulmonol 2013 Epub May 15.

ABSTRACT

INTRODUCTION

Although the role of TNF-α in tumor development is not fully understood, an increased risk of malignancy with TNF-α- inhibitors, such as infliximab has been suggested.

CASE-PRESENTATION

We present a 54 year old non smoking female sarcoidosis patient. After seven months of infliximab therapy a T1aN0M0 larynx carcinoma of the right vocal cord was found and excised. Within a year, whilst still on treatment, a second larynx carcinoma of the opposite vocal cord appeared.

DISCUSSION

A bilateral vocal cord tumor is rare, especially in a never smoker. Evidence on the role of infliximab in carcinogenesis is inconclusive. To date, there are no follow-up studies evaluating malignancy risk of infliximab therapy in sarcoidosis patients. No studies in other diseases focus on laryngeal carcinomas during infliximab use. We argue that infliximab treatment might have attributed to the rapid progression of vocal cord carcinomas in this patient with an a priori low risk tumor profile. This case illustrates that caution remains warranted in patients with previous malignancies when considering initiation of TNF-α-inhibitors.

INTRODUCTION

Tumor necrosis factor-α (TNF-α) inhibitors such as infliximab are used in treatment of various diseases like rheumatoid arthritis, Crohn's disease, ankylosing spondylitis and psoriasis. Today, infliximab is also an upcoming therapeutic option for cases of severe pulmonary and/or extra-pulmonary sarcoidosis refractory to standard therapy [1].

Besides its key role in inflammation, TNF-α has several qualities that may have impact on carcinogenesis, tumor growth and the time-point of clinical detection of malignancies. Although the role of TNF-α in tumor development is not fully understood, an increased risk of malignancy with TNF-α- inhibitors has been suggested in other diseases than sarcoidosis [2-4].

CASE REPORT

We present the case of a 54 year-old female patient who was diagnosed with five years earlier with Scadding stage II sarcoidosis. Diagnosis was biopsy proven with non-caseating granulomas found in endobronchial biopsies. Pulmonary complaints were dyspnoea on exertion and coughing. Moreover, her sarcoidosis was accompanied by severe small fibre neuropathy which was invalidating in every day life. Other extra-pulmonary symptoms were extrathoracic lymphnodes. A trial of prednisone failed due to severe psychological side effects. The severity of her symptoms and established disease activity on 18F-fluorodeoxyglucose positron emission tomography (^{18}F-FDG PET) scan prompted the decision to initiate infliximab therapy. Infliximab was administered at a monthly dose of 5 mg/kg bodyweight accompanied by a low dose of methotrexate (7.5 mg/week). She visited a Ear Nose Throat (ENT) specialist for complaints of varying hoarseness. She had never smoked and did not consume alcohol. There was no history of passive smoking or occupational exposure as she had an office job. Biopsy of the vocal cord did not reveal an underlying malignancy

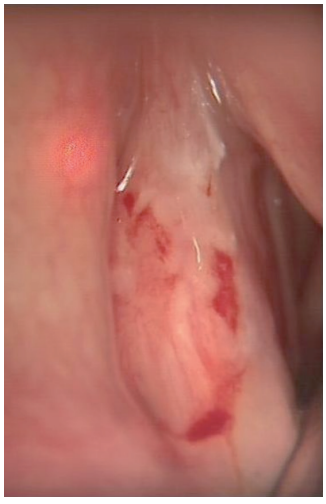

Figure 1: Left sided T1aN0M0 vocal cord carcinoma under surgical microscope

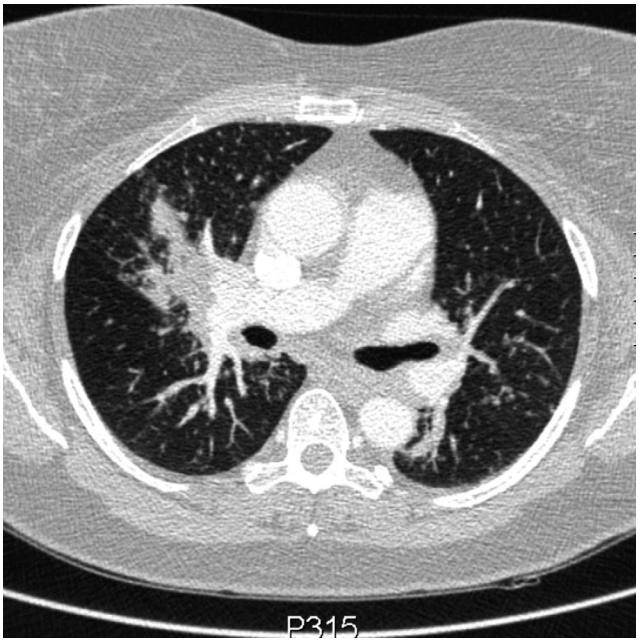

Figure 2: HRCT of the thorax after discontinuation of infliximab showing increased consolidation and nodular abnormalities

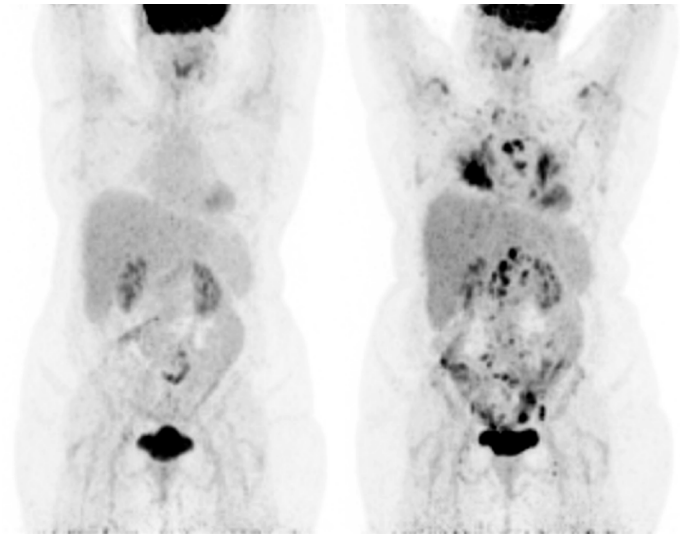

Figure 3: ^{18}F-FDG PET scan monitoring sarcoidosis activity level
In the left frame is shown the ^{18}F-FDG PET scan during treatment with infliximab and in the right frame the ^{18}F-FDG PET scan after discontinuation of infliximab revealing reactivation of sarcoidosis as black spots in lungs and lymph nodes

and it was diagnosed as chronic laryngitis. She was treated with speech therapy, proton pump inhibitors and antifungal therapy.

Seven months after initiation of infliximab therapy she revisited her ENT-specialist with increasing vocal problems. Biopsy of the right true vocal cord revealed a squamous cell carcinoma with extensive necrosis, which was radically excised using laser surgery. After staging of the lymph nodes and exclusion of pulmonary metastases by chest X-ray, it was classified as a T1aN0M0 larynx carcinoma of the right true vocal cord. After elaborate discussions, she continued infliximab therapy because of the unlikely relation with the vocal cord tumor, full recovery from her vocal cord malignancy after ENT-surgery and the positive effect on her sarcoidosis with increased quality of life. Within a year her voice turned increasingly hoarse and a second larynx carcinoma on the opposite vocal cord was discovered (Fig 1). This vocal cord tumour was treated with laser excision as well. Infliximab therapy was now discontinued on behalf of the uncertain but possible relationship between infliximab and the bilateral larynx carcinomas. At the moment of the second ENT surgery her sarcoidosis symptoms were stable. Unfortunately, half a year after infliximab discontinuation the small fibre neuropathy and pulmonary symptoms returned as shown on High Resolution CT of the chest (Fig 2) and on ^{18}F-FDG PET scan which revealed extensive systemic reactivation of sarcoidosis (Fig 3).

DISCUSSION

Laryngeal cancer is generally uncommon in males and very rare in females being the 22nd most frequent cancer in females. Vocal fold cancer constitutes for half of laryngeal cancers. The highest rate in females is found in the United States African American population and is 3/100.000 [5]. Vocal fold squamous cell carcinomas most often develop on healthy mucosa, but precancerous lesions can occur. The main risk factor is smoking of tobacco, alcohol consumption further increases the risk especially when combined with smoking [5].

A bilateral vocal cord carcinoma is extremely rare, especially in a patient without risk factors such as smoking or alcohol use. In patients with a larynx carcinoma, the rate of development of a second metachronous primary cancer of the upper aerodigestive tract is around 1% per year. Patients with a history of smoking by far had the highest risk in this group [6].

To date, there are no follow-up studies evaluating malignancy risk of infliximab therapy in sarcoidosis patients. No studies in other diseases focus on laryngeal carcinomas during infliximab use. Studies on the possible increased risk of malignancy by infliximab have been performed in other diseases with varying outcomes. Some studies and case reports did suggest a relationship between infliximab and malignancy [2-4]. Several larger studies performed in Crohn's disease, rheumatoid arthritis and psoriasis did not find an increased risk [7-9]. One large meta-analysis including 22.904 patients treated with different TNF-α-inhibitors only found an increased risk of non-melanoma skin cancer but no increased risk of other cancers [10].

Because the association between infliximab and malignancy has not been elucidated, we did not stop treatment with infliximab after the first vocal cord carcinoma occurred. This is in line with the

Dutch guideline on responsible use of biologicals which does not determine previous malignancy a strict contra-indication. Moreover she fully recovered after the first ENT-surgery and infliximab was the only therapy that significantly improved the patients symptoms of sarcoidosis. However, in retrospect this might not have been the right decision for this patient.

It could be possible that the carcinogenic effect of infliximab only affects certain patients with an underlying genetic susceptibility. We argue that infliximab treatment might have contributed to the rapid development of vocal cord carcinomas in this patient with an à priori low risk tumor profile, but currently unknown genetic susceptibility. In this apparently malignancy prone patient we therefore did not restart infliximab therapy for the relapse of her sarcoidosis symptoms.

This case illustrates that even though guidelines do not provide strict contra-indication, caution is warranted in patients developing their first malignancy shortly after initiation of infliximab therapy and in patients with previous malignancies when considering initiation of TNF-α-inhibitors. More knowledge should be gained on tumor risk after infliximab therapy in patients with previous malignancies, possibly discouraging use of TNF-α-inhibitors in future cases.

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CHAPTER 12

SUMMARY AND GENERAL DISCUSSION

SUMMARY

The main goal of this thesis was to investigate various second and third-line systemic therapeutic options in severe sarcoidosis, that is refractory to first-line therapy, and to evaluate which patients benefit most from the different treatment options in order to find predictive markers for clinical decision making.

In **chapter 2**, a complete overview of all currently available therapeutic strategies in sarcoidosis is given. Corticosteroids (e.g. prednisone) remain the mainstay of therapy in sarcoidosis since their first use in this disease in the 1950s. A second-line therapeutic is often added to the treatment regimen in case of intolerable side effects, inefficacy or prolonged use of steroids. Methotrexate is considered by many to be the first choice drug in second-line therapeutics of sarcoidosis, but other frequently used second-line drugs are azathioprine and leflunomide. No large trials comparing different treatment options have been performed in sarcoidosis. In patients with severe disease, who do not respond well to first and second-line therapy, biologicals such as infliximab can be promising. In addition to an overview of current therapies, gaps in current literature regarding sarcoidosis treatment are depicted to underline the importance of research in this mostly empiric field of medicine. Furthermore, we highlight future medicine in sarcoidosis with emphasis on the role of personalised medicine.

In **chapter 3**, we evaluated second-line therapy in the first study comparing the two most often used second-line drugs. In a retrospective international cohort-study, we included 200 patients from St Antonius Hospital, Nieuwegein, the Netherlands and University Hospital Leuven, Belgium of whom 145 received methotrexate and 55 received azathioprine. We demonstrated a decrease in prednisone daily dose by a mean of 6.32 mg per year ($P<0.0001$) whilst on therapy, with a similar steroid-sparing capacity for methotrexate and azathioprine. Of all patients completing 1 year of therapy, 70% had a reduction in daily prednisone dose of at least 10 mg. Furthermore, methotrexate and azathioprine had a similar positive effect on lung function, and comparable side effects, except for a higher infection rate in the azathioprine group.

Chapter 4 investigates the use of serum activity markers angiotensin converting enzyme (ACE) and soluble interleukin-2 receptor (sIL-2R) during methotrexate therapy, as currently the clinical value of these markers is still under debate. We analysed 114 sarcoidosis patients who used methotrexate for six months and found that high baseline levels of ACE correlated significantly with lung function improvement after treatment. After six months of methotrexate, mean ACE and sIL-2R decreased significantly ($p<0.0001$). Decreases in both ACE and sIL-2R correlated with an increase in lung function. The strongest correlation was found between change in ACE and change in diffusion capacity of the lung for carbonmonoxide (DLCO) ($R=0.63$, $P<0.0001$). Measurements of these biomarkers are recommended when monitoring treatment effects in sarcoidosis patients and are most suited for follow-up.

In **chapter 5**, the off-label use of infliximab, a monoclonal antibody targeted against tumor necrosis factor alpha (TNFα) was evaluated as third-line therapeutic option. Although the effect of infliximab therapy in rheumatoid arthritis and Crohn's disease has been well documented, literature on

infliximab therapy in sarcoidosis is scarce. In this study we therefore aimed to evaluate change in function, disease activity and quality of life (QoL) after infliximab treatment in patients with refractory sarcoidosis. In this retrospective cohort study consisting of 48 patients, a significant decrease in the serum markers ACE and sIL-2R and of Maximum Standardised Uptake value (SUVmax) on ^{18}F-fluorodeoxyglucose by positron emission tomography (^{18}F-FDG PET) was found. Furthermore, a significant improvement in fatigue severity and physical functioning scores was observed. In patients with a pulmonary treatment indication, a significant and clinically relevant improvement in vital capacity (VC) (7.6%;$p<0.0001$), forced expiratory volume in one second (FEV1) (7.9%; $p<0.0001$) and DLCO (3.5%; $p=0.011$) was found. This leads us to conclude that infliximab improves disease activity, lung function and QoL in patients with refractory sarcoidosis. From research in other immune mediated inflammatory diseases (IMIDs), such as Crohn's disease, psoriasis and rheumatoid arthritis, we know that formation of antinuclear antibodies (ANAs) can occur during infliximab therapy. The ANA level at baseline as well as development of ANAs during treatment are suggested to correlate with decreased treatment response and formation of antibodies towards infliximab (ATIs). To date, it has not been studied whether ANA formation during infliximab therapy occurs in sarcoidosis patients. **Chapter 6** presents our experience with formation of ANAs during infliximab therapy in sarcoidosis patients. In a retrospective cohort study of 39 sarcoidosis patients treated with infliximab we found ten ATI-positive patients (29%). However, only one patient had increased ANA reactivity at baseline (titre 1:320). A total of only four patients developed modest ANA reactivity during treatment of whom only one was ATI-positive. Our study therefore shows that, in sarcoidosis patients, the presence of antibodies towards infliximab is not reflected by the presence nor development of antinuclear antibody reactivity. This suggests that antinuclear antibody formation during infliximab treatment might be disease specific.

In **chapter 7**, results of the first prospective open-label study on infliximab in sarcoidosis were described extensively. In this trial, 56 patients with sarcoidosis refractory to conventional treatment and persistent disease activity on ^{18}F-FDG PET were prospectively included. Response was measured on three dimensions: the functional response, the inflammatory response and response in QoL. A considerable improvement in forced vital capacity (FVC) of 6.6% predicted was seen in patients with a pulmonary treatment indication. Furthermore, Maximum Standardised Uptake Value (SUVmax) of pulmonary parenchyma on ^{18}F-FDG PET decreased by 3.93 ($p<0.0001$). High SUVmax of the pulmonary parenchyma on ^{18}F-FDG PET at baseline predicted FVC improvement ($R=0.62$; $p=0.0004$). This is the first prospective study in a real life clinical design showing a high response rate to infliximab therapy in selected patients with active sarcoidosis. These results suggest that ^{18}F-FDG PET is useful in therapeutic decision making in complex sarcoidosis.

Although infliximab appears beneficiary in the short run, a question is raised on long-term infliximab efficacy and relapse rate. In **chapter 8** we provide evidence for a high relapse rate after discontinuation of infliximab therapy in sarcoidosis patients. In the retrospective cohort study including 47 sarcoidosis patients who started infliximab therapy, Kaplan–Meier analysis revealed a median time to relapse of 11.1 months and showed that 25% of the cohort had relapsed within

4 months. The majority of patients who discontinued infliximab therapy relapsed within the follow-up period (62%). High serum sIL-2R (hazard ratio 2.24, p=0.033) and high SUVmax on ^{18}F-FDG PET (hazard ratio 4.33, p<0.001) at initiation of therapy were significant predictors of relapse. These results suggest that close monitoring of patients in this category is indicated upon discontinuation of infliximab treatment.

TNFα is an important cytokine in the inflammatory process of sarcoidosis which signals through binding of two TNF-receptors (TNFRs). In **chapter 9** data is presented regarding differential expression of TNFR1 and TNFR2 on subpopulations of human monocytes in sarcoidosis patients and healthy controls. Sarcoidosis patients had a significantly higher frequency of intermediate monocytes than healthy controls. In both sarcoidosis patients and healthy controls, intermediate monocytes show the highest expression level of TNFR1 among monocyte subpopulations and nonclassical monocytes show the highest expression level of TNFR2. These new findings provide evidence for the existence of two functionally-distinct CD16 expressing monocyte subpopulations, that might be of relevance in granuloma genesis and/or infliximab responsiveness.

Elaborating on the previous findings of monocyte subsets and TNFRs, **chapter 10** presents preliminary results of our research on changes in monocyte subsets during infliximab therapy in a prospective cohort of refractory sarcoidosis patients. The effect of TNF blockade on monocytes in sarcoidosis has not been studied before. We collected blood of 38 patients with severe and active sarcoidosis and also of 18 healthy controls and found that the percentage of monocytes at baseline correlated with ACE and sIL-2R. During therapy, the percentage of non-classical monocytes decreased from 15.6% to 12.3% (p=0.0063). Furthermore, the percentage of classical monocytes expressing TNFR1 decreased significantly (p=0.0065), while TNFR2 expression remained stable throughout therapy. Mean chemokine receptor CCR5 expression increased significantly on both intermediate monocytes and on non-classical monocytes (p=0.001). Both the change in expression of chemokine receptors CCR5 and CCR2 on non-classicals correlated with response to treatment. Our findings suggest that each monocyte subset has a different role in sarcoidosis. Further investigation of their function may help to understand the disease process and predict response to therapy.

In **Chapter 11** two special cases illustrate clinical practice of sarcoidosis treatment with infliximab. **Chapter 11.1** shows that sarcoidosis is a disease that can have diverse manifestations, such as sarcoidosis of the earlobe. In this case, the granulomatous inflammation of the earlobe caused severe pain which was a reason for therapy. After topical therapy and first- and second-line systemic therapy had failed, the patient started with infliximab therapy. The ^{18}F-FDG PET scan before initiation showed high uptake in the earlobe which disappeared after infliximab therapy. Furthermore, infliximab treatment decreased his symptoms. This case shows that even relatively small extrapulmonary localisations of sarcoidosis can be visualised by ^{18}F-FDG PET, and that a decrease of FDG-uptake correlates well with clinical improvement upon infliximab treatment.

In **Chapter 11.2** we illustrate that infliximab therapy possibly can have severe side effects in susceptible patients. A case of severe sarcoidosis, refractory to standard first and second-line

treatment, is presented. After seven months of infliximab therapy a T1aN0M0 larynx carcinoma of the right vocal cord was found and excised. Within a year, whilst still on treatment, a second larynx carcinoma of the opposite vocal cord appeared.
A bilateral vocal cord tumor is rare, especially in a never smoker. However, evidence on the role of infliximab in carcinogenesis is currently inconclusive. We argue that infliximab treatment might have attributed to the rapid progression of vocal cord carcinomas in this patient with a priori low tumor risk. This case illustrates that caution remains warranted in patients with previous malignancies when considering initiation of TNF-inhibitors.

GENERAL DISCUSSION

Sarcoidosis is a rare granulomatous disease of unknown origin. The disease can have a wide variety of clinical manifestations and outcomes, and is self remitting in the majority of patients [1]. The answer to the question why innovation and knowledge on treatment of sarcoidosis is far behind from other diseases, can directly be derived from those two sentences which constitute its definition.
First, the widely accepted hypothesis is that sarcoidosis is an exaggerated granulomatous reaction after exposure to unidentified antigens in individuals who are genetically susceptible, but the true cause of sarcoidosis is unknown [1,2]. As a result, no curative treatment exists and all currently used drugs are used in sarcoidosis after extrapolation of positive results in other inflammatory diseases [3].
Second, sarcoidosis has a very heterogeneous disease character. Sarcoidosis aetiology is known to have a genetic component, but also exposure to various organic and inorganic substances is thought to be causally involved in its pathogenesis [4,5]. Furthermore, large differences exist between various phenotypes of sarcoidosis, for example fibrotic pulmonary disease, cardiac sarcoidosis and small fibre neuropathy. These different disease phenotypes and possible heterogeneity in underlying mechanisms likely require different treatment strategies.
And last, sarcoidosis is a rare disease and with a prevalence of less than 1 per 2,000 inhabitants in the Netherlands it is categorised as an orphan disease [6]. Moreover, the fact that only a minority of sarcoidosis patients require systemic treatment, results in small sample sizes in the few available trials on severe sarcoidosis. These and other aspects regarding sarcoidosis treatment will be discussed into more detail in the following section.

DEFINING RESPONSE TO THERAPY IN SARCOIDOSIS

Studies investigating therapy in sarcoidosis struggle with the absence of a validated composite endpoint of response, which is applicable for both pulmonary and all forms of extrapulmonary sarcoidosis.
Hard endpoints in pulmonary function tests such as FVC are often used in pulmonary sarcoidosis research as they are easy to use and reproducible [7]. The minimal clinically important difference for FVC in sarcoidosis, however, is not known. Extrapolating from its use in another interstitial

lung disease, idiopathic pulmonary fibrosis (IPF), a minimal important change of 5% of predicted is likely useful [3,8]. A downfall of pulmonary function tests, is that they do not cover the various aspects of extrapulmonary sarcoidosis.

In other IMIDs, composite scores of clinical and laboratory tests have been successfully implemented into research and clinical settings. The Disease Activity Score with 28 joint counts (DAS28) enables rheumatologists to easily compare the treatment effects found in various randomised controlled trials (RCTs) [9,10] and the Psoriasis Area and Severity Index (PASI) is used in most trials in psoriasis because it is an easy and validated test [11]. In an attempt to objectify extrapulmonary sarcoidosis outcomes onto a similar scale, some proposals have been made, but none have found their way into clinical practice on a global scale. Judson and colleagues proposed the extrapulmonary Physician Organ Severity Tool (ePOST), which was used in post-hoc analysis of extrapulmonary involvement in the largest trial of infliximab in sarcoidosis [12]. This tool has not been validated nor used in other studies and has also received some criticism, because it does not differentiate between asymptomatic involvement, morbidity without danger and major and potentially life-threatening organ involvement [13]. Another instrument, the WASOG Sarcoidosis Organ assessment Tool, was recently adjusted and establishes criteria for sarcoidosis organ involvement, however this tool is not suited for follow-up of patients during therapy and measure response [14].

In chapter 7, we investigated infliximab therapy in a prospective cohort and, in addition to demonstrating a response in organ function, we used a composite scoring system based on three dimensions of response: function, inflammation and QoL. Whether this composite overall response score could be the basis for a scoring system similar to DAS28 in rheumatoid arthritis [9,10] should be investigated further in a larger cohort of sarcoidosis patients preferably with various disease severities.

Another response endpoint which has gained increasing attention over the past decade is QoL [15]. The scarce RCTs that are conducted in sarcoidosis treatment mostly include this topic as secondary endpoint. However, lack of uniformity in questionnaires used, hinders the possibility to compare various studies. Most studies have measured quality of life in sarcoidosis by means of the generic questionnaire 36-Item Short-Form Health Survey [15]. However, the fatigue assessment scale (FAS), is the only questionnaire especially designed for sarcoidosis [16], but only covers complaints of fatigue.

Another approach is to define treatment response based on cellular changes during therapy. In chapter 10 a first attempt was made describing cellular responses in monocyte subsets during infliximab therapy. How the changes seen in monocyte subsets translate into clinical practice has not been determined yet. The recently found positive results with monocyte and granulocyte apheresis confirm an important role for these cells [17]. More in vitro and human research on cellular responses during therapy should be performed to better understand inter-patient variability, but also to possibly gain insight into etiological mechanisms in sarcoidosis.

TIMING AND DURATION OF TREATMENT

The vast majority of papers regarding any type of sarcoidosis treatment concern short-term treatment outcome, with no available information on the long-term effectiveness [18]. Because all available therapeutics have side effects and toxicity as downside [19], physicians ideally attempt to keep patients as stable as possible, with as little therapy as needed. However, no guidelines specifically describe when to start tapering second and third line therapy and which tapering regimen to use [2,18,20]. In chapter 8 we describe that sarcoidosis patients often relapse after infliximab therapy has been discontinued [21], and we suggest that in patients with high disease activity on ^{18}F-FDG PET and sIL-2R in particular, therapy should not be discontinued hastily. Unfortunately, the optimal duration of therapy in this group of patients has not been studied yet.

Research in other IMIDs, such as rheumatoid arthritis, is far ahead of the field of sarcoidosis. Although most used drugs in sarcoidosis have been extrapolated from rheumatoid arthritis or inflammatory bowel disease (IBD), the approach to initiation of treatment is different. In sarcoidosis, a step-up treatment regimen is followed with increasing treatment intensity after first and second-line treatment have failed [2,19]. In rheumatoid arthritis the 'hit hard, hit early' strategy has been introduced. Instead of 'wait and see' and 'step-up' regimens, patients are treated with biologicals in an early stage of disease to prevent disease progression and concomitant morbidity [22]. Because sarcoidosis is such a heterogeneous disease and most patients recover without treatment, it would be difficult to select the right patients for this strategy. Furthermore, whether a more aggressive treatment strategy would prevent selected severe sarcoidosis patients from eventually deteriorating in the long run, should be subject of new investigations.

The idea that tempering the exaggerated immune response is beneficial in sarcoidosis is attractive. However, for none of the therapeutic interventions currently employed in sarcoidosis treatment, it is known whether they can render a truly positive effect on prevention of fibrosis or mortality [2]. One of the hypotheses is that treatment does not alter the natural disease evolution of a patient. It could be genetically determined whether patients develop end stage fibrotic disease, in the same manner as wound healing function and dermal scar formation are genetically influenced [23,24]. A comparison can also be drawn with IBD, which consists of two different diseases with different phenotypes. Crohn's disease is very susceptible to scar formation and Colitis Ulcerosa is not [25]. An implication of genetic variation of TGF-beta3 has been suggested to be associated with fibrosis developing in sarcoidosis patients [26]. With this hypothesis in mind, immunosuppressive therapy would not prevent fibrosis formation in genetically or otherwise fibrosis prone sarcoidosis patients. However, sarcoidosis patients with a 'fibrotic phenotype' could possibly present a new target group for the antifibrotic drugs pirfenidone and nintedanib, which currently create promising expectations in treatment of IPF [27,28].

A way of investigating whether new biological interventions alter long-term outcomes in sarcoidosis would be to conduct a large international trial in which disease outcomes in a historically matched cohort (e.g. before introduction of biologicals) would be compared to biological therapy.

PERSONALISED MEDICINE

'Personalised medicine' is a term which is used to indicate the selection of the most appropriate pharmacological therapy for an individual patient implying maximal effectiveness with minimal side-effects [29]. As sarcoidosis can have various disease phenotypes and an unpredictable clinical course, response to pharmacological treatment is diverse.

Little is known on whether specific patient and disease characteristics can possibly predict response to currently available therapy in sarcoidosis. A recent review of literature and expert opinion provides some handles to appoint a specific sarcoidosis organ involvement to the most appropriate therapy [30]. The review also covers therapy in sarcoidosis associated pulmonary hypertension, in which anti-inflammatory drugs do not work.

In chapter 3 we showed that patients with the highest levels of ACE and sIL-2R generally achieve the highest gain in pulmonary function after six months of methotrexate therapy. Furthermore, in chapter 7 we show that patients with high activity on ^{18}F-FDG PET, have the largest improvement in pulmonary function after six months of infliximab therapy. These combined results show that in general, the largest effect of immunosuppressive therapy can be expected in patients with high disease activity.

To date, studies in severe sarcoidosis treatment have focused mostly on therapy with anti-inflammatory drugs. It is possible that instead of anti-inflammatory treatment, also anti-fibrotic, antibiotic and anti-mycotic drugs might be beneficial to the appropriate patients [27,28,31-33]. Moreover, a personally guided combination of anti-inflammatory, anti-fibrotic, antibiotic, anti-mycotic and/or anti-pulmonary hypertension therapy could be the future in sarcoidosis treatment.

Besides the exploration of these phenotypic predictors of response, the principles of pharmacogenetics also become increasingly important in personalised medicine. An example is Thiopurine Methyltransferase (TPMT)-genotyping, which is used in daily practice in sarcoidosis patients starting azathioprine therapy. The lower the TPMT activity, the higher the risk of developing toxicity, especially myelosuppression [34]. Recently, absence of the TNF -308 variant A-allele in sarcoidosis patients refractory to conventional treatment was shown to be associated with better response to TNF-α inhibitors, suggesting a possible use for TNF G-308A polymorphism genotyping when optimising therapy [35]. However, these results should be interpreted with caution because the TNF gene is located closely to and in strong linkage with the HLA-DRB1 gene [36]. Concluding, although in other pulmonary fields, such as pulmonary oncology, pharmacogenetics have a major role [37], in sarcoidosis the value of pharmacogenetics when tailoring pharmacotherapy still needs further exploring.

OFF-LABEL USE IN AN ORPHAN DISEASE

As described above, sarcoidosis is an orphan disease with less than 1 per 2000 inhabitants of The Netherlands affected [6]. Only half of patients require therapy for their disease at any point in time [30]. The category of patients refractory to standard first and second line therapy, who require

the use of biologicals, comprises an even smaller portion of less than 5% of sarcoidosis patients. Due to small numbers of patients with severe disease, most available evidence comes from retrospective cohort studies and expert opinions [3,18]. Unfortunately, pharmaceutical companies are hesitant to further invest in the field of orphan diseases, especially when a drug has already been approved for other treatment indications [38]. To date, none of all drugs described in this thesis have been registered for use in sarcoidosis [39]. As most used first and second-line drugs, such as corticosteroids or methotrexate, are generically available and therefore less expensive, these drugs do not tend to generate endorsement problems. The more expensive biological therapies such as infliximab or adalimumab, however, are still not generally endorsed by health care insurance companies in many countries (including The Netherlands) and their use remains off-label [39]. Due to low patient numbers, treatment of rare diseases will never meet the criteria used for registration of new drugs for more common diseases. Reimbursement of drugs for rare diseases should therefore be facilitated more easily, for example when efficacy could be expected based on pathophysiology or effect in other diseases [40]. Financial endorsement for off-label use of biologicals in a selected group of severe sarcoidosis patients needs to be better regulated, especially because this number is estimated to be of low occurrence (< 1:150000 inhabitants of the Netherlands). Care for severe sarcoidosis patients should be clustered in authorised expertise centres of excellence, which can register patients and conduct research on treatment outcomes. Only through cooperation of pharmaceutical companies, health insurances companies and centres of excellence, especially in combination with outcome research on efficacy of these drugs, progress can be made.

CONCLUDING REMARKS

This thesis was written as an attempt to increase insight into systemic treatment strategies in sarcoidosis and improve current practice in complicated and/or life-threatening sarcoidosis.

International cooperation with uniform registration of treatment outcome and side effects in severe sarcoidosis patients, could lead to higher numbers in (retrospective) trials on sarcoidosis treatment. These registries are the only correct way to determine long term efficacy and possibly provide answers to questions on timing and duration of treatment. Furthermore, convincing efficacy data of existing biologicals and new therapies are needed to persuade insurance companies to endorse biological therapies for selected patients, refractory to standard therapy.

Functional cellular and molecular research gives more insight into key players and determinators of this puzzling disease. With possible triggers and key cellular players as main targets of interest, potential new treatment strategies might be found.

Finally, although we have not found one simple parameter that directs any individual patient to the correct treatment strategy, this thesis does underline the importance of the presence of severe and inflammatory active disease to achieve an optimal treatment effect from second or third-line therapy. Therefore, we propose that in addition to symptoms, functional tests and radiological imaging, activity markers in serum and ^{18}F-FDG PET should have a pivotal role in clinical decision

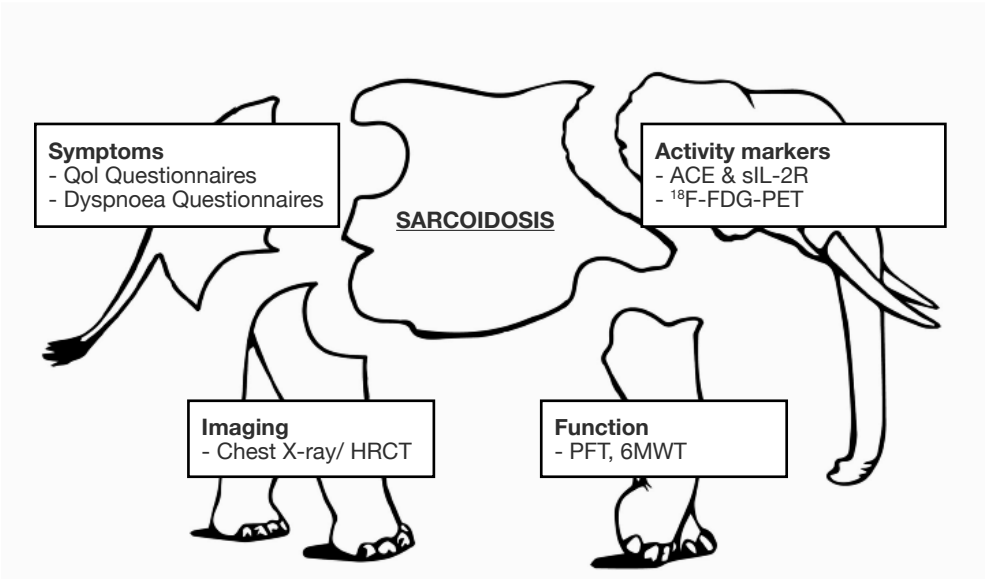

Figure 1: Schematic proposition of clinical decision making in pulmonary sarcoidosis
Sarcoidosis management can be compared to the ancient Indian tale of the six blind men and the elephant. One needs to be able to see all parts of the puzzle to get a complete overall image of disease severity and need for therapeutical intervention.

QoI= Quality of life, HRCT= high resolution computed tomography, ACE= angiotensin converting enzyme, sIL-2R= serum Interleukin-2 receptor, ^{18}F-FDG PET= ^{18}F-fluorodeoxyglucose by positron emission tomography, PFT= pulmonary function test, 6MWT= six minute walking test.

making in severe sarcoidosis treatment. Sarcoidosis management can be compared to the ancient Indian tale of the six blind men and the elephant (Figure 1). The physician needs to see all parts of the puzzle to determine it is in fact sarcoidosis and to establish disease severity and need for therapeutic intervention.

The data presented in this thesis will hopefully encourage further investigation of new and existing therapeutic strategies for severe sarcoidosis. Ideally, international cooperation could play a pivotal role in establishing treatment effects in severe and refractory sarcoidosis. The key may lie in implementing different treatment strategies for patients with different phenotypes or even genotypes. One step at a time, global cooperative research will lift sarcoidosis treatment from largely experience and eminence based medicine towards more evidence based medicine.

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DUTCH SUMMARY /
NEDERLANDSE SAMENVATTING

AFFILIATIONS OF THE AUTHORS

LIST OF SCIENTIFIC
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PRESENTATIONS

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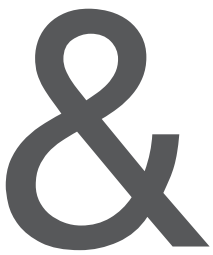
CURRICULUM VITAE



NEDERLANDSE SAMENVATTING / DUTCH SUMMARY

SYSTEMISCHE BEHANDELSTRATEGIEËN VOOR ERNSTIGE SARCOÏDOSE

INTRODUCTIE

Sarcoïdose is een ziekte van nog onbekende oorzaak waarbij ontstekingen ontstaan in verschillende onderdelen van het lichaam. In bijna alle organen kunnen deze ontstekingen voorkomen, maar ze zijn het vaakst te vinden in de longen. Andere organen waarin de ziekte kan voorkomen zijn bijvoorbeeld de ogen, het hart en het zenuwstelsel. De klachten bij sarcoïdose kunnen zeer uiteenlopend zijn en zijn afhankelijk van het orgaan waarin de ontstekingen zich bevinden. De meest voorkomende klachten zijn moeheid, koorts, droge hoest, benauwdheid, pijn op de borst en gewichtsverlies. Sarcoïdose komt voor bij zowel mannen als vrouwen en bij mensen van alle thniciteiten. Patiënten met een donkere huidskleur hebben echter vaker een ernstigere vorm van sarcoïdose.

Hoewel de ziekte al meer dan 100 jaar bekend is, blijft het stellen van de diagnose een uitdaging. Dit gebeurt op meestal op basis van passende symptomen en/of verschijnselen, een passend radiologisch beeld, een biopt met daarin granulomen (ophopingen van ontstekingscellen) en door uitsluiten van andere oorzaken van granuloomvorming in het lichaam zoals bijvoorbeeld infecties. Aangezien sarcoïdose bij het merendeel van de patiënten een gunstig beloop heeft en vanzelf over kan gaan, hoeft de ziekte niet altijd behandeld te worden. Er is echter een groep patiënten bij wie de ziekte zeer ernstig is en kan leiden tot orgaanschade en in sommige gevallen zelfs overlijden. Deze patiënten met ernstige ziekte moeten zeker behandeld worden. Omdat de oorzaak van de ziekte nog niet bekend is, zijn er op dit moment geen medicijnen die de ziekte kunnen genezen. Wel zijn er middelen die het immuunsysteem, en daarmee de klachten, kunnen onderdrukken of remmen. Het is bij sarcoïdose echter nog niet goed onderzocht welk medicijn het beste resultaat geeft.

Aangezien bij 90% van alle patiënten de longen in meer of mindere mate zijn aangedaan, is het nuttig om tijdens het verloop van de ziekte en tijdens therapie herhaaldelijk de longfunctie te meten. De longfunctiemeting vertrelt hoe goed de longen werken qua in en uitstroom van lucht en ook qua opname van zuurstof. De longfunctie is echter niet bij alle vormen van sarcoïdose een goede maat voor ernst van ziekte, zoals bijvoorbeeld bij sarcoïdose van het hart of zenuwstelsel. Bij deze vormen zullen ook andere testen gedaan moeten worden.

Naast orgaanfunctie wordt in de specialistische praktijk ook gekeken naar de mate van de avtiviteit van de ontstekingscomponent van de ziekte. Een actieve sarcoidose, waarbij veel ontstekingscellen in het lichaam actief zijn, kan vergeleken worden met een brandend bos. In dit soort gevallen heeft het nut om de ontstekingscellen te remmen, oftewel de ontsteking te “blussen”. Na behandeling kan de actieve sarcoïdose tot rust komen en veranderen in een zogenoemde sarcoïdose in remissie, wat vergeleken kan worden met een uitgeblust bos. Er kan soms wel littekenvorming achter blijven, ook wel fibrose genoemd, maar er kan ook restloze genezing optreden. Om per patiënt onderscheid te kunnen maken tussen actieve sarcoïdose en sarcoïdose in remissie, maken we gebruik van bloedmarkers, meetbare stoffen in het bloed, die ziekte activiteit kunnen opsporen

en ook van een scan die ziekte activiteit in beeld kan brengen, de 18F-FDG PET scan. Dit is een scan waarbij door middel van radioactief suiker kan worden bepaald waar in het lichaam veel energie gebruikt wordt en dus de ziekte actief is. De waarde van deze bloedmarkers en 18F-FDG PET scan in de monitoring van sarcoïdose behandeling zijn echter nog niet goed onderzocht.
Het doel van dit promotieonderzoek is het in kaart brengen van de verschillende behandelmogelijkheden voor patiënten met een ernstige vorm van sarcoïdose die niet reageert op de standaard therapie. Verder wordt in dit proefschrift gekeken of het mogelijk is te voorspellen bij welke patiënten de verschillende medicijnen het beste resultaat geven.

Hoofdstuk 2 geeft een overzicht van de verschillende stappen in de behandeling van sarcoïdose. Bij het merendeel van alle patiënten gaan de symptomen van sarcoïdose binnen 2-3 jaar vanzelf over, er is echter ook een groep patiënten bij wie de ziekte chronisch wordt. In deze groep van patiënten kan de ziekte zeer ernstig en zelfs dodelijk zijn. Indien er sprake is van sterk verminderde kwaliteit van leven of bedreiging van orgaanfunctie is er een reden tot starten van behandeling.
Omdat de oorzaak van sarcoïdose niet bekend is bestaat er nog geen genezende therapie. Prednison, een algemeen ontstekingsremmend medicijn, is het eerste middel dat gegeven wordt. Een nadeel is dat het bij chronisch gebruik veel bijwerkingen geeft, zoals dunne huid, gewichtstoename en ontregeling of ontstaan van suikerziekte. Wanneer prednison niet effectief is of te veel bijwerkingen geeft wordt een tweedelijns middel toegevoegd aan de behandeling. Methotrexaat is wereldwijd het meest gebruikte tweedelijns middel bij sarcoïdose. Gebruik van methotrexaat maakt het mogelijk de dosis prednison te verlagen en verbetert de longfunctie. Andere tweedelijns middelen zijn azathioprine en leflunomide, maar het is niet bekend welk van deze middelen de beste keus is. Bij patiënten met ernstige sarcoïdose bij wie tweedelijns therapie niet werkt of veel bijwerkingen geeft kan met een biologisch medicijn zoals infliximab gestart worden. Biologische medicijnen zijn nieuwe medicijnen die, anders dan normale medicijnen, gemaakt worden in een levend organisme in plaats van via een scheikundige bewerking. Biologische medicijnen kunnen een specifiek deel van het immuunsysteem blokkeren, in plaats van het hele immuunsysteem remmen zoals bijvoorbeeld prednison. Welke patiënten het meeste baat hebben bij biologische therapie met infliximab is nog niet bekend. In dit hoofdstuk bespreken we daarnaast de beperkingen van de op dit moment beschikbare therapieën voor sarcoïdose. In de toekomst zal door meer internationale samenwerking een beter inzicht moeten komen in welke therapie voor welke sarcoïdose patiënt het meest geschikt is.

In **Hoofdstuk 3** wordt de tweedelijns therapie van sarcoïdose beschreven en worden resultaten van de eerste vergelijkende studie tussen twee middelen, methotrexaat en azathioprine, gepresenteerd. Omdat chronisch gebruik van prednison veel bijwerkingen geeft, wordt bij ernstig zieke patiënten vaak een tweedelijns middel toegevoegd. Het is echter niet bekend welk van deze medicijnen het meest effectief is. Het doel van deze studie was het vergelijken van de twee meest gebruikte tweedelijns middelen. In een internationale samenwerking tussen het St.

Antonius Ziekenhuis Nieuwegein, Nederland en de Katholieke Universiteit Leuven, België, werden alle sarcoïdosepatiënten die behandeld werden met methotrexaat of azathioprine vergeleken in een retrospectieve cohort studie. In totaal werden 200 patiënten geïncludeerd, waarvan 145 patiënten methotrexaat kregen en 55 patiënten azathioprine. De belangrijkste uitkomst was het prednison sparend effect, oftewel het vermogen om prednison af te bouwen na starten van het tweedelijns medicijn. Hieruit bleek dat de dagelijkse prednison dosering significant daalde met 6.32 mg/jaar ($p<0.0001$) in beide groepen. Daarnaast lieten de longfunctieparameters een kleine maar significante stijging zien. Wel traden er bij beide middelen veel bijwerkingen op, waarbij maag-darm klachten het meest voorkwamen in beide groepen. Er traden significant meer infecties op in de azathioprine groep (34.6 vs 18.1%, $p=0.01$).
Concluderend laat deze retrospectieve studie zien dat zowel methotrexaat als azathioprine effectief zijn als tweedelijns middel bij sarcoïdose, aangezien ze een prednison sparend effect hebben en ook een verbetering geven van longfunctie. In de azathioprine groep traden echter vaker infecties op.

Hoofdstuk 4 wordt beschreven welke patiënten de meeste verbetering in longfunctie hebben na het starten van methotrexaat. Ziekteactiviteit kan gemeten worden door middel van de bloedmarkers 'angiotensin converting enzyme' (ACE) and 'soluble Interleukin-2 Receptor' (sIL-2R). In een studie met 114 patiënten die met het tweedelijns middel methotrexaat behandeld werden zagen we dat de markers ACE en sIL-2R beiden sterk daalden onder therapie ($p<0.0001$). Ook was er een correlatie tussen daling in ACE en verbetering van diffusiecapaciteit van de long ($R=0.63$, $p<0.0001$). Daarnaast was een hoge waarde van ACE voorafgaand aan starten van therapie een voorspeller van een grotere longfunctie verbetering na therapie ($R=0.57$, $p<0.0001$). Hieruit blijkt dat deze biomarkers kunnen helpen bij het maken van moeilijke beslissingen rondom starten van therapie bij ernstige sarcoïdose patiënten.

In **Hoofdstuk 5** beschrijven we de ervaring in ons centrum met het biologische derdelijns middel infliximab. Dit middel wordt gegeven wanneer een patiënt ondanks eerste en tweedelijns therapie nog steeds ernstige of progressieve ziekte heeft. Uit resultaten van één eerdere grote studie kwam naar voren dat infliximab een kleine verbetering in longfunctie kan geven. In het onderzoek beschreven in dit hoofdstuk wilden we daarnaast kijken of infliximab ook de ziekte-activiteit verlaagt en de kwaliteit van leven verbetert. Voor deze studie werden 47 patiënten die behandeld waren met infliximab retrospectief geanalyseerd. Na een half jaar infliximab therapie steeg de longfunctieparameter VC significant met 7.6% ($p<0.0001$). Deze verbetering van de longfunctie is groter dan eerder in de literatuur beschreven werd. Daarnaast zagen we een significante afname van de ziekteactiviteit zoals gemeten in bloed middels de markers ACE en sIL-2R. Ook zagen we een significante afname in ziekte activiteit van 2.7 ($p<0.00005$) op de 18F-FDG PET scan. Na een half jaar infliximab therapie zagen we significante verbetering op twee subschalen van kwaliteit van leven: de subschaal moeheid en de subschaal fysiek functioneren.

Concluderend is infliximab therapie niet alleen effectief in het verbeteren van longfunctie, maar vermindert infliximab ook de ziekte activiteit en verbetert de kwaliteit van leven.

Infliximab therapie wordt ook gebruikt bij andere ziekten die te maken hebben met over activiteit van het immuunsysteem zoals de ziekte van Crohn, psoriasis of reuma. Hierbij is gezien dat er tijdens de therapie soms antistoffen gevormd worden tegen de celkern. Het hebben van deze antinucleaire antistoffen (ANA's) heeft mogelijk een relatie met respons op de therapie en met de vorming van antilichamen gericht tegen infliximab. Antistoffen tegen infliximab zijn van klinisch belang omdat ze infliximab onwerkzaam maken. Het is niet bekend of bij sarcoïdose ook ANAs gevormd worden tijdens de behandeling met infliximab.
In **hoofdstuk 6** beschrijven we een retrospectief cohort onderzoek bij 39 sarcoïdose patiënten die langere tijd met infliximab behandeld zijn. Slechts één patiënt had een verhoogde ANA waarde voorafgaand aan therapie. Tijdens therapie stegen slechts bij vier patiënten in milde mate de ANA waarden. Wel werden tijdens de behandelperiode bij tien patiënten (28.6%) antistoffen gevonden gericht tegen infliximab. Dit onderzoek laat zien dat hoewel een relatief hoog percentage van de patiënten antistoffen ontwikkelde tegen infliximab, dit niet samen hangt met verhoogde ANA waardes bij aanvang van therapie noch met de ontwikkeling van verhoogde ANA waarden tijdens therapie. De vorming van ANAs tijdens infliximab therapie is dus mogelijk ziekte specifiek.

In **Hoofdstuk 7** worden de resultaten van een prospectieve studie naar het effect van infliximab behandeling bij ernstige sarcoïdose gepresenteerd. Er werden 56 patiënten geïncludeerd welke eerder niet reageerden op standaard eerste- en tweedelijns therapie. Na zes maanden infliximab therapie werd een goede respons gevonden met een gemiddelde verbetering van de longfunctiemarker FVC van 6.6% van voorspeld (p=0.0007). De spiegel van infliximab in het bloed was hoog bij bijna alle patiënten maar er was geen relatie tussen de infliximabspiegel en respons op therapie. Wel werd een verband gevonden tussen een hoge mate van ziekte activiteit op de 18F-FDG PET scan en een goede verbetering van de longfunctie (R=0.62; p=0.0004). Deze studie laat zien dat 18F-FDG PET kan bijdragen aan klinische besluitvorming rondom de behandeling van ernstige sarcoïdose met infliximab.

In **Hoofstuk 8** wordt dieper ingegaan op de derdelijns therapie met infliximab bij ernstige sarcoïdose. In eerdere hoofdstukken zagen we dat dit medicijn een gunstig effect heeft op de longfunctie na een half jaar therapie. Het is echter niet bekend hoe lang infliximab gegeven moet worden en hoeveel patiënten een recidief van ziekte ontwikkelen na staken van dit middel. In deze retrospectieve studie werden 47 patiënten geïncludeerd die na minstens 6 maanden behandeling gestopt waren met infliximab. Na het stoppen met infliximab therapie ontwikkelde 62% van alle patiënten een recidief van ziekte. De mediane tijd tot optreden van het recidief was 11.1 maanden, waarbij 25% van alle patiënten al een recidief had binnen 4 maanden. Voorspellers van de recidiefkans waren hoge activiteit op 18F-FDG PET scan met een hazard ratio van 3.77 (p<0.001)

en een hoge waarde van serum sIL-2R in het bloed met een hazard ratio (HR) van 2.24 (p=0.033). In multivariate analyse, bleek hoge activiteit op 18F-FDG PET scan een onafhankelijke voorspeller voor de kans op een recidief (HR 4.33, p<0.001). Deze resultaten laten zien dat patiënten met veel ziekte activiteit bij aanvang van de infliximab therapie nauwlettend gecontroleerd dienen te worden indien deze behandeling gestaakt wordt.

Tumor necrosis factor (TNF) is een signaaleiwit dat een belangrijke rol speelt in het ontstekingsproces van sarcoïdose. TNF geeft signalen door aan cellen via receptoren die aanwezig zijn op de celwand. Infliximab is een biologisch medicijn specifiek gericht tegen TNF. In **Hoofdstuk 9** worden de resultaten beschreven van een onderzoek naar het aantal TNF-receptoren (TNFRs) op de buitenkant van een bepaalde soort witte bloedcellen, de monocyten. Binnen de monocyten worden sinds kort drie subpopulaties onderscheiden: de 'classical', 'intermediate' en 'non-classical' monocyten. Zowel bij sarcoïdosepatiënten als bij gezonde vrijwilligers hadden de 'intermediate' monocyten op hun celoppervlak de hoogste expressie van TNFR1 en de 'non-classical' monocyten hadden de hoogste expressie van TNFR2. Verder was opvallend dat patienten een hoger aantal 'intermediate' monocyten hadden dan gezonde vrijwilligers. Deze bevindingen suggereren dat de verschillende subpopulaties van monocyten ook functioneel van elkaar verschillen. Mogelijk kan dit samenhangen met het effect van anti-TNF therapie zoals infliximab bij sarcoïdose.

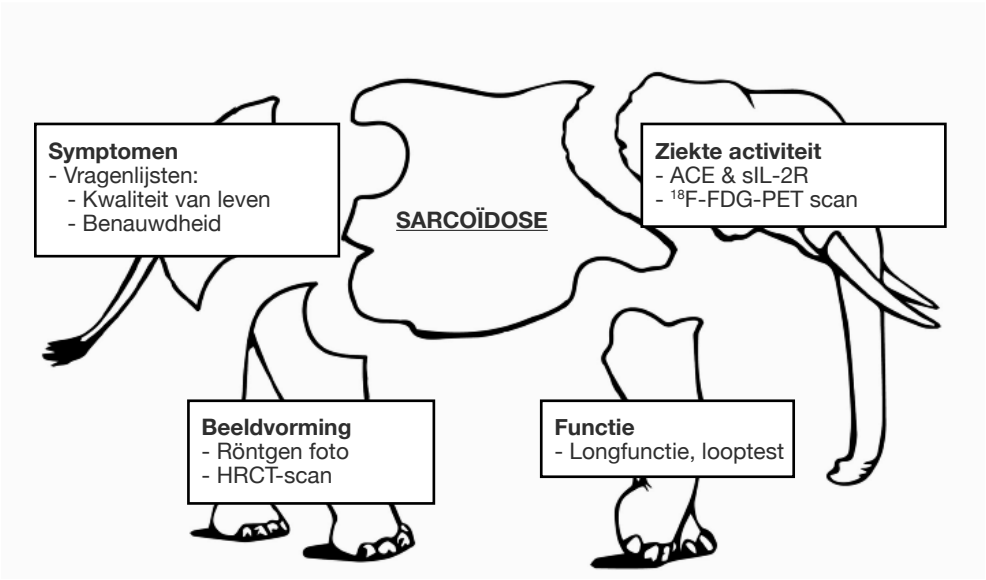
Hoofdstuk 10 onderzoekt wat er gebeurt met monocyten en TNFR expressie tijdens infliximab therapie. Hiervoor is bloed verzameld van 38 sarcoïdose patiënten die gedurende zes maanden een behandeling met infliximab ondergingen en van 18 gezonde controles. Het percentage monocyten bij aanvang van de behandeling bleek verband te houden met de hoogte van biomarkers ACE en sIL-2R. Verder werd gezien dat een subpopulatie van de monocyten, de 'non-classical' monocyten, na 6 maanden therapie sterk daalde van 15.6% naar 12.3% (p=0.0063). Daarnaast daalde het percentage van 'classical' monocyten dat TNFR1 tot uiting bracht (p=0.0065), terwijl expressie van TNFR2 stabiel bleef. Bij de analyse van een ander receptor op het celoppervlak, de chemokine receptor CCR5, bleek dat de gemiddelde expressie hiervan op 'non-classical' en 'intermediate' monocyten significant steeg gedurende de therapie. Ook werd een relatie gevonden tussen CCR5 en CCR2 op 'non-classical' monocyten en mate van algehele respons op therapie. Deze bevindingen wijzen op een verschillende rol voor de drie monocyt subpopulaties tijdens therapie en mogelijk ook tijdens het ontstaan van sarcoïdose.

In **Hoofdstuk 11** worden twee casus gepresenteerd uit de praktijk van de behandeling van ernstige sarcoïdose met het biologische medicijn infliximab. **Hoofdstuk 11.1** laat zien dat sarcoïdose een ziekte is die zich in vele organen of lichaamsdelen kan uiten zoals in dit geval de oorlel. De ontstoken oorlel was bij de patiënt zo pijnlijk dat hij hiervoor behandeld moest worden. Omdat lokale injecties en behandeling met eerste en tweedelijns therapie niet hielpen werd overgegaan tot behandeling met infliximab. Op de 18F-FDG PET scan voorafgaand aan de therapie was duidelijk te zien dat in

de oorlel hoge ontstekingsactiviteit aanwezig was. Na behandeling waren de activiteit alsmede de klachten van de patiënt verdwenen. Deze casus illustreert zowel de soms bijzondere manifestaties van sarcoïdose en illustreert de waarde van de 18F-FDG PET scan om lokalisaties van sarcoïdose buiten te longen, en de reactie op infliximab, objectief aan te tonen.
In **Hoofdstuk 11.2** wordt getoond dat infliximab soms ernstige bijwerkingen kan hebben in patiënten die daarvoor uitzonderlijk gevoelig zijn. We beschrijven de casus van een patiënte met ernstige sarcoïdose, niet reagerend op standaard therapie. Kort nadat zij is gestart met infliximab therapie krijgt zij een kwaadaardige tumor op één van haar stembanden welke werd verwijderd. Omdat dit zo snel is ontstaan na het starten van therapie wordt na multidiciplinair beraad een relatie tussen infliximab en het ontstaan van de tumor niet aannemelijk geacht. Later ontstaat een tweede tumor op de andere stemband welke eveneens wordt verwijderd. Aangezien deze patiënte geen risicofactoren had voor het ontwikkelen van een keeltumor zoals roken of alcohol gebruik is een verband met de gebruikte anti-sarcoïdose medicatie niet uit te sluiten. Voorzichtigheid lijkt daarom geboden bij patiënten met kwaadaardige ziekten in de voorgeschiedenis wanneer infliximab gestart wordt.

CONCLUSIE

In dit proefschrift hebben we verschillende therapeutische opties voor moeilijk behandelbare sarcoïdose onderzocht. Hieruit komt naar voren dat tweedelijns immuunsysteem onderdrukkende middelen het best werken bij sarcoidosepatiënten die bewezen ontstekingsactiviteit hebben zoals bijvoorbeeld te meten is met de biomarkers ACE en sIL-2R in het bloed. Ook laat dit proefschrift zien dat derdelijns therapie met het biologische medicijn infliximab beter werkt dan tot nu toe gedacht, met name bij patiënten met veel ziekte activiteit zoals te meten met een 18F-FDG PET scan. Daarnaast hebben juist deze patiënten met hoge activiteit op de 18F-FDG PET scan veel kans om een terugval te krijgen wanneer infliximab gestopt wordt.
Om sarcoïdose in de toekomst beter te kunnen behandelen is meer wetenschappelijk inzicht nodig. De moeilijkheid bij de behandeling van deze ziekte zit in meerdere factoren. Ten eerste is de oorzaak van sarcoïdose nog steeds niet gevonden. Bovendien lijken meerdere oorzaken bij verschillende patiënten een rol te spelen in het ontstaan van het ziekteproces. Hierdoor is een genezende behandeling momenteel niet voorhanden.
Een ander probleem is het ontbreken van duidelijke richtlijnen voor monitoren van behandeleffect en klinische beslissingen rondom behandelindicaties. Omdat bij sarcoïdose nog niet één allesomvattende klinische, radiologische of laboratorium parameter is die duidelijk richtinggevend is, wil ik naar aanleiding van dit proefschrift de vergelijking maken met het oude Indiase verhaal van de zes blinde mannen en de olifant (Figuur 1). De moraal van dit verhaal is dat men het complete beeld moet kunnen zien, om een goede inschatting te kunnen maken. Meerdere delen van een puzzel moeten hiervoor bij elkaar komen. Voor het beoordelen van de ernst van de sarcoïdose, de mate van ziekte activiteit en de impact die de ziekte heeft op het leven van de patiënt, heeft de behandelend arts daarom het totaalpakket nodig van functionele parameters, activiteitsparameters en maten van kwaliteit van leven.



Figuur 1: Schematisch voorstel voor klinische besluitvorming rondom long sarcoïdose
Dit voorstel werd gebaseerd op het verhaal van zes blinde mannen en de olifant. Om een goed totaalbeeld te krijgen van de toestand van de patiënt met long sarcoïdose, zullen alle facetten beoordeeld moeten worden.

HRCT-scan = high resolution computed tomography scan, ACE= angiotensin converting enzyme, sIL-2R= serum interleukin-2 receptor, 18-FDG PET scan= 18F-fluorodeoxyglucose by positron emission tomography scan.

Tenslotte is de groep patiënten met ernstige sarcoïdose welke niet op standaard therapie met prednison reageert relatief klein. Dit genereert onvoldoende aandacht van farmaceutische bedrijven die voor innovatie zouden kunnen zorgen en veroorzaakt kleine patiënten aantallen in de spaarzame studies die verricht worden. De meeste kennis komt op dit moment voort uit observationele cohortstudies en meningen van experts. Hierdoor is de behandeling van sarcoïdose nog lang geen ‘evidence based medicine’. Meer (internationale) samenwerking zou er in de toekomst voor moeten zorgen dat er juist voor deze kleine groep patiënten met een zeer slechte prognose meer goede behandelopties komen.

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POSTER PRESENTATIONS

2014 Dynamic changes in monocyte subsets during infliximab therapy in sarcoidosis. World Association of Sarcoidosis and other Granulomatous Disorders conference, Kusadasi, Turkey.

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CHAIR

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European Respiratory society international congress, Barcelona, Spain.

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Het boek is klaar, en nu? Eerst maar eens verder met de opleiding tot longarts, maar de wetenschap is nog niet van me af….

CURRICULUM VITAE

Renske (Adriane Dore Marie) Vorselaars, was born on August 11th 1984 in Tilburg. After finishing Gymnasium ‘Cum Laude’ at Theresia Lyceum Tilburg, she started Medicine at Utrecht University in 2002.

During her studies she did an internship at the department of paediatrics at Hospital Kuala Lumpur, Malaysia and volunteered at Young Africa Skills centre, Beira, Mozambique.

From June 2009 she worked as a resident at the department of pulmonology, Hofpoort ziekenhuis Woerden, where her interest in pulmonology was generated. In June 2010 she started as a resident at the department of pulmonology of St Antonius Hospital, Nieuwegein (head Prof. dr. J.M.M. Van den Bosch†). From January 2011 she worked as a research fellow in the Center of Interstitial Lung Diseases (head Prof. dr. J.C. Grutters), to study treatment options in refractory sarcoidosis, which was the foundation of this thesis. For a part of this research she worked at the pulmonary department of University Hospitals Leuven, Belgium (head Prof. dr. W.A. Wuyts).

In May 2013 she started her specialist training in pulmonary medicine at St Antonius Hospital, Nieuwegein (head Dr. F.M.N.H Schramel), which commenced with two years of training in internal medicine (head Dr. A.B.M. Geers). Her specialist training was interrupted for six months in 2014 to finish this thesis. During her residency she was active as a board member of the hospitals Resident society ‘De Antoniaan’ and as a founding member of the hospitals PhD-club ‘De Promovendiclub’. Part of her research was awarded with an International Trainee scholarship Award of the American Thoracic Society at the annual ATS Conference 2014 in San Diego.